22nd Century Medical Center Donation Course

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Donation company: Kaatsu Japan Co., Ltd



Course opened : October 2004 ~ September 2014 (10-year maturity)

◆ Donation course name

Ischemic Circulatory Physiology, Kaatsu Training

◆Donation course instructor

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♦Purpose of course establishment

The course carries out multifaceted examination and verification research on innovative exercise methods represented by KAATSU training. Based on the results, we promote health maintenance and promotion activities in Japan, and widely advocate a model for reducing medical expenses as a social contribution.

Research

It goes without saying that prolonging healthy life span is a social issue in Japan, where the aging of society is rapidly progressing. Under the keywords of health promotion, disease prevention, and long-term care prevention, it is urgent that industry, government, and academia work together to reduce social security burden. It is a social role that is required for all organizations in the health and medical field. In such a situation, the usefulness of exercise is high from the medical point of view of prevention and treatment, and development and diffusion of a science-based exercise method with strong intention more than ever is strongly desired.

KAATSU training is performed by pressurizing the lower limbs or upper limbs with a pneumatic pressure band, and exercise with moderate blood flow restriction is expected to have a muscle hypertrophic effect even under short-term and light loads. It is a rehabilitation method suitable for society. In addition, since the endocrine system is activated, including growth hormone, depending on the type of disease, a direct improvement effect is expected. Furthermore, the pooling action of the lower extremity blood by pressurization has the same standing stimulation effect as lower body negative pressure (LBNP), so it seems to have the cardiovascular system deconditioning preventing effect for astronauts and long-term bedridden patients. As a result, muscle hypertrophy effect (improvement of disuse atrophy) is expected in patients with various diseases as well as healthy people. Furthermore, this method can be widely used in sports, nursing prevention, clinical and space medicine in Japan. It is also expected to be applied to cardiac rehabilitation muscle strength training and orthostatic dysregulation (OD) patients.

Animal experiments are also conducted to elucidate the mechanism of KAATSU training. As its underlying mechanism, promotion of muscle protein synthesis, and suppression of degradation systems have been reported, but the details are unknown. Therefore, we aim to elucidate the molecular mechanism of the effect of this method. We also examined the chronic effects and their molecular mechanisms. KAATSU ttraining was considered to be a useful method in measures against sarcopenia in the elderly.

◆ 22nd Century Medical Center (Excerpt from Tokyo University

Hospital website)

At the University of Tokyo Hospital, a variety of initiatives aimed at advanced medical research and development are underway. As part of that, in order to research

and develop new clinical medicine or medical related services in the field of the University of Tokyo Hospital, the establishment of a 22nd century medical center is planned in 2002. In 2004, the course was opened and activities started, and in April 2006, it was officially launched as the center of the University of Tokyo Hospital. The Central Medical Center 2 is the base of activities from September 2006, and consists of a donation course and a social cooperation course.

Research activities include the development of new diagnostic methods and treatments, the spread and verification of therapeutic methods, preventive medicine, medical policy, medical equipment and facilities provided through actual clinics and services. These research activities occupy a position close to practical use and social return among medical and medical research, and play a central role in translational research promoted by the University of Tokyo Medical School and Medical School at the Hongo Campus. In addition, by promoting industry-academia collaboration and various joint research, it plays a role to improve the global presence.

Major clinical achievements

I. About the effect and safety of KAATSU training

• Because KAATSU muscle training promotes secretion of growth hormone in acute exercise and is a special training method called blood flow restriction, we examined the influence on hemodynamics during training.

Takano H, Morita T, Iida H, Asada K, Kato M, Uno K, Hirose K, Matsumoto A, Takenaka K, Hirata Y, Eto F, Nagai R, SatoY, Nakajima T 2005. Hemodynamic and hormonal responses to a short-term low-intensity resistance exercise with the reduction of muscle blood flow. Eur J Appl Physiol. 95: 65-73.

We investigated the hemodynamic and hormonal responses to a short-term low-intensity resistance exercise (STLIRE) with the reduction of muscle blood flow. Eleven untrained men performed bilateral leg extension exercise under the reduction of muscle blood flow of the proximal end of both legs pressure-applied by a specially designed belt (a banding pressure of 1.3 times higher than resting systolic blood pressure, 160-180 mmHg), named as Kaatsu. The intensity of STLIRE was 20% of one repetition maximum. The subjects performed 30 repetitions, and after a 20-seconds rest, they performed three sets again until exhaustion. The superficial femoral arterial blood flow and hemodynamic parameters were measured by using the ultrasound and impedance cardiography. Serum concentrations of growth hormone (GH), vascular endothelial growth factor (VEGF), noradrenaline (NE), insulin-like growth factor (IGF)-1, ghrelin, and lactate were also measured. Under the conditions with Kaatsu, the arterial flow was reduced to about 30% of the control. STLIRE with Kaatsu significantly increased GH (0.11+-0.03 to 8.6+-1.1 ng/ml, P < 0.01), IGF-1 (210+-40 to 236+-56 ng/ml, P < 0.01), and VEGF (41+/-13 to 103+/-38 pg/ml, P < 0.05). The increase in GH was related to neither NE nor lactate, but the increase in VEGF was related to that in lactate (r = 0.57, P < 0.05). Ghrelin did not change during the exercise. The maximal heart rate (HR) and blood pressure (BP) in STLIRE with Kaatsu were higher than that without Kaatsu. Stroke volume (SV) was lower due to the decrease of the venous return by Kaatsu, but, total peripheral resistance (TPR) did not change significantly. These results suggest that STLIRE with Kaatsu significantly stimulates the exercise-induced GH, IGF, and VEGF responses with the reduction of cardiac preload during exercise, which may become a unique method for rehabilitation in patients with cardiovascular diseases.

Pub med is the first dissertation to be searched first in KAATSU, and so far more than 100 citations have been seen.

• Research to verify the safety of KAATSU training

a) Research from national questionnaire survey

Nakajima T et al. 2006. Use and safety of KAATSU training: Results of a national survey. Int J KAATSU Training Res 2: 5-14.

KAATSU training is a novel training, which is performed under conditions of restricted blood flow. It can induce a variety of beneficial effects such as increased muscle strength, and it has been adopted by a number of facilities in recent times. The purpose of the present study is to know the present state of KAATSU training in Japan and examine the incidence of adverse events in the field. The data were obtained from KAATSU leaders or instructors in a total of 105 out of 195 facilities where KAATSU training has been adopted. Based on survey results, 12,642 persons have received KAATSU training (male 45.4%, female 54.6%). KAATSU training has been applied to all generations of people including the young (80 years old). The most popular purpose of KAATSU training is to strengthen muscle in athletes and to promote the health of subjects, including the elderly. It has been also applied to various kinds of physical conditions, cerebrovascular diseases, orthopedic diseases, obesity, cardiac diseases, neuromuscular diseases, diabetes, hypertension and respiratory diseases. In KAATSU training, various types of exercise modalities (physical exercise, walking, cycling, and weight training) are used. Most facilities have used 5-30 min KAATSU training each time, and performed it 1-3 times a week. Approximately 80% of the facilities are satisfied with the results of KAATSU training with only small numbers of complications reported. These results indicate that the KAATSU training is a safe and promising method for training athletes and healthy persons, and can also be applied to persons with various physical conditions.

b) Examination about influence of KAATSU training on coagullaion, and fibrinolytic system

The following studies were conducted to further confirm the safety. Although the subject was a healthy person, the study was added under several conditions. Conducted as a joint research with Japan Aerospace Exploration Agency (JAXA).

Nakajima T, et al 2007. Effects of KAATSU training on haemostasis in healthy subjects. Int J KAATSU Training Res 3: 11-21.

Purposes: The KAATSU training is performed under the reduction of muscle blood flow by a specially designed belt (KAATSU belt), which induces blood pooling in capacitance vessels by restricting venous return. However, no prior studies have examined the effects of KAATSU training on haemostasis. The purpose of the present study was to investigate acute effects of KAATSU training on haemostasis including fibrinolytic responses in healthy subjects. Methods: Two protocols have been performed. (1) 6 healthy men (mean age= 48 ± 5 yr) performed KAATSU (160 mmHg) of both thighs for 15 minutes and then KAASTU training combined with low-intensity leg and foot aerobic exercises for ~ 10 minutes in hypobaric chamber, which mimics 8000 feet in airflight. (2) Another 7 men (mean age= 30 ± 4 yr) performed leg press exercises (30 % 1 RM) with and without KAATSU of both thighs 24 h after bed rest. Blood samples were taken at rest, immediately after KAATSU, and exercises with or without KAATSU, and after exercise. For the investigation of blood fibrinolysis, determinations of tissue-type plasminogen activator (tPA) activity or antigen, plasminogen activator inhibitor (PAI)-1 activity or antigen, fibrin degradation product (FDP) and D-dimer were used. Prothrombin time (PT) and platelet counts were also measured. Results: (1) In hypobaric chamber, KAATSU by itself significantly increased tPA activity, while PAI-1 activity was unchanged. Furthermore, immediately after the exercise, tPA activity increased significantly. (2) During the exercises combined with KAATSU 24 h after bed rest, tPA antigen significantly increased, compared with control exercises, but PAI-1 antigen was unchanged. In both cases, KAATSU training did not induce fibrin formation as assessed by fibrin D-dimer and FDP. Conclusions: This study indicates that potentially favorable changes occur in fibrinolytic factors after KAATSU and KAATSU training in healthy subjects.

•Examination of influence of hemodynamics by KAATSU training of lower limbs

The above-mentioned study revealed the effect of KAATSU on venous blood pooling in the lower limbs. This led to the following study. In outer space, weightlessness causes the blood to shift to the head and body center, which is responsible for the deconditioning of the circulatory system. Therefore, we examined the effects of KAATSU on hemodynamics and the effects on various hormones. As a result, depending on the applied pressure, blood accumulation in the lower limbs is observed, and at 150 mmHg or more, accumulation in the lower limbs occurs more than standing stimulation, and hemodynamically standing stimulation is given in the prone position.

Iida H, Kurano M, Takano H, Oonuma H, Imuta H, Kubota N, Morita T, Meguro K, Sato Y, Abe T, Yamazaki Y, Nakajima T. 2006. Can KAATSU be used for an orthostatic stress in astronauts?: A case study. Int J KAATSU Training Res 2: 45-52.

The application of an orthostatic stress such as lower body negative pressure (LBNP) during exercise has been proposed to minimize the effects of weightlessness on the cardiovascular system and subsequently to reduce the cardiovascular deconditioning. The KAATSU training is a novel method for strength training to induce muscle strength and hypertrophy. KAATSU induces venous pooling of blood in capacitance vessels by restricting venous blood flow. Therefore, to investigate whether KAATSU can be used as an orthostatic stress, we examined the effects of KAATSU on the hemodynamic, autonomic nervous and hormonal parameters in one subject. The several parameters were measured by impedance cardiography; heart rate (HR), mean blood pressure (mBP), stroke volume (SV), cardiac output (CO), total peripheral resistance (TPR), and heart rate variability (HRV). These data were obtained before (pre), during and after (post) pressurization (50 and 200 mmHg) on both thighs with KAATSU mini belts, and compared with those in standing. The serum concentration of noradrenaline (NA) and vasopressin (ADH), and plasma rennin activity (PRA) were also measured. The application of 200 mmHg KAATSU decreased SV, which was almost equal to the value in standing. HR and TPR increased in a similar manner as standing with slight change of mBP. High frequency (HFRR), a marker of parasympathetic nervous activity, decreased during both 200 mmHg KAATSU and standing, while LFRR/HFRR, a quantitative marker of sympathetic nervous activity, increased significantly. During KAATSU and standing, NA, PRA

and ADH increased. These results indicate that the application of KAATSU on both thighs simulates systemic cardiovascular effects of orthostasis in one gravity (1G), and that KAATSU training appears to be a useful method for potential countermeasure like lower body negative pressure (LBNP) against orthostatic intolerance in space flight as well as strength training to induce muscle strength and hypertrophy.

Iida H, Kurano M, Takano H, Kubota N, Morita T, Meguro K, Sato Y, Abe T, Yamazaki Y, Uno K, Takenaka K, Hirose K, Nakajima T. 2007. Hemodynamic and neurohumoral responses to the restriction of femoral blood flow by KAATSU in healthy subjects. Eur J Appl Physiol 100: 275-285.

The application of an orthostatic stress such as lower body negative pressure (LBNP) has been proposed to minimize the effects of weightlessness on the cardiovascular system and subsequently to reduce the cardiovascular deconditioning. The KAATSU training is a novel method to induce muscle strength and hypertrophy with blood pooling in capacitance vessels by restricting venous return. Here, we studied the hemodynamic, autonomic nervous and hormonal responses to the restriction of femoral blood flow by KAATSU in healthy male subjects, using the ultrasonography and impedance cardiography. The pressurization on both thighs induced pooling of blood into the legs with pressure-dependent reduction of femoral arterial blood flow. The application of 200 mmHg KAATSU significantly decreased left ventricular diastolic dimension (LVDd), cardiac output (CO) and diameter of inferior vena cava (IVC). Similarly, 200 mmHg KAATSU also decreased stroke volume (SV), which was almost equal to the value in standing. Heart rate (HR) and total peripheral resistance (TPR) increased in a similar manner to standing with slight change of mean blood pressure (mBP). High-frequency power (HF(RR)) decreased during both 200 mmHg KAATSU and standing, while low-frequency/high-frequency power (LF(RR)/HF(RR)) increased significantly. During KAATSU and standing, the concentration of noradrenaline (NA) and vasopressin (ADH) and plasma renin activity (PRA) increased. These results indicate that KAATSU in supine subjects reproduces the effects of standing on HR, SV, TPR, etc., thus stimulating an orthostatic stimulus. And, KAATSU training appears to be a useful method for potential countermeasure like LBNP against orthostatic intolerance after spaceflight.

Examination about the usefulness as a training of astronauts

Thus, KAATSU was considered to have new application as a training method for astronauts, including the prevention of muscle atrophy in space. Therefore, joint research with two JAXA was conducted.

a) Examination about influence on hemodynamics and various hormones of leg press by the presence or absence of KAATSU in environment simulating weightlessness 24 hours after -6 degrees bed rest

Kubota N, Takano H, Tsutsumi T, Kurano M, Iida H, Yasuda T, Meguro K, Morita T, Sato Y, Kawashima S, Yamazaki Y, Ohshima H, Tachibana S, Ishii N, Abe T, Nakajima T.2008. Resistance exercise combined with KAATSU during simulated weightlessness Int J KAATSU Training Res 4:9-15.

The application of a gravity-specific stress (e.g. LBNP), in combination with exercise, prevents cardiovascular deconditioning in space flight. KAATSU training is a method to induce blood pooling in capacitance vessels by restricting venous return (as with LBNP) and which

when combined with low-intensity resistance (RE) exercise produces remarkable muscle mass and muscle strength gains. The purpose of this study was to investigate the hemodynamic and neurohumonal responses induced by KAATSU in combination with leg RE (30 % 1 RM), during simulated weightlessness (6°head-down tilt for 24 h, n=7). Following 24 h bed rest 6° head-down tilt, body mass was decreased from 75.3 \pm 3.9 to 73.3 \pm 3.8 Kg (P<0.01). Blood volume (BV) and plasma volume (PV) were reduced by $-4.4 \pm 1.4\%$ and $-7.9 \pm 2.5\%$, respectively. During RE, BV and PV were significantly decreased; the changes with KAATSU induced a lower-body venous pooling, resulting in a sustained decrease in stroke volume (SV; from 77.0 \pm 4.4 ml to 55.9 \pm 5.1 ml; P<0.01) that was comparable to resting SV while standing. Consequently, RE heart rate (HR) was greater with KAATSU. The serum concentrations of plasma renin activity (PRA), vasopressin (ADH), noradrenaline (NOR), and lactate were also significantly elevated during RE with KAATSU as compared to control RE. These hemodynamic and neurohumoral responses following head-down tilt and during RE closely approximate the gravity-specific stress observed with LBNP. Thus, when used in combination with RE, KAATSU may be a useful countermeasure in microgravity

Nakajima T et al. 2008. Hemodynamic responses to simulated weightlessness of 24-h head-down bed rest and KAATSU blood flow restriction. Eur J Appl Physiol. 104: 727-737.

The KAATSU training is a unique method of muscle training with restricting venous blood flow, which might be applied to prevent muscle atrophy during space flight, but the effects of KAATSU in microgravity remain unknown. We investigated the hemodynamic responses to KAATSU during actually simulated weightlessness (6 degrees head-down tilt for 24 h, n = 8), and compared those to KAATSU in the seated position before bed rest. KAATSU was applied to the proximal ends of both the thighs. In the seated position before bed rest, sequential incrementing of KAATSU cuff pressure and altering the level of blood flow restriction resulted in a decrease in stroke volume (SV) with an increase in heart rate (HR). KAATSU (150-200 mmHg) decreased SV comparable to standing. Following 24-h bed rest, body mass, blood volume (BV), plasma volume (PV), and diameter of the inferior vena cava (IVC) were significantly reduced. Norepinephrine (NOR), vasopressin (ADH), and plasma renin activity (PRA) tend to be reduced. A decrease in SV and CO induced by KAATSU during the simulated weightlessness was larger than that in the seated position before bed rest, and one of eight subjects developed presyncope due to hypotension during 100 mmHg KAATSU. High-frequency power (HF(RR)) decreased during KAATSU and standing, while low-frequency/high-frequency power (LF(RR)/HF(RR)) increased significantly. NOR, ADH and PRA also increased during KAATSU. These results indicate that KAATSU blood flow restriction reproduces the effects of standing on HR, SV, NOR, ADH, PRA, etc., thus stimulating a gravity-like stress during simulated weightlessness. However, syncope due to lower extremity blood pooling and subsequent reduction of venous return may be induced during KAATSU in microgravity as reported in cases of lower-body negative pressure.

b. Examination of the effect on aerobic capacity and muscle hypertrophy by KAATSU ergometer

In space, an ergometer exercise lasting two and a half hours is performed to prevent muscle atrophy, but it has been found that even such long-term exercise does not sufficiently prevent muscle atrophy. Therefore, a study was conducted on the effect of aerobic exercise and muscle hypertrophy with and without KAATSU by bicycle ergometer exercise for 2 months.

Abe T, Fujita S, Nakajima T, Sakamaki M, Ozaki H, Ogasawara R, Sugaya M, Kudo M, Kurano M, Yasuda T, Sato Y, Ohshima H, Mukai C, Ishii N. Effects of low-intensity cycle training with restricted leg blood flow on thigh muscle volume and VO2max in young men. Journal of Sports Science and Medicine 2010; 9, 452-458.

Concurrent improvements in aerobic capacity and muscle hypertrophy in response to a single mode of training have not been reported. We examined the effects of low-intensity cycle exercise training with and without blood flow restriction (BFR) on muscle size and maximum oxygen uptake (VO2max). A group of 19 young men (mean age \pm SD: 23.0 \pm 1.7 years) were allocated randomly into either a BFR-training group (n=9, BFR-training) or a non-BFR control training group (n=10, CON-training), both of which trained 3 days/wk for 8 wk. Training intensity and duration were 40% of VO2max and 15 min for the BFR-training group and 40% of VO2max and 45 min for the CON-training group. MRI-measured thigh and quadriceps muscle cross-sectional area and muscle volume increased by 3.4-5.1% (P < 0.01) and isometric knee extension strength tended to increase by 7.7% (p < 0.10) in the BFR-training group. There was no change in muscle size ($\sim 0.6\%$) and strength ($\sim 1.4\%$) in the CON-training group. Significant improvements in VO2max (6.4%) and exercise time until exhaustion (15.4%) were observed in the BFR-training group (p < 0.05) but not in the CON-training group (-0.1 and 3. 9%, respectively). The results suggest that low-intensity, short-duration cycling exercise combined with BFR improves both muscle hypertrophy and aerobic capacity concurrently in young men. Key pointsConcurrent improvements in aerobic capacity and muscle hypertrophy in response to a single mode of training have not been reported. In the present study, low-intensity (40% of VO2max) cycle training with BFR can elicit concurrent improvement in muscle hypertrophy and aerobic capacity.

• Examination about the influence on muscle activity and metabolic system by KAATSU training

Under KAATSU training (12 healthy people), despite the low intensity load, muscle activity and blood pH and lactic acid production comparable to high intensity are observed, which is thought to be one of the mechanisms of muscle hypertrophy effect.

Yasuda T, Fujita T, Miyagi Y, Kubota Y, Sato Y, Nakajima T, Bemben MG, Abe T. 2006. Electromyographic responses of arm and chest muscle during bench press exercise with and without KAATSU. Int J KAATSU Training Res 2: 15-19.

The purpose of this study was to compare the EMG activity of blood flow restricted (limb) and nonrestricted (trunk) muscles during multi-joint exercise with and without KAATSU. Twelve (6 women and 6 men) healthy college students [means (SD) age: 24.1 (3.5) yrs] performed 4 sets (30, 15, 15, and 15 reps) of flat bench press exercise (30% of a predetermined one repetition maximum, 1-RM) during two different conditions [with KAATSU and without KAATSU (Control)]. In the KAATSU condition, a specially designed elastic cuff belt (30 mm wide) was placed at the most proximal position of the upper arm and inflated to a pressure of 100% of individual's resting systolic blood pressure. Surface EMG was recorded from the muscle belly of the triceps brachii (TB) and pectoralis major (PM) muscles, and mean integrated EMG (iEMG) was analyzed. During 4 sets of the exercise, gradual increases in iEMG were

observed in both TB and PM muscles for the KAATSU condition. The magnitude of the increases in iEMG in the TB and PM muscles were higher (P<0.05) with KAATSU compared to the Control condition. In the first set, the mean exercise intensity from normalized iEMG was approximately 40% of 1-RM in both Control and KAATSU conditions. However, the mean exercise intensity of both muscles were 60-70% of 1-RM for the KAATSU condition and only about 50% of 1-RM for the Control condition, respectively, during the fourth set. We concluded that increases in iEMG in the trunk muscle during KAATSU might be an important factor for KAATSU training-induced trunk muscle hypertrophy.

Yasuda T, Abe T, Brechue WF, Iida H, Takano H, Meguro K, Kurano M, Fujita S, Nakajima T. Venous blood gas and metabolite response to low-intensity muscle contractions with external limb compression. Metabolism. 2010 Oct;59(10):1510-9. doi: 10.1016/j.metabol.2010.01.016. Epub 2010 Mar 4.

The effect of low-intensity resistance exercise with external limb compression (100 [EC100] and 160 [EC160] mm Hg) on limb blood flow and venous blood gas-metabolite response was investigated and compared with that of high-intensity resistance exercise (no external compression). Unilateral elbow flexion muscle contractions were performed at 20% (75 repetitions, 4 sets, 30-second rest intervals) and 70% of 1-repetition maximum (1-RM; 3 sets, each set was until failure, 3-minute rest intervals). Precontraction brachial arterial blood flow (Doppler ultrasound) was reduced with EC100 or EC160 (56% and 39% of baseline value, respectively) compared with no external compression (control). At 20% 1-RM, brachial arterial blood flow increased after contractions performed with EC160 (190%), but not with the others. Decreases in venous oxygen partial pressure (P(v)O(2)) and venous oxygen saturation (S(v)O(2)) were greater during EC100 and EC160 than control (mean [SE]: P(v)O(2), 28 [3] vs 26 [2] vs 33 [2] mm Hg; S(v)O(2), 41% [5%] vs 34% [4%] vs 52% [5%], respectively). Changes in venous pH (pH(v)), venous carbon dioxide partial pressure (P(v)CO(2)), and venous lactate concentration ([L(-)](v)) were greater with EC160 than EC100 and/or control (pH(v), 7.19 [0.01] vs 7.25 [0.01] vs 7.27 [0.02]; P(v)CO(2), 72 [3] vs 64 [2] vs 60 [3] mm Hg; [L(-)](v), 5.4 [0.6] vs 3.7 [0.4] vs 3.0 [0.4] mmol/L, respectively). Seventy percent 1-RM contractions resulted in greater changes in pH(v) (7.14 [0.02]), P(v)CO(2) (91 [5] mm Hg), and [L(-)](v) (7.0 [0.5] mmol/L) than EC100 and EC160, but P(v)O(2) (30 [4] mm Hg) and S(v)O(2) (40% [3%]) were similar. In conclusion, changes in pH(v), P(v)CO(2), and [L(-)](v), but not in P(v)O(2) and S(v)O(2), are sensitive to changes in relative, "internal" intensity of low-intensity muscle contractions caused by reduced blood flow (EC160) or high-intensity muscle contractions. Given the magnitude of the changes in pH(v), P(v)CO(2), and [L(-)](v), it appears plausible that they may be involved in stimulating the observed increase in muscle activation via group III and IV afferents

Yasuda T, Fukumura K, Fukuda T, Iida H, Imuta H, Sato Y, Yamasoba T, Nakajima T. Effects of low-intensity, elastic band resistance exercise combined with blood flow restriction on muscle activation. Scand J Med Sci Sports. 2014 Feb;24(1):55-61. doi: 10.1111/j.1600-0838.2012.01489.x. Epub 2012 Jun 27.

We examined the effects of blood flow-restricted, low-intensity resistance exercise (termed kaatsu) using an elastic band for resistance on muscle activation. Nine men performed triceps extension and biceps flexion exercises (four sets respectively) using an elastic band for

resistance with blood flow restriction (BFR) or CON (unrestricted blood flow). During a BFR session, subjects wore pressure cuffs inflated to 170-260 mmHg on the proximal region of both arms. Surface electromyography (EMG) was recorded from the triceps brachii and biceps brachii muscles, and mean integrated EMG (iEMG) was analyzed. Blood lactate concentration was obtained before (Pre) and immediately after two exercises (Post). During triceps extension and biceps flexion exercises, muscle activation increased progressively (P<0.05) under BFR (46% and 69%, respectively) but not under CON (12% and 23%, respectively). Blood lactate concentration at Post was higher (P<0.05) under BFR than under CON (3.6 and 2.1 mmol/L, respectively). Blood lactate concentration at Post was significantly correlated with increased iEMG in both triceps extension (r=0.65, P<0.01) and biceps flexion exercises (r=0.52, P<0.05). We conclude that kaatsu training using elastic bands for resistance enhances muscle activation and may be an effective method to promote muscle hypertrophy in older adults or patients with a low level of activity.

II. Application to patients with various diseases

The KAATSU training is performed for patients with various diseases centering on heart disease, and the effects as rehabilitation, direct or secondary effects on diseases are compared with the effects of conventional rehabilitation methods. Although over 1000 cases in total of KAATSU training have been conducted since March 2007, no serious side effects have been observed, and it has been found to be safe for patients with disease.

• Effects of low-intensity KAATSU resistance training on skeletal muscle size/strength and endurance capacity in patients with ischemic heart disease

T. Nakajima, M. Kurano, F. Sakagami, H. Iida, K. Fukumura, T. Fukuda, H. Takano, H. Madarame, T. Yasuda, T. Nagata, Y. Sato, T. Yamasoba, T. Morita. Effects of low-intensity KAATSU resistance training on skeletal muscle size/strength and endurance capacity in patients with ischemic heart disease. Int J KAATSU Training Res 2010; 6: 1-7

KAATSU training induces muscle hypertrophy and strengthens muscle in athletes and healthy subjects through short-term and low-intensity exercise. However, it remains uninvestigated whether lowintensity KAATSU resistance training (LIKRT) induces muscle strength and hypertrophy in patients with cardiovascular diseases. We examined the effects of LIKRT on skeletal muscle size/strength and endurance capacity in patients with ischemic heart disease (IHD). Seven male patients with stable IHD performed three kinds of resistance exercises (leg press, leg curl and leg extension) with their femoral muscle blood flow restricted by KAATSU belt two times/week for three months. We measured one RM (1-RM) in each resistance exercises, and evaluated muscle cross-sectional areas (CSA) by MRI before training and after the training. We used cardiopulmonary examinations to measure endurance capacity (Peak VO2 (VO2peak), VO2 at anaerobic threshold (VO2AT)). We performed blood sampling to measure resting plasma level of insulin growth factor-1 (IGF-1) and serum high-sensitive C-reactive protein (hsCRP). LIKRT significantly increased leg press (15%), leg curl (18%) and leg extension (17%) 1-RM strength. Increases of muscle CSA in quadriceps femoris at the proximal lower leg (30%), the mid-thigh (50%), and the proximal lower leg (70%) were 5.1%, 4.6% and 10.4%, respectively. Similarly, hamstring and adductor CSA were also increased by LIKRT. LIKRT significantly increased VO2peak and VO2AT by 10.7% and 10.9%,

respectively. IGF-1 and hsCRP were not altered before or after the training. These results suggest that LIKRT increases muscle strength/mass and endurance capacity in patients with IHD. LIKRT appears to be a promising and effective resistance method in cardiac rehabilitation.

• Study on the influence of KAATSU walking on venous compliance

Iida H, Nakajima T, Kurano M, Yasuda T, Sakamaki M, Sato Y, Yamasoba T, Abe T. Effects of Walking with Blood Flow Restriction on Limb Venous Compliance in Elderly Subjects. Clin Physiol Funct Imaging. 2011 Nov;31(6):472-476.

Venous compliance declines with age and improves with chronic endurance exercise. KAATSU, an exercise combined with blood flow restriction (BFR), is a unique training method for promoting muscle hypertrophy and strength gains by using low-intensity resistance exercises or walking. This method also induces pooling of venous blood in the legs. Therefore, we hypothesized that slow walking with BFR may affect limb venous compliance and examined the influence of 6 weeks of walking with BFR on venous compliance in older women. Sixteen women aged 59-78 years were partially randomized into either a slow walking with BFR group (n=9, BFR walk group) or a non-exercising control group (n=7, control group). The BFR walk group performed 20-min treadmill slow walking (67 m min(-1)), 5 days per week for 6 weeks. Before (pre) and after (post) those 6 weeks, venous properties were assessed using strain gauge venous occlusion plethysmography. After 6 weeks, leg venous compliance increased significantly in the BFR walk group (pre: 0.0518 ± 0.0084 , post: 0.0619 ± 0.0150 ml 100 mmHg(-1), P<0.05), and maximal venous outflow (MVO) at 80 mmHg also increased ml(-1)significantly after the BFR walk group trained for 6 weeks (pre: $55 \cdot 3 \pm 15 \cdot 6$, post: $67 \cdot 1 \pm 18 \cdot 9$ ml 100ml(-1) min(-1), P<0.01), but no significant differences were observed in venous compliance and MVO in the control group. In addition, there was no significant change in arm compliance in the BFR walk group. In conclusion, this study provides the first evidence that 6 weeks of walking exercise with BFR may improve limb venous compliance in untrained elder female subjects.

• Evaluation of hemodynamics of dizziness-associated with orthostatic hypotension and examination of effectiveness of KAATSU training

2009-2011 Challenging sprouting research representative Tatsuya Yamasoba (share Toshiaki Nakajima)

Evaluation of hemodynamics of dizziness-associated with orthostatic hypotension and treatment by KAATSU trainin

Dizziness and balance disorder associated with orthostatic hypotension is a frequent disease at the Otorhinolaryngology and Cardiovascular Internal Medicine Departments, but its diagnosis is measurement of blood pressure and pulse rate at supine and standing positions (Sherong test). It is not based on a detailed and accurate diagnosis of the hemodynamics, and the cause has not been sufficiently evaluated. Moreover, the treatment for this disease is symptomatic, such as administration of a hypertensive agent, and no radical treatment has been performed. In this study, diagnosis and evaluation focused on hemodynamics were performed on cases diagnosed with orthostatic hypotension and visited for dizziness. At the same time, muscle training under blood flow restriction (KAATSU training) is performed, and it is determined whether orthostatic hypotension can be improved by increasing the muscle mass of the lower leg called the second heart, and circulation The influence on the behavior was also examined. Although some showed improvement trends, it seemed necessary to conduct large-scale prospective research in the future.

Elucidation of molecular mechanism of skeletal muscle hypertrophy by KAATSU training and application to elderly people sarcopenia

2012-2015 Grant-in-Aid for Scientific Research (B) Toshiaki Nakajima (Representative) Elucidation of molecular mechanism of skeletal muscle hypertrophy by KAATSU training and application to elderly sarcopenia

This study aimed to elucidate the molecular mechanism of muscle hypertrophy in KAATSU training using the rat KAATSU model as well as the effects on muscle hypertrophy and muscle strengthening in elderly people with KAATSU training. We examined muscle strengthening and muscle hypertrophy in low-intensity KAATSU training in elderly people. As a result of training of upper or lower limbs and loading method, a machine or an elastic band was used. Furthermore, the rat KAATSU model was used to investigate the effect of acute blood flow restriction on muscle oxygen partial pressure and the effect on muscle protein synthesis system. We also examined the chronic effects and their molecular mechanisms. KAATSU training was considered to be a useful method in measures against sarcopenia in the elderly.

Nakajima T, Koide S, Yasuda T, Hasegawa T, Yamasoba T, Obi S, Toyoda S, Nakamura F, Inoue T, Poole DC, Kano Y. Muscle hypertrophy following blood flow-restricted, low-force isometric electrical stimulation in rat tibialis anterior: role for muscle hypoxia. J Appl Physiol (1985). 2018 Jul 1;125(1):134-145. doi: 10.1152/japplphysiol.00972.2017. Epub 2018 Mar 22.

Low-force exercise training with blood flow restriction (BFR) elicits muscle hypertrophy as seen typically after higher-force exercise. We investigated the effects of microvascular hypoxia [i.e., low microvascular O2 partial pressures (P mvO2)] during contractions on muscle hypertrophic signaling, growth response, and key muscle adaptations for increasing exercise capacity. Wistar rats were fitted with a cuff placed around the upper thigh and inflated to restrict limb blood flow. Low-force isometric contractions (30 Hz) were evoked via electrical stimulation of the tibialis anterior (TA) muscle. The P mvO2 was determined by phosphorescence quenching. Rats underwent acute and chronic stimulation protocols. Whereas P mvO2 decreased transiently with 30 Hz contractions, simultaneous BFR induced severe hypoxia, reducing P mvO2 lower than present for maximal (100 Hz) contractions. Low-force electrical stimulation (EXER) induced muscle hypertrophy (6.2%, P < 0.01), whereas control group conditions or BFR alone did not. EXER+BFR also induced an increase in muscle mass (11.0%, P < 0.01) and, unique among conditions studied, significantly increased fiber cross-sectional area in the superficial TA (P < 0.05). Phosphorylation of ribosomal protein S6 was enhanced by EXER+BFR, as were peroxisome proliferator-activated receptor gamma coactivator-1 α and glucose transporter 4 protein levels. Fibronectin type III domain-containing protein 5, cytochrome c oxidase subunit 4, monocarboxylate transporter 1 (MCT1), and cluster of differentiation 147 increased with EXER alone. EXER+BFR significantly increased MCT1 expression more than EXER alone. These data demonstrate that microvascular hypoxia during contractions is not essential for hypertrophy. However, hypoxia induced via BFR may potentiate the muscle hypertrophic response (as evidenced by the increased superficial fiber cross-sectional area) with increased glucose transporter and mitochondrial biogenesis, which contributes to the pleiotropic effects of exercise training with BFR that culminate in an improved capacity for sustained exercise. NEW & NOTEWORTHY We investigated the effects of low microvascular O2 partial pressures (P mvO2) during contractions on muscle hypertrophic signaling and key elements in the muscle adaptation for increasing exercise capacity. Although demonstrating that muscle hypoxia is not obligatory for the hypertrophic response to low-force, electrically induced muscle contractions, the reduced P mvO2 enhanced ribosomal protein S6 phosphorylation and potentiated the hypertrophic response. Furthermore, contractions with blood flow restriction increased oxidative capacity, glucose transporter, and mitochondrial biogenesis, which are key determinants of the pleiotropic effects of exercise training.

Nakajima T, Yasuda T, Koide S, Yamasoba T, Obi S, Toyoda S, Sato Y, Inoue T, Kano Y. Repetitive restriction of muscle blood flow enhances mTOR signaling pathways in a rat model.Heart Vessels. 2016 Oct;31(10):1685-95. doi: 10.1007/s00380-016-0801-6. Epub 2016 Feb 1.

Skeletal muscle is a plastic organ that adapts its mass to various stresses by affecting pathways that regulate protein synthesis and degradation. This study investigated the effects of repetitive restriction of muscle blood flow (RRMBF) on microvascular oxygen pressure (PmvO2), mammalian target of rapamycin (mTOR) signaling pathways, and transcripts associated with proteolysis in rat skeletal muscle. Eleven-week-old male Wistar rats under anesthesia underwent six RRMBF consisting of an external compressive force of 100 mmHg for 5 min applied to the proximal portion of the right thigh, each followed by 3 min rest. During RRMBF, PmvO2 was measured by phosphorescence quenching techniques. The total RNA and protein of the tibialis anterior muscle were obtained from control rats, and rats treated with RRMBF 0-6 h after the stimuli. The protein expression and phosphorylation of various signaling proteins were determined by western blotting. The mRNA expression level was measured by real-time RT-PCR analysis. The total muscle weight increased in rats 0 h after RRMBF, but not in rats 1-6 h. During RRMBF, PmvO2 significantly decreased (36.1 ± 5.7 to 5.9 ± 1.7 torr), and recovered at rest period. RRMBF significantly increased phosphorylation of p70 S6-kinase (p70S6k), a downstream target of mTOR, and ribosomal protein S6 1 h after the stimuli. The protein level of REDD1 and phosphorylation of AMPK and MAPKs did not change. The mRNA expression levels of FOXO3a, MuRF-1, and myostatin were not significantly altered. These results suggested that RRMBF significantly decreased PmvO2, and enhanced mTOR signaling pathways in skeletal muscle using a rat model, which may play a role in diminishing muscle atrophy under various conditions in human studies.

• Elucidation of muscle hypertrophy mechanism of KAATSU training and its application

2013 Young Scientist Research Program (B) Yasuda T Elucidation of muscle hypertrophy mechanism of KAATSU training and its application: Comparison of shortening and extensibility contraction

Development of a muscle strength improvement program that takes advantage of KAATSU training

• 2011-2012 fiscal year research program young research (B) Yasuda T Development of a

muscle strength improvement program that takes advantage of KAATSU training

Yasuda T, Fukumura K, Uchida Y, Koshi H, Iida H, Masamune K, Yamasoba T, Sato Y, Nakajima T. Effects of Low-Load, Elastic Band Resistance Training Combined with Blood Flow Restriction on Muscle Size and Arterial Stiffness in Older Adults. J Gerontol A. Biol Sci Med Sci. 2015 Aug;70(8):950-8. doi: 10.1093/gerona/glu084. Epub 2014 Jun 10.

Yasuda T, Nakajima T. Response to the Letter "Blood Flow Restricted Training in Older Adults: Consider Standardized Methodology for Future Investigations?". J Gerontol A Biol Sci Med Sci. 2016 May;71(5):620-1. doi: 10.1093/gerona/glv178. Epub 2015 Oct 15.

We examined the effect of low-load, elastic band resistance training with blood flow restriction (BFR) on muscle size and arterial stiffness in older adults. Healthy older adults (aged 61-85 years) were divided into BFR training (BFR-T, n = 9) or non-BFR training (CON-T, n = 8) groups. Both groups performed low-load arm curl and triceps down exercises (four sets, total 75 repetitions for each) using an elastic band, 2 d/wk for 12 weeks. The BFR-T group wore inflated pneumatic elastic cuffs (120-270 mm Hg) on both arms during training. Magnetic resonance imaging-measured muscle cross-sectional area of the upper arm, maximum voluntary isometric contraction of the elbow flexors and extensors, cardio-ankle vascular index testing, and ankle-brachial pressure index were measured before and 3-5 days after the final training session. Muscle cross-sectional area of the elbow flexors (17.6%) and extensors (17.4%) increased, as did elbow flexion and elbow extension maximum voluntary isometric contraction (7.8% and 16.1%, respectively) improved (p < .05) in the BFR-T group, but not in the CON-T group. In cardio-ankle vascular index and ankle-brachial pressure index testing, there were no changes between pre- and post-results in either group. In conclusion, elastic band BFR-T improves muscle cross-sectional area as well as maximal muscle strength but does not negatively affect arterial stiffness in older adults.

Yasuda T, Fukumura K, Fukuda T, Uchida Y, Iida H, Meguro M, Sato Y, Yamasoba T, Nakajima T. Muscle size and arterial stiffness after blood flow-restricted low-intensity resistance training in older adults. Scand J Med Sci Sports. 2014 Oct;24(5):799-806. doi: 10.1111/sms.12087. Epub 2013 Jun 3.

Previous studies have shown that blood flow-restricted low-intensity resistance training (BFR-RT) causes muscle hypertrophy while maintaining arterial function in young adults. We examined the effects of BFR-RT on muscle size and arterial stiffness in older adults. Healthy subjects (ages 61-84 years) were divided into BFR-RT (n=9) or non-training control (CON; n=10) groups. The BFR-RT group performed 20% and 30%, respectively, of one-repetition maximal (1-RM) knee extension and leg press exercises, 2 days/wk for 12 weeks. The BFR-RT group wore elastic cuffs (120-270 mmHg) on both legs during training. Magnetic resonance imaging-measured muscle cross-sectional area (CSA), 1-RM strength, chair stand (CS) test, and cardio-ankle vascular index testing (CAVI), an index of arterial stiffness, were measured before and 3-5 days after the final training session. Muscle CSA of the quadriceps (8.0%), adductors (6.5%), and gluteus maximus (4.4%), leg extension and leg press 1-RM strength (26.1% and 33.4%), and CS performance (18.3%) improved (P < 0.05) in the BFR-RT group, but not in the CON group. In CAVI testing, there were no changes in both two groups. In conclusion, BFR-RT improves muscle CSA as well as maximal muscle strength, but does not negatively affect arterial stiffness or humeral coagulation factors in older adults.

Yasuda T, Fukumura K, Iida H, Nakajima T. Effects of detraining after blood flow-restricted low-load elastic band training on muscle size and arterial stiffness in older women. Springerplus. 2015 Jul 15;4:348. doi: 10.1186/s40064-015-1132-2. eCollection 2015.

BACKGROUND: We examined the effects of detraining after blood flow-restricted (BFR) low-load elastic band training on muscle size and arterial stiffness in older women.

FINDINGS: Fourteen women were divided into BFR training (BFR-T) or non-BFR training (CON-T). Each group participated in 12 weeks of arm curl and press down training using an elastic band either with (BFR-T) or without BFR (CON-T). Muscle cross-sectional area (CSA) and maximum voluntary isometric contraction (MVIC) for upper arms and cardio-ankle vascular index (CAVI) were evaluated before and after the 12-week training period and also after 12 weeks of detraining. CSA and MVIC were higher at post and detraining (CSA: 16.3% (p < 0.01) and 6.9% (p < 0.01) for elbow flexion and 17.1% (p < 0.01) and 8.7% (p > 0.05) for elbow extension; MVIC: 7.3 and 3.9% (both p > 0.05) for elbow flexion and 17.6 and 15.1% (both p < 0.01) for elbow extension) than at pre for the BFR-T, but not for the CON-T. There was no change in CAVI for the two groups.

CONCLUSIONS: Increased muscle strength/size following 12 weeks of elastic band BFR-T was well maintained with a low risk of arterial stiffness after 12 weeks of detraining in older women.

 Application of KAATSU muscle training in respiratory rehabilitation of COPD patients 2009-2011 Grant-in-Aid for Scientific Research (C) Nakaga T ((Representative) (Shared Nakajima T, H21-23) Application of KAATSU Muscle Training in Respiratory Rehabilitation for COPD Patients

In COPD patients, the effects of arm curl exercise with free weights on muscle activity were examined. The application of blood flow restriction enhanced muscle activity on the electromyography and increased blood lactate concentration and Borg scale, but did not reduce SpO2. The KAATSU training was considered to enhance muscle activity safely and effectively in COPD patients, even with low strength and easy exercise modes such as using free weights and rubber bands. Finally, the chronic effects of KAATSU training were examined in COPD patients. Leg extension under KAATSU confirmed the improvement of QOL and the increase of muscle strength and muscle mass, and also the maximum inspiratory pressure (PImax) and the maximum expiratory pressure (PEmax) were also improved. Not only COPD but also old age is important as a cause of COPD patients causing sarcopenia. Therefore, in the elderly, the effects of KAATSU walk (walk under KAATSU) on QOL, muscle strength, etc. were examined. The KKATSU training sgnificantly increased muscle strength, and improved function of timed up & go and chair stand test was also observed. In COPD patients with sarcopenia, pressure walks may also be a safe and effective strength training. In the future, it seems necessary to study more cases.

• Case report papers

1) H. Iida, M. Kurano, H. Takano, H. Oonuma, H. Imuta, N. Kubota, T. Morita, K. Meguro, Y. Sato, T. Abe, Y. Yamazaki, T. Nakajima. Can KAATSU be used for an orthostatic stress in

astronauts?: A case study. Int J KAATSU Training Res 2, 45-52, 2006.

The application of an orthostatic stress such as lower body negative pressure (LBNP) during exercise has been proposed to minimize the effects of weightlessness on the cardiovascular system and subsequently to reduce the cardiovascular deconditioning. The KAATSU training is a novel method for strength training to induce muscle strength and hypertrophy. KAATSU induces venous pooling of blood in capacitance vessels by restricting venous blood flow. Therefore, to investigate whether KAATSU can be used as an orthostatic stress, we examined the effects of KAATSU on the hemodynamic, autonomic nervous and hormonal parameters in one subject. The several parameters were measured by impedance cardiography; heart rate (HR), mean blood pressure (mBP), stroke volume (SV), cardiac output (CO), total peripheral resistance (TPR), and heart rate variability (HRV). These data were obtained before (pre), during and after (post) pressurization (50 and 200 mmHg) on both thighs with KAATSU mini belts, and compared with those in standing. The serum concentration of noradrenaline (NA) and vasopressin (ADH), and plasma rennin activity (PRA) were also measured. The application of 200 mmHg KAATSU decreased SV, which was almost equal to the value in standing. HR and TPR increased in a similar manner as standing with slight change of mBP. High frequency (HFRR), a marker of parasympathetic nervous activity, decreased during both 200 mmHg KAATSU and standing, while LFRR/HFRR, a quantitative marker of sympathetic nervous activity, increased significantly. During KAATSU and standing, NA, PRA and ADH increased. These results indicate that the application of KAATSU on both thighs simulates systemic cardiovascular effects of orthostasis in one gravity (1G), and that KAATSU training appears to be a useful method for potential countermeasure like lower body negative pressure (LBNP) against orthostatic intolerance in space flight as well as strength training to induce muscle strength and hypertrophy.

2) Madarame H, Kurano M, Takano H, Iida H, Sato Y, Ohshima H, Abe T, Ishii N, Morita T and Nakajima T. Effects of low-intensity resistance exercise with blood flow restriction on coagulation system in healthy subjects. Clinical Physiology and Functional Imaging 2010 May;30(3):210-3.

Churg-Strauss syndrome (CSS) is an eosinophilic vasculitis, which involves multiple organ systems, including the lungs, heart, skin and neuromuscular system. Since CSS is an inflammatory disease, administration of systemic glucocorticoids has been a standard therapy for the disease. However, even if the inflammation has been controlled by glucocorticoids, neuromuscular disorders often do not improve. Thus, we aimed in this study to evaluate the efficacy of low-intensity exercise with blood flow restriction (BFR) on CSS-induced muscle atrophy and weakness. A 40-year-old female ballet dancer with CSS-induced muscle atrophy in her right lower leg underwent a 14-wk training program, which consisted of low-intensity resistance exercise (plantar flexion, leg extension and leg curl) and walking exercise with BFR. To measure cross-sectional area (CSA) of lower leg muscles, magnetic resonance images (MRI) of both lower legs were obtained before and after the 14-wk training period. After the 14-wk training period, muscle CSA of the right lower leg showed 29.1% increase, though it was still 23.4% smaller than that of the left side. In conclusion, this case study showed that low-intensity exercise with BFR induced muscle hypertrophy in a female ballet dancer with CSS. It is suggested that low-intensity exercise with BFR can be a valuable rehabilitation tool for patients with CSS to counteract muscle atrophy and weakness.

3) Uchida Y, Morita T, Fukumura K, Otsuka T, Fukuda T, sato Y, Nakajima T. Effects of KAATSU training on a patient with benign fasciculation syndrome. Int J KAATSU Training Res 2012; 8: 9-12.

[Objective] Benign fasciculation syndrome (BFS) is a neurological disorder characterized by involuntary and repeated contractions of synergetic muscles and commonly occurs in the evelids, arms and legs. BFS is also associated with pain, which may interfere with everyday activities. This report describes the case of a BFS patient who performed KAATSU exercise, a type of exercises performed under the conditions of restricting muscle blood flow. [Methods] The patient performed KAATSU exercise of the bilateral upper and lower extremities at a frequency of twice a week for 3 months. Knee extensor strength as measured with a hand-held dynamometer (HHD), QOL scores as assessed by the SF-36v2 questionnaire form, and bilateral femoral muscle mass as measured by MRI were compared before and after exercise. [Results] After a 3-month KAATSU exercise program, the right and left knee extensor strength as measured with a HHD increased by about 26% from 30.9 to 38.8 kgf and by about 44% from 39.9 to 57.4 kgf, respectively, and the bilateral femoral muscle mass as measured by MRI increased by about 23% from 8,730 to 10,709 cc. Overall improvement in QOL was also observed, as assessed by the SF-36v2 questionnaire form. [Conclusion] For patients with neurological disorders with pain, such as the present patient, active introduction of KAATSU-based exercise is likely to result in improved health-related QOL, as well as increased muscle mass and strength.

4) Iwashita H, Morita T, Sato Y, Nakajima T. KAATSU training in a case of patients with periventricular leukomalacia (PVL). International Jouranal of KAATSU Training Research 2014; 10: 7-11.

[Objective] The effectiveness of KAATSU training has been reported in wide-ranging fields from sports medicine to rehabilitation, and KAATSU training has been clinically applied. However, there have been only limited reports on pediatric cases. We performed KAATSU training in a pediatric patient with periventricular leukomalacia (PVL) and examined its effectiveness and safety for cerebral palsy. We report here the results of this examination. [Methods]KAATSU training was performed on a PVL patient on an outpatient basis. This training was performed once a week for 14 weeks and involved mainly three specified motions of the upper and lower limbs. Evaluation was performed using the Gross Motor Function Measure (Gross Motor Function Classification System: GMFM) and videos. In this report, the effectiveness of short-term KAATSU training was compared with that of intensive physical therapy using data in the literature. [Results]QOL improved due to short-term reduction of muscle tonus and increased acquired movements resulting from KAATSU training. [Conclusion]The results of this report suggest that KAATSU training can be effective in PVL patients. Further examination is necessary with increased cases and evaluation methods.

5) Nakajima T, Yasuda T, Fukumura K, Kurano M, Imanishi T, Morita T, Sato Y, Hiraizumi Y. KAATSU training as a new exercise therapy for femoral head avascular necrosis: A case study. Int J KAATSU Training Res 2015;11:1-6.

Femoral head avascular necrosis is a condition in which part of the femoral head undergoes necrosis due to decreased blood flow. The femoral head gradually disintegrates causing pain and even today, there are no effective rehabilitation methods other than symptomatic treatment such as decreasing the load on the hip joints with the use of a cane or walker. We herein describe our insights into this condition based on our experience with a case of femoral head avascular necrosis caused by steroid use in which KAATSU training was found to be highly effective. The patient was a 34-year-old woman (154 cm tall and weighing 50 kg, a radiologist). Since the age of 23, this patient had been receiving steroid treatment to control her refractory asthma. She later developed pain in her right hip and gradually suffered hip joint deformation, restricted range of motion, and difficulty in walking. MRI revealed Association Research Circulation Osseous (ARCO) stage IV disease. She suffered marked pain of the right hip joint every time she walked, occasionally falling and required a cane to walk. At the patient's own request, she received KAATSU training including KAATSU walking over a period of 3 months (total 28 sessions). Various assessments were carried out before and after training to determine the effects of KAATSU training. QOL was determined by SF-36v2, and marked improvement of role physical, body pain, general health, vitality, and social functioning were noted. Before training, the Japanese Orthopaedic Association (JOA) hip scores for pain were 10 (right), 40 (left), walking ability 16 (right), 20 (left), while 3 months after training, these scores were markedly improved in the affected side. Furthermore, not only did muscle strength on the affected side show marked improvement, but the MRI also revealed a tendency for improvement of the right femoral head avascular necrosis. DEXA showed signs of a clear increase in bone mineral density. Based on the above, these results suggest that KAATSU training is extremely useful as a rehabilitation method in patients with femoral head avascular necrosis. But, further larger scale investigations should be carried out in the future to support our findings.

6) Hiraizumi Y, Nakajima T, Sato Y, Imanishi T. KAATSU training as a new effective exercise therapy in a case of femoral medical condyle osteonecrosis. Int J KAATSU Training Res 2016;12:1-4

Idiopathic osteonecrosis of the medial condyle of the femur, a relatively common disease among Japanese women 60 years or older, is often progressive and requires surgical treatment. In this paper, we report on a case where KAATSU training was effective for a 71-year-old woman with bone necrosis in the left femoral medial condyle revealed by magnetic resonance imaging (MRI). At the first visit, the patient was suffering severe pain, and unable to walk without a cane. After 1 to 2 months of KAATSU training, her pain was mitigated and in about 3 months she was able to walk without a cane. In 6 months she could go upstairs and downstairs using the handrails and about 2 to 3 months later, she no longer needed to use the handrails. MRI revealed marked shrinkage (to 10 mm x 15 mm) of the necrotized region as a result of bone tissue remodeling. From these results, KAATSU training seems to be useful as a new method of rehabilitation for patients with idiopathic osteonecrosis of the medial condyle of the femur.

7) Yasuda T, Oosumi S, Sugimoto S, Morita T, Sato Y, Ishii M, Nakajima T. Effect of KAATSU training on thigh muscle size and potential safety for a patient with knee meniscectomy over 3 years. Int J KAATSU Training Res 2017;13:11-14.

[Objective] It is well known that knee meniscectomy is one of the major knee surgeries, which induces thigh muscle atrophy. However, it is unclear whether thigh muscle size after knee

meniscectomy can be improved with KAATSU training. We examined effect of KAATSU training on thigh muscle size and safety for a patient with knee meniscectomy. [Methods] The patient was a 57-year-old woman (standing height 159 cm and body weight 52 kg). The KAATSU training composed of 7 types of resistance exercise and one type of cycling exercise was provided for a total of 125 sessions over approximately 3 years. Transverse scans were carried out for mid-thigh length. Thigh muscle cross-sectional area (CSA) in affected-leg and unaffected-leg was measured by the CT scan before, 63 weeks, and 152 weeks after the training. [Results] Thigh muscle CSA was highly increased for affected-leg, and the attained level was exactly similar for both legs after the 152 weeks training period. [Conclusion] The long-term KAATSU exercises were a highly safe and effective training method for a patient with knee meniscectomy

8) Ooshima A, Ishizaka Y, Katayanagi S, Arakawa C, Takahashi R, Nozawa N, Mizushima Y, Matsumoto K, Sawaguchi T, Haruyama A, Toyoda S, Ogawa H, Shibasaki I, Yagi H, Yamaguchi S, Uematsu A, Yasuda T, Sato Y, Fukuda H, Inoue T, Nakajima T. Combination of KAATSU training® and BCAA intake for a patient after aortic valve replacement surgery: A case study. Int J KAATSU Training Res 2018;12:1-4 $_{\circ}$

We report a case in which a favorable course was obtained by using KAATSU training® and BCAA intake early after aortic valve replacement surgery. The patient was a 43-year-old male with low cardiac function who underwent aortic valve replacement surgery. Heart failure and hypotension persisted while waiting for surgery. He tended to line on a bed all day, so that disuse muscular atrophy advanced. Therefore, in addition to the postoperative rehabilitation program, KAATSU training and BCAA intake were performed postoperatively and continued in outpatient rehabilitation. The KAATSU training was performed using knee extension twice or three times a week. For BCAA intake (2.5 g), one pack of jelly containing BCAA (Reha-Time Jelly, Clinico Co., Ltd.) was taken within 30 minutes after the training. About 3 months later, the thigh circumference (+ 7.3 cm), the maximum voluntary isometric contraction of knee extension (+ 20 kgf), the quadriceps muscle thickness (+ 1 cm) evaluated by a B-mode ultrasound, the muscle mass of the lower limb (+1 kg), and a marked increase in thigh muscle cross-sectional area as measured by CT scan were observed. No deterioration of circulatory hemodynamics and side effects were observed during the course. In conclusion, the combined use of KAATSU training and BCAA intake early after cardiac operation seems to be a safe and effective way to obtain muscle hypertrophy and muscle strengthening, but further studies are needed to clarify it.

• Paper presentation (English)

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J Cardiovasc Pharmacol 47, 314-321, 2006.

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ANTI-AGING MEDICINE

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Review Article Effects of Exercise and Anti-Aging

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Abstract

As demographic aging continues in Japan, the number of very elderly individuals aged 75 years or older is increasing rapidly, as is the number of bedridden, elderly individuals, with ramifications extending to economic problems such as health care costs and insurance for long-term care. Consequently, there is a great importance to questions of how to prevent age-related loss of muscle (sarcopenia) to prevent bedridden states, and further to improve quality of life (QOL) and maintain active lifestyles. Exercise is the most effective means for preventing and addressing sarcopenia. Regular exercise is also reported to prevent progression of arteriosclerosis, prevent lifestyle diseases, and delay onset of dementia. However, the effects of exercise are known to differ substantially for different types of exercise. Regular walking and other aerobic exercise improves cardiovascular endurance, but among the elderly, loss of muscular strength, muscular atrophy, and other diminished physical functions have implications for falling and fractures, and it is not uncommon to see a consequent aggravation of disuse syndrome due to inactivity, leading to a bedridden state. In this light, strength training is also important for elderly individuals, to increase muscular strength and muscle mass. It is also highly important for elderly individuals to eat a diet, particularly amino acids, that enhances the effects of exercise. Here we present an overview of aerobic exercise, resistance training, and "kaatsu training" (*i.e.*, training under pressure-restricted blood flow to the extremities) representing anti-aging exercise methods. We likewise discuss the importance of diet for exercise.

KEY WORDS: Kaatsu training, anti-aging, resistance exercise, amino acids, sarcopenia

Introduction

Muscular strength and muscle mass are well known to decline with age. This phenomenon is called sarcopenia. When an individual passes age 30, muscle mass decreases approximately 0.3-0.5% every year, and after age 60, the rate of decrease is very substantial. At age 80, muscle mass is thought to decrease to 50% or lower than during the prime years. In bed, muscles also decrease by 0.6% per day. Consequently, hospital admission for cerebrovascular accident, fracture, or other such reasons can lead, in 3 weeks of bed rest, to even a 12% reduction in muscle mass, and particularly for elderly individuals or sarcopenia patients, such an instance often leads to a bedridden state or disuse syndrome. Additionally, agerelated sarcopenia not only increases the risk of falling; it can also be a factor in decreased independence or frailty of the elderly due to insulin resistance or glucose metabolism disorder associated with decreased muscle mass. Routine measures to maintain muscular strength and muscle mass and prevent sarcopenia are therefore highly important.

In gross distinction, exercise can be divided into strength training versus aerobic exercise such as walking (treadmill) and bicycling (ergometer)¹⁾. *Fig. 1* presents differences in the effects of aerobic exercise versus strength training. The effects of aerobic exercise are more apparent than those from strength training in terms of peak oxygen uptake, extension of exercise endurance time, and other improvements in cardiovascular endurance. However, aerobic exercise does not increase muscle mass, and its effect of increasing bone density is reputedly

lower than that of strength training. In contrast, resistance (muscular strength) training has a stronger effect than aerobic exercise on enhancing muscular strength and muscle size and likewise acts strongly to increase bone density. Resistance training also elevates basal metabolism. Each type of exercise has an apparently similar effect of improving glucose metabolism and fat metabolism, and both are reputedly useful for improving insulin resistance, in particular, decreasing insulin response to glucose loading.

American College of Sports Medicine (ACSM) guidelines report that elderly individuals show sarcopenia and diminished muscle strength in nearly all cases and state that resistance training is needed to enhance muscle strength and maintain muscle mass. Strength training is also seen to improve insulin resistance, increase bone density, and improve QOL and is recommended for progressive, age-related osteoporosis, a cause of fractures. However, the guidelines also state that elderly individuals should not engage in high-intensity strength training with weights. While muscle strength and muscle mass clearly diminish with age, peak oxygen uptake is also known to decrease. Myers et al. studied the association between peak oxygen uptake and prognosis by comparing individuals with peak oxygen uptake at or lower than 5 METS with those at higher levels; they reported a distinct difference in long-term prognosis both among healthy individuals free from obvious disease and among patients with cardiovascular disease²⁾. Recently, the incidence of dementia has also increased rapidly, and after 10 years, 10% of those age 65 years or older are said

	Aerobic exercise	Resistance training
Body composition		
Bone density	1	1 1 1
% Body fat	$\downarrow \downarrow$	Ļ
Muscle strength/muscle mass	-	1 1 1
Glucose metabolism		
Insulin response to glucose loading	↓ ↓	↓ ↓
Insulin level (fasting)	Ļ	Ļ
Insulin sensitivity	1 1	↑ ↑
Serum lipids		
HDL	↑ —	↑ —
LDL	↓ -	↓ -
Resting heart rate	↓ ↓	_
Resting blood Systolic	↓ ↓	↓ ↓
pressure Diastolic	↓ ↓	Ļ
VO-max		 ↑ —
Sub-peak, peak endurance time		l † †
Resting basal metabolism	↑	1 Ť Ť

Fig. 1. Comparative effects of aerobic exercise and strength training (cf. Reference 1)

to develop dementia. In contrast, routine exercise is reported to improve QOL in dementia³⁾, and results from a prospective cohort study state that routine exercise may also delay onset of dementia⁴⁾. In this light, anti-aging exercise is the joint pursuit of both types of exercise to manifest their independent effects: aerobic exercise to improve peak oxygen uptake and impart endurance, and strength training to improve muscle strength, and this regimen is also recommended for its anticipated complementary effects.

Here we provide an overview of aerobic exercise, resistance training, and kaatsu training, together representing anti-aging exercise. Kaatsu training is a new training method reported to increase muscle size and muscular strength even with lowintensity loading, in a manner not achieved by conventional resistance training. We also discuss diet, and particularly amino acid intake, highly important elements for enhancing the effect of exercise in elderly individuals.

I. Aerobic exercise and strength training

Ia. Aerobic exercise

Aerobic exercise (*e.g.*, walking, treadmill, bicycle ergometer) known to improve cardiopulmonary endurance is fundamental to exercise therapy. Evidence as to its multifaceted effects proves the usefulness of exercise therapy ^{5,6}). In particular, 1) improved exercise endurance is shown by improved anaerobic threshold (AT) and peak oxygen uptake, which increases patient QOL. 2) Symptomatic improvement in identical ADL tasks and 3) improved life expectancy are shown by numerous meta-analyses indicating that cardiovascular death and total deaths decrease by 20-30%. 4) Improved lipid metabolism and glucose metabolism and improvement in obesity are demonstrated by further improvement with concomitant dietary guidance and weight loss. 5) Decreased smoking rates and 6) improved psychosocial satisfaction and decreased stress are psychological improvements. Other

reported effects include decreased blood pressure at rest and during exercise, decreased heart rate, decreased myocardial oxygen consumption during exercise, increased mass of active muscles, and increased muscular mitochondrial activity. In this light, routine aerobic exercise is also useful for preventing progression of arteriosclerosis, preventing lifestyle disease, and at the same time improving QOL of the elderly.

Actual aerobic exercise is often prescribed by determining load intensity using a treadmill or bicycle (ergometer)^{7,8)}. The effects of exercise are closely related to exercise intensity, and the effect of an increase in peak oxygen uptake (an index of exercise endurance) increases with increasing exercise intensity. In general, improvement in aerobic capacity requires exercise at an intensity of 50-80% of peak oxygen uptake (VO₂max) for 20 min. or longer, 3-5 times per week. However, for considerations of safety among elderly and other such individuals, the load intensity used is, for example, 40-60% of peak oxygen uptake and 50-70% of maximum heart rate as measured by an exercise load tester, and AT as prescribed (intensity of 70-80% AT or 1 minute prior to AT). Exercise with a somewhat challenging target value (Borg scale 13) as a subjective symptom is often pursued for 20-60 min. per day, 2-5 days per week. The effects of exercise are also manifest more readily at low levels of physical capability and activity. In a study of the effect of cardiac rehabilitation (aerobic exercise) on peak oxygen uptake among patients participating in cardiac rehabilitation at our facility⁹⁾, a mean 15% increase in peak oxygen uptake was observed after 3 months of 40 minutes of aerobic exercise (ergometer) performed 2-3 times per week. The patients involved were age 34-85 years and thus included very elderly individuals. In this respect, ongoing, appropriate aerobic exercise is regarded as a safe means of exercise pursued actively even among elderly individuals. Recent reports state that repetitive training alternating between high and low loads, i.e., interval training and hypoxic training involving exercise in a low-oxygen environment¹⁰, increases cardiopulmonary endurance more than ordinary aerobic exercise, and future applications are also anticipated.

Ib. Strength training

As described, appropriate aerobic exercise is useful for improving cardiopulmonary endurance even in the elderly. However, sarcopenia and diminished muscular strength are seen in virtually all elderly individuals, and resistance training is also needed to enhance muscular strength and increase muscle mass. Strength training is also known to have effects of improving insulin resistance, increasing bone density, and improving QOL. Strength training is therefore recommended as an anti-aging measure to increase muscular strength and muscle mass ⁷⁾. Resistance training is strength-enhancing exercise of the extremities or trunk using free weights, rubber bands, or machines and is performed with careful setting of loads for each exercise. The recommended resistance exercise regimen is 2-3 times per week, and for the most part, 2 times per week is used. The procedures used come from a strength training menu of several categories (8 categories) for the major muscle groups and include chest press, shoulder press, triceps extension, biceps curl, pull-down (upper back), lower back extension, abdominal crunch/curl-up, leg extension or leg press, leg curls (hamstrings), and calf raises, with load intensity at 40-60% of 1RM. In strength training using training machines, the load intensity is preferably 30-40% for upper extremity exercise and 40-50% for lower extremity exercise. In most cases, 1 set comprises 8-15 repetitions, and 1-2 sets are performed. However, effective improvement of the structure and function of skeletal muscle, for example, to increase muscle size and muscular strength, generally requires a high intensity exceeding 70% of maximum weight lift potential (1RM), to the point of muscular fatigue, i.e., 3 sets, 2-3 times per week. In a systematic review of the literature published to date concerning the sarcopenia-improving effect of strength training, Michiya et al.¹¹⁾ reported that even moderate intensity could have the effect of increasing muscular strength, but high-intensity training is required to increase muscle mass. Accordingly, conventional strength training pursued at an intensity of 40-60% 1RM often simply does not produce increases in muscle size. However, high-intensity loading entails a risk of injury to the moving organs or circulatory system in elderly individuals. High-intensity strength training studied in healthy, young subjects is also reported to increase arterial stiffness, an index of arteriosclerosis 12-14), and among elderly individuals too, high-intensity strength training is often not recommended due to increased stress on the joints.

II. Kaatsu training

In ordinary resistance training, muscle size is known not to increase unless loading is applied at an intensity of 65-70% 1RM or higher, and a rehabilitation technique with the effect of increasing muscle size safely at low intensity would represent a highly revolutionary training method with application for patients with various diseases, including the elderly. Kaatsu training is a new strength training method which stimulates numerous muscle fibers and can produce increased muscle size and strength even with low-intensity loading ^{15,16}. At present, kaatsu training is used by persons such as healthy individuals and athletes to increase strength, but the training has also been applied in rehabilitation to increase strength among patients with various diseases ^{17,18}. Here we briefly describe kaatsu training and its mechanism and also summarize actual applications.

IIa. About kaatsu training

Kaatsu training is strength training in which specialized pressure-applying belts are used to apply pressure at the base of the extremities, and training is performed in a state of restricted blood flow ^{15,16,19-23)}. The training is distinctive for producing increases in muscle size and strength through short-duration, low load-intensity training. While there is considerable research on the load intensity used in ordinary strength training and its principal effects, load intensity, essentially, mechanical stress, is thought to be the most crucial element for effecting increases in muscle size. In ordinary exercise procedures, a load intensity of 65-70% 1RM or greater is needed to increase muscle strength and size; however, an intensity of 65% 1RM or greater is often difficult for elderly individuals and patients presenting muscular atrophy. In addition, resistance training at high-intensity raises blood pressure, and due care is required. In contrast, because one characteristic of kaatsu training is production of increased muscle size and strength at low load-intensity that cannot be realized through conventional resistance training, larger muscle size and greater strength are produced even at the low intensity of 20%-40% 1RM, i.e., nearly a routine level of activity. Takarada, Ishii, et al.²⁰⁾ studied the effects of kaatsu training (30%-50% 1RM, 3 sets, 2×/week, 4 months) of upper arm flexors among subjects comprising healthy females of mean aged 60 years old, and reported in their results that both cross-sectional area and muscle strength of the biceps brachii increased by a mean of approximately 20.3%, an effect equivalent to training at an intensity of 80% 1RM. Conversely, there was virtually no observed effect in the same training at an intensity of 30%-50% 1RM when kaatsu pressure was not applied, suggesting that the effect of increased muscle size was attributable to kaatsu pressure. Ishii et al.²³⁾ also stated that leg extension kaatsu training (20-30 RM [sic], 5 sets, 2×/week, for 2 months) among top athletes also produced an approximate mean of 10% increase in muscle size and 15% increase in strength, but muscle size and strength did not increase in training at the same intensity without kaatsu pressure. These results suggest that kaatsu training has a substantial effect without regard to the age or training history of the subject, and that the primary factor in such effects is kaatsu pressure itself. Wernorm²⁴⁾ recently studied the domestic and foreign literature concerning high-intensity strength training and low-intensity training under restricted blood flow and reported that the relative increase in quadriceps size from leg extension exercise was similar in each case. In high-intensity strength training (80% 1RM, 3-4 sets, 6-10 repetitions, 60-120 second pause), the increase was 0.03-0.26%/day and 1-7%/month; in the low-intensity training under restricted blood flow (20-50% 1RM, 3-4 sets, 15-30 repetitions, 30-60 second pause), the increase was 0.04-0.22%/day and 1.2-6%/month.

In high-intensity strength training, muscle fatigue also precludes consecutive-day training for healthy individuals who are non-athletes, and patients as well, but the use of lowintensity loading in kaatsu training allows consecutive-day training. Yasuda *et al.* reported that muscle cross-sectional area increased approximately 8% in the short duration of 2 weeks when kaatsu training (intensity approximately 20% 1RM) was performed twice per day (morning and evening) every day by healthy individuals using the lower extremities ²⁵). Kaatsu training is also not limited to resistance training using strength machines and can also be applied in aerobic exercise such as walking or ergometer use ^{26,27}). Abe *et al.*²⁶ reported observing increased lower limb strength and muscle size in young, healthy individuals after 3 weeks of walking at a normal pace (walking at approximately 20% of peak oxygen uptake). In this fashion, kaatsu training can also be applied in aerobic training methods and can be performed ordinarily by anyone, and the training can also be adjusted according to individual abilities. By inducing effects of increased muscle strength and size at a low intensity of 20% 1RM, kaatsu resistance training allows development of individual muscle groups without use of special machines, and if machines are used, even more effective strength training can be accomplished.

There are several recent reports concerning the effects of kaatsu training among elderly individuals. One concerning research using strength training machines reports that increased muscle strength akin to that in high-intensity strength training was observed²⁸⁾. We likewise studied the effects of kaatsu strength training on muscle strength and size among 7 patients with stable ischemic heart disease (52±4 years, 5 pPCI, 2 pCABG)²⁹⁾. The low-load strength training was performed with lower leg extremity only (leg extension, leg press, leg curl) at 20-30% 1RM, 4 sets. The interval between sets was 30 seconds, and the training duration was 2×/week for 3 months. Fig. 2 presents the effects of kaatsu training with regard to increased muscle size and strength. After kaatsu training, effects of a significant increase in muscle size (A) and muscle strength (B) were observed. Additionally, during the training, no particular problematic adverse effects were observed.

Abe et al. reported that increased muscle size was observed in a research study concerning kaatsu-walk training conducted with elderly individuals ^{30,31}). High-intensity strength training has been reported to increase arterial stiffness, an index of arteriosclerosis ^{12,13}), but in kaatsu-walk training, arterial stiffness was instead seen to improve ^{32,33}. Venous stiffness as well as arterial stiffness has been reported to increase with aging, and we studied the effects of kaatsu-walk training by elderly individuals on venous stiffness. In the results, we reported that kaatsu-walk training by elderly individuals also improved venous stiffness ³⁴⁾. This series of results suggests that in kaatsu-walk training, an effect of increased muscle size is observed even among elderly individuals only with low intensity loads at which conventional training does not produce such an effect, and likewise, that vascular compliance may be improved, and that progression of arteriosclerosis may be prevented.

IIb. Muscle growth mechanism of kaatsu training

Through various mechanisms, kaatsu training can increase muscle size and strength effectively ^{16,19}. Kaatsu training involves exercising while pressure is applied to the base of the extremities with specialized pressure-application



Fig. 2. Effect of kaatsu training on ischemic heart disease patients (cf. Reference 29)

A: Change in muscle cross-section after kaatsu training. Relative increase in size of separate muscle groups, thigh region, according to MRI. Relative increase in cross-section versus pre-training value for separate muscle groups 30% down from top of thigh region, at central 50%, and 70% down from top of thigh region. Quadriceps femoris (QF), hamstring (HAM), adductor (ADD).

B: Relative increase in 1 RM for various strength training exercises (leg extension, leg press, leg curl). p < 0.05 vs. control.

belts to restrict primarily venous blood flow in the muscles (intramuscular blood pooling)^{21,22)}. Oxygen supply to the muscles is then reduced and clearance of lactic acid and other metabolic products is obstructed. As a result, it is thought that a large number of motor units are mobilized during exercise in order to maintain strength ¹⁶). This mobilization of a large number of motor units during exercise is regarded as one cause of the increase in muscle size, and in reality, kaatsu training under low-intensity load has been shown to accelerate protein synthesis³⁵⁾. Fujita et al. also carried out leg extension exercise at 20% 1RM and reported that muscle protein synthesis was stimulated by a change in the mRNA translation initiation system³⁵⁾. A report³⁶⁾ based on electromyogram analysis also states that activity in a large number of muscle fibers has also been observed under kaatsu pressure, as in high-intensity training, in spite of the light load applied.

Ohta et al. studied the effect of kaatsu training on muscle fibers and reported that both Type I (slow-twitch fibers) and Type II fibers (fast-twitch fibers) each demonstrated a trend of increased size in muscle biopsy performed after 8 weeks of kaatsu rehabilitation following reconstruction of the anterior cruciate ligament^{37,38)}. Type II fibers are also reported to increase in size in kaatsu training by healthy individuals²⁵⁾. In general, it is Type II fibers that show conspicuous enlargement due to muscle training. Consequently, the muscle-enlarging effect of kaatsu training is thought to result mainly from enlargement of fast-twitch fibers, but further studies to include the effect on slow-twitch fibers are needed. As fast-twitch fibers also decrease markedly with aging, and this phenomenon is closely related to decreased muscular strength in the elderly, kaatsu training may be effective for increasing muscle size and strength in elderly individuals.

Growth hormone (GH) and other such humoral factors are said to contribute to muscle growth through resistance training. One mechanism of the muscle size-increasing effect of kaatsu training is thought to be the role of increased growth hormone secretion. GH is secreted by the pituitary gland, is an import growth factor affecting the muscles, bones, and other effectors, and is known to have various functions. Secretion of GH pursuant to exercise depends on the intensity, type, and duration of exercise and other related factors and requires at least 10 minutes of high-intensity exercise or exercise exceeding the anaerobic threshold (AT); at lower loading, distinct secretion does not occur. In low-intensity kaatsu training (20% 1RM) studied in the thigh muscles, the level of GH in blood was reported to increase markedly immediately after exercise ³⁹⁾. Our investigation also found distinct secretion under kaatsu pressure, at low-intensity resistance loading of 20% 1RM⁴⁰. This effect was observed not only with resistance loading of the extremities, but also in ordinary walking²⁶⁾. In kaatsu training, accumulation of lactic acid and other such substances stimulates multifunctional afferent nerves distributed in skeletal muscle, and triggers a metabolic reflex mediated by the hypothalamicpituitary system, which is considered to play a crucial role in secretion of G.H.

Localized mechanisms may also contribute to the overall mechanism of muscle growth. These include growth factor secreted by muscle fibers or their surrounding cells. In an experiment using a kaatsu animal model (rat with surgically-induced selective blocking of veins from muscles of the posterior extremities), Kawada *et al.*⁴¹⁾ reported that fast-twitch muscle fibers were selectively enlarged approximately 10 days after restriction of blood flow. These enlarged muscles demonstrated characteristics including 1) decreased myostatin expression, 2) increased active hepatocyte growth factor (HGF), 3) increased nitric oxide synthase (NOS-1), and 4) increased

muscular glycogen. Myostatin in particular is a factor which strongly restricts muscular growth, and the fact that this change is regarded as a factor accelerating muscular growth means that the changes in such local factors due to kaatsu pressure may cause muscle growth.

IIc. Effect of kaatsu training on trunk muscles

Like the lower extremities, the trunk muscles are known as skeletal muscles readily susceptible to sarcopenia ^{42,43}. Sarcopenia in these muscles, which play a crucial role in daily activities and walking, can lead to diminishment of bodily functions required for activities of daily living. We therefore summarize the effects of kaatsu training on the trunk muscles.

Investigation of the effect of kaatsu training at 30% 1RM on the trunk muscles using primarily the bench press, a multiarticular exercise, showed that the amount of muscular activity in the pectoral muscles and triceps brachii during kaatsu exercise increased in both muscle groups as the number of lifting repetitions increased, and at the last stage of the final set, activity reached a high value of 110-120% versus the start of exercise in both muscle groups. Kaatsu exercise also produced a significantly higher level of muscular activity than exercise without kaatsu pressure⁴⁴⁾. This finding represented results similar to those in single-articular exercise moving only the limbs, and in low-intensity kaatsu bench press exercise, large mechanical stress in excess of the loading weight lifted was found to bear on the muscles of the trunk, not merely on those of the arms^{45,46)}. The stimulation from kaatsu training is thus regarded to substantially affect even the trunk muscles, where blood flow is not restricted.

Changes in muscle thickness have also been observed in the triceps and pectoral muscles during 2-week kaatsu training performed $2\times/day$ (morning and afternoon, *Fig. 3*)⁴⁷⁾. Typically, muscle thickness at these locations demonstrates an extremely high correlation with muscle cross-sectional area and muscle volume, therefore, any changes in muscle thickness can be a primary indication of changes in muscle mass. Muscle thickness of the pectorals measured prior to the morning training increased approximately 11% in the first 1-week period, did not decrease even with the interruption of a break on Sunday, and was observed to increase approximately 6% further in Week 2. These changes in muscle thickness did not decline even 3 weeks after the completion of training. However, virtually no changes in muscle thickness were observed throughout the 2-week period in training without kaatsu pressure. Such changes in muscle thickness were also observed in the triceps, and the limbs and trunk demonstrated similar changes. Next, to determine whether any changes in cross-sectional area of the pectorals would be observed under ordinary training conditions, the effects of kaatsu training were observed during 6 weeks (3×/week) of bench press exercise. As a result, muscle cross-sectional area measured by MRI showed an approximate 5% increase in the triceps muscle and an approximate 8% enlargement of the pectoral muscles ^{48,49)}. These results therefore show that kaatsu training may also enlarge the muscles of the trunk. As stated previously, a marked increase in muscular activity was observed in the pectorals, and not only the triceps, during low-intensity bench press exercise with kaatsu pressure. It is possible that when kaatsu pressure fatigues the muscle groups of the arms, the thoracic muscle groups, as cooperative muscles, compensate and work more actively than usual, and that this stimulation is one important factor causing an increase in the size of the trunk muscles. Dramatic activation of the endocrine system has also been observed in kaatsu exercise, and in conjunction, effects



outside the local area where blood flow is restricted have also been reported (*i.e.*, effect migration), which may be another substantial factor⁵⁰.

Because pressure is applied at the base of the extremities in kaatsu training, it is readily but incorrectly thought that perhaps only the extremities grow. But in reality, as such purely local enlargement occurs, a similar response has also been found to occur in muscles of the trunk which contribute to motion of the shoulder joints and hip joints ^{47-49,51,52}). Consequently, kaatsu training performed at low intensity can also enlarge the muscles of the trunk, and not only those of the extremities, indicating that this training is extremely effective for frail and elderly individuals and may hold substantial promise as a supportive method to correct sarcopenia in particular.

IId. Potential effect of kaatsu training on muscular atrophy

Cachexia and sarcopenia are known as particularly conspicuous states of muscular atrophy. Sarcopenia refers to a decrease in muscle size leading to a distinct reduction in muscle strength. The diseases engendering this state are most often chronic obstructive pulmonary disease (COPD) and heart failure but also include senility, rheumatoid arthritis, and cancer. Various factors contribute to cause sarcopenia, including degeneration of muscle fibers, inflammation due to increased levels of tumor necrosis factor (TNF)- α and other cytokines, nutrition, and disuse. Resistance training, as well as aerobic exercise, are useful in preventing muscular atrophy, but improvement is often slow. Here we present an actual case where kaatsu training proved useful for such a patient. A 39-year-old female experienced decreased muscle strength and muscular atrophy in the lower limbs due to allergic

granulomatous angiitis, making plantar flexion and dorsal flexion impossible in this case. Before training started, lower limb circumference at the distal portion was 26.9 cm in the right leg and 31.7 cm in the left leg, showing clear atrophy on the right side. Kaatsu training was performed 2 times per week with an attachment pressure of 40 mmHg applied to the lower limbs, and setting pressures of 200 mmHg and 150 mmHg applied to the right and left legs respectively at the beginning of the training. Training comprised light, low-load exercise including plantar flexion and dorsal flexion of the hands and feet (3-point set), non-loaded leg extensions (alternating), nonloaded squats, and kaatsu-walk training. The fact that even pressures of 200-300 mmHg do not cause hemostasis is a particular feature of the specialized kaatsu belts used, though individual differences and setting pressures produce different results. Training was then changed to lower limb-only exercise, and the exercise level was raised with increased loads. The 22nd training comprised kaatsu exercise of the lower limbs only. The attachment pressure used was 45 mmHg, and setting pressures were 300 mmHg and 180 mmHg for the right and left legs respectively. Three-point sets of leg extensions with a 5 kg load and leg curls with a 14 kg load applied deliberately to the right leg were performed, followed by calf raises under kaatsu pressure and kaatsu-walk training. Fig. 4 presents MRI findings for the lower limbs before inception of kaatsu training and 3 months later. The individual also perceived an increase in muscle strength and muscle size, and plantar flexion and dorsal flexion also became possible. Kaatsu training was thus determined to have a clearly effective improvement, even in a patient presenting muscular atrophy⁵³⁾.

As stated above, recent investigations of the effect of kaatsu training on muscle strength and mass among elderly individuals have reported a distinct effect²⁸⁻³³⁾.

III. Exercise and nutritional intervention for correction of sarcopenia

Measures have been studied to maintain muscle mass and prevent atrophy in occurrences of sarcopenia, a phenomenon among the elderly in which muscular atrophy leads to decreased muscle mass, resulting in impaired mobility. In particular, nutritional intake and consumption of essential amino acids including branched chain amino acids (BCAA) are crucial for rapidly increasing synthesis of skeletal muscle proteins. The amount of muscle proteins present is determined by the balance of muscle protein synthesis and decomposition. Fig. 5 presents the effects of exercise (resistance exercise) and diet (amino acid intake) on muscle protein synthesis and decomposition ⁵⁴⁾. Muscle proteins are continually and repeatedly synthesized and decomposed, and at rest, decomposition of muscle proteins outweighs synthesis, and the net balance of muscle protein addition and subtraction produces no increase. At the same time, muscle protein synthesis is regulated by amino acid concentrations in the blood, and when such concentrations decline, muscle protein synthesis decreases rapidly; conversely, when such concentrations increase, muscle protein synthesis also increases. Consequently, when amino acids are administered at rest, muscle protein synthesis increases (Fig. 5). In addition, when amino acids are consumed after exercise, muscle protein synthesis is accelerated, while muscle protein decomposition caused by exercise is inhibited, moving the net balance of muscle proteins distinctly toward increase and improvement. However, when amino acids are not administered, decomposition of muscle proteins is not inhibited, and the balance does not become positive. Thus, consumption of BCAA is thought to decrease the amount of amino acids liberated from muscle proteins, inhibit decomposition of muscle proteins, and in this way incline the balance toward an



Fig. 4. Effect of kaatsu training in a case of decreased strength and muscular atrophy, lower extremities, due to allergic angiitis (cf. Reference 53). MRI findings before/after kaatsu training shown. Clearly atrophied right lower thigh muscle is perceptibly larger after kaatsu training.


Fig. 5. Effect of amino acid (AA) administration at rest and during exercise on muscle protein synthesis and decomposition (from Reference 54)

 $F_{O.M.}$ (intracellular amino acid utilization (protein synthesis)), $F_{M.O.}$ (intracellular amino acid appearance (proteolysis)), N.B. (net amino acid balance across the leg (negative numbers indicate net release)). Muscle protein synthesis at rest is increased by administration of amino acids. In exercise, amino acid administration makes that balance positive, and synthesis of muscle proteins overcomes decomposition. See reference for details.

increase in muscle proteins. As a result, if strength training is performed on an empty stomach, unless the acceleration of muscle protein synthesis exceeds the rate of decomposition, it is difficult to trigger an increase in muscle size. The combination of extrinsic BCAA and resistance training are thus considered as an important, complementary relationship moving the balance of muscle proteins toward a net increase, and habitual exercise (resistance training) in combination of extrinsic BCAA is thought to induce increases in muscle size. In this acceleration of muscle protein synthesis by amino acids, leucine, which is categorized as branched-chain amino acid (BCAA), plays an important and unique role as compared to other essential amino acids⁵⁵. BCAA have been shown to accelerate synthesis of muscle proteins by increasing the translation rate of mRNA (*Fig. 6*).

Consuming a regular meal and then before exercise consuming a large amount of proteins and amino acids is reportedly effective for enhancing the effect of amino acids. Further, in young individuals, the addition of sugars to amino acids increases the rate of protein synthesis synergistically. Consumption of sugars together with proteins elevates insulin secretion, and insulin is a hormone with a proteinassimilating effect which stimulates muscle protein synthesis by incorporating amino acids in the blood into muscle tissue and changing the transcription initiation system of mRNA. An adequate supply of amino acids in the blood is also crucial for stimulation of muscle protein synthesis by insulin. In sum, nutritional intervention is regarded as an extremely important factor for increasing muscle size.

Many factors are regarded as causes of sarcopenia among the elderly, including age-related changes in endocrine secretion function, inactivity, and inadequate nutrition, particularly inadequate amino acid intake. Irregular meals or amino acid intake and inactivity is theorized to lead to a negative balance of muscle protein addition and subtraction and cause muscle atrophy. However, resistance training has been proven to accelerate synthesis of skeletal muscle proteins even among the elderly, just as in young individuals ^{56,57}). Volpi et al.⁵⁸⁾ investigated muscle protein metabolism as a function of amino acid intake among elderly individuals. Their results showed that the accelerating effect on muscle protein synthesis was equivalent when complete amino acids were provided and when essential amino acids were provided, and essential amino acids produced a muscle protein synthesis-accelerating effect even among elderly individuals ⁵⁸⁾. Thus, exercise and nutritional intervention are regarded as useful for correction of sarcopenia even among elderly individuals, just as among younger individuals.



Fig. 6. Effect on 70-kDa S6 protein kinase (p70^{S6K}) Ser⁴²⁴/Thr⁴²¹ phosphorylation in skeletal muscle from administration of essential amino acids (BCAA) at rest and during exercise. Phosphorylation at 1 and 2 hours after exercise is distinctly increased by administration of BCAA versus non-administration (from Reference 55).

Conversely, Volpi *et al.*⁵⁹⁾ investigated the effects of amino acids and sugars on muscle protein assimilation among elderly individuals, and reported that protein assimilation produced by amino acids alone was clearly lowered when sugar was consumed. This effect is deemed related to factors such as insulin resistance⁶⁰⁾, but it can also be assumed that the capability for dietary-based protein synthesis is reduced among the elderly. Fujita *et al.*⁶¹⁾ also reported that increase in intramuscular blood flow is highly important for acceleration of muscle protein synthesis by insulin. Consequently, when insulin resistance or other such factors preclude increase in muscular blood flow, muscle protein synthesis is also thought to decline. In the elderly, such nutritional deficiency may contribute to sarcopenia, and further study of effective nutritional intervention for the elderly is needed.

Conclusion

As societal aging continues, questions of how to prevent age-related muscle loss (sarcopenia) to prevent bedridden states, and further to improve QOL and maintain active lifestyles are crucial issues. Exercise therapy is highly useful in these respects. Our paper discusses aerobic exercise, strength training, and kaatsu training in particular as anti-aging exercise. Diet that enhances the effects of exercise, and amino acid intake in particular, are regarded as extremely important measures to correct sarcopenia among the elderly. Further study is needed on prevention and improvement of sarcopenia through multifaceted programs combining exercise and dietary intervention.

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KAATSU training[®] as a new exercise therapy for femoral head avascular necrosis: A case study

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Femoral head avascular necrosis is a condition in which part of the femoral head undergoes necrosis due to decreased blood flow. The femoral head gradually disintegrates causing pain and even today, there are no effective rehabilitation methods other than symptomatic treatment such as decreasing the load on the hip joints with the use of a cane or walker. We herein describe our insights into this condition based on our experience with a case of femoral head avascular necrosis caused by steroid use in which KAATSU training was found to be highly effective. The patient was a 34-year-old woman (154 cm tall and weighing 50 kg, a radiologist). Since the age of 23, this patient had been receiving steroid treatment to control her refractory asthma. She later developed pain in her right hip and aradually suffered hip joint deformation, restricted range of motion, and difficulty in walking. MRI revealed Association Research Circulation Osseous (ARCO) stage IV disease. She suffered marked pain of the right hip joint every time she walked, occasionally falling and required a cane to walk. At the patient's own request, she received KAATSU training including KAATSU walking over a period of 3 months (total 28 sessions). Various assessments were carried out before and after training to determine the effects of KAATSU training. QOL was determined by SF-36v2, and marked improvement of role physical, body pain, general health, vitality, and social functioning were noted. Before training, the Japanese Orthopaedic Association (JOA) hip scores for pain were 10 (right), 40 (left), walking ability 16 (right), 20 (left), while 3 months after training, these scores were markedly improved in the affected side. Furthermore, not only did muscle strength on the affected side show marked improvement, but the MRI also revealed a tendency for improvement of the right femoral head avascular necrosis. DEXA showed signs of a clear increase in bone mineral density. Based on the above, these results suggest that KAATSU training is extremely useful as a rehabilitation method in patients with femoral head avascular necrosis. But, further larger scale investigations should be carried out in the future to support our findings.

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Key words: Femoral head avascuar necrosis, KAATSU training, Bone density, Pain, Rehabilitation

Introduction

Femoral head avascular necrosis is a condition in which part of the femoral head suffers necrosis due to a decrease in blood flow. Although the cause remains unknown, possibilities include thrombosis due to abnormalities in the blood coagulation system, and injuries of the vascular endothelium (Chang et al., 1993). Risk factors include the administration of steroids (Weinstein, 2011). Steroid administration leads to decreases in blood flow due to increases in the internal pressure of the femoral head resulting from hypertrophy and proliferation of adipocytes. Together with decrease in blood flow, it has been reported that direct inhibition of osteoblasts and osteocytes may also be involved (Cruess, 1976; Kerachian et al., 2009; Ding et al., 2015). Pain occurs as the femoral head disintegrates, and other than symptomatic treatment such as using canes and walkers to reduce weight bearing stress upon the hip joint, there are no effective rehabilitation methods (Wang et al., 2014). Muscle

strength training is often used in the rehabilitation exercises but there are cases where pain will make it impossible to exercise while weight-bearing is not possible and so this is often ineffective. Therefore, even today, it is difficult to prevent the progression of disease with conservative treatment alone, and prognosis is poor so that surgery is eventually required.

KAATSU training (Takarada et al., 2000; Sato et al., 2007) allows for muscle growth when blood flow is restricted, even with low stress training, and major increases in muscle mass can be achieved. It is indeed a useful rehabilitation method for patients with various diseases and our aging society (Nakajima, 2010; Abe et al., 2010; Ozaki et al., 2011; Nakajima et al., 2011; Yasuda et al., 2014). Exercise methods include no load, elastic band (Yasuda et al., 2015), and in addition to free weight, walking (Abe et al., 2006), and ergometer (Abe et al., 2010). In this way KAATSU training is being featured as a method of rehabilitation in cases where high exercise loads are to be avoided.

In addition, KAATSU training with restricted blood flow causes decreases in the intramuscular oxygen partial pressure during exercise and hypoxemia. With active refilling of vasculature in response to belt release, reactive hyperemia develops and vascular endothelial growth factor (VEGF) and nitric oxide (NO) production are enhanced (Takano et al., 2005; Horiuchi and Okita, 2012) and this may lead to improvements in both endothelial function and circulation. In this way, KAATSU training appears to be effective as a rehabilitation method in patients with femoral head avascular necrosis, but there are few reports regarding the effects of KAATSU training in these patients until now.

We experienced a case of femoral head avascular necrosis in one patient and would like to report our findings.

1. Case introduction

Case report: A 34-year-old woman (height 154 cm, weight 50 kg, profession radiologist)

Diagnosis: femoral head avascular necrosis

Chief complaint: pain of the hip joint during ambulation, gait disturbance.

Family history: no notable findings.

History of present illness: bronchial asthma with an onset during youth. Since experiencing a severe attack of aspirin-induced asthma at age 15, the patient became dependent on steroids and repeatedly required hospitalization because of poor control and had continued to take a large dose of steroids. As of age 23, HOT was introduced, and since age 25, as soon as her clinical rotations started, pain in the right hip joint began to manifest. Thereafter, deformation of the right hip joint progressed until at age 33, and she developed femoral head avascular necrosis (OA). Gradually, the hip joint pain worsened with the limitations in her range of motion (ROM), and abduction external rotation contracture appeared. The patient suffered extreme pain in the right pelvis during walking and in order to reduce the joint load during walking had been using a cane. She presented at our hospital hoping to receive exercise treatment with KAATSU training. The patient had been taking prednisolone 3 mg/day, theophylline, montelukast sodium, and salmeterol/fluticasone.

This research was approved by the Institutional Review Board at the University of Tokyo and performed after receiving patient consent.

2. KAATSU training protocol

A KAATSU belt is wrapped around the base of the thigh and a KAATSU Master device (KAATSU Japan, Co., Ltd) was used. KAATSU training was provided for a total of 28 sessions over 3 months. Base pressure was 45 – 50 SKU. The optimal pressure started at 100

- 140 SKU and while repeatedly pressurizing and depressurizing, it gradually increased in 20 SKU increments, until an optimal pressure was reached. The training menu was as follows. 1) 3 point set: Toe curls, ankle dorsiflexion, and ankle plantar flexion. 2) KAATSU walk: gradually increased distance to approximately 150 to 300m during each session until a comfortable walking speed was achieved. 3) Non-KAATSU walk: belts were removed and the patient was allowed to walk for 75m to check for symptoms. In late stage intervention, calf-raises and squats were added, and the patient extended her comfortable walking distance to approximately 300m. Calf raises and squats were performed standing and bearing one's own weight. The KAATSU side in the early stage of intervention was the affected leg only, starting with a KAATSU pressure of 300-320 SKU. In the latter stages, in addition to the affected right thigh, the healthy left thigh (160-300 SKU) exercise therapy under KAATSU condition was also conducted.

3. Evaluation

The following evaluations were conducted before and after training.

- 1) Magnetic resonance imaging (MRI)
- 2) Life functioning: 36-item short form health survey (SF–36v2).
 - The effect of training on life functioning was investigated using the SF-36v2 evaluation survey. Role physical, body pain, general health, vitality, social functioning, role emotional, and mental health were assessed.
- 3) Muscle strength evaluation using Cybex
- Measurements were taken with the subject seated in a chair with the trunk and thighs immobilized. Exercise involved bending the knee joint 90°, from a flexed position to extension and flexion. Bilateral lower limb muscle strength (isomeric knee extension strength: knee joint 75°, isokinetic knee extension and flexion muscle strength: 30°/sec, 90°/sec, 180°/ sec) was determined.
- 4) Functional analysis of the hip joint by Japanese Orthopaedic Association (JOA) hip scores as shown in Table 1 (Takatori et al., 2010)
- 5) Dual energy x-ray absorbed absorptiometry (DEXA) bone mineral density measurement device.

4. Results and clinical course

MRI findings before training are shown in Fig. 1A (left). T1 and T2 weighted images of the right femoral head weight-bearing areas revealed areas of low intensity signals. The surrounding areas showed high signals in the T2 weighted image, suggesting bone necrosis and an edematous change of the surrounding marrow. Her right hip joint showed joint space narrowing and formation of bone spurs, while the femoral head showed signs of flattening leading to

<u>I. Pain</u>			point
No complaints regarding the hip joint			40
Indefinite complaints (feeling strange, fatigue) present but no pain			35
No pain during walking (however at the start of walking or after walking for long distan	ces there is so	metimes pain	ı) 30
No spontaneous pain. Pain during walking, but disappears with short rests			20
Spontaneous pain is sometimes present. Pain during walking but alleviates with rest			10
Continuous spontaneous pain or nocturnal pain			0
II. Range of motion assessment			
Flexion: Joint angle counted in 10° intervals, one point per 10°. However, all points bey	ond 120° are	counted	
as 12 points (if joint contracture is present this is subtracted and evaluations based on m	ovable range)	•	
Abduction: Joint angles are measured in 10° increments and each 10° is counted as 2 pc	oints.		
However any angle over 30° is counted as 8 points			
III. Walking ability			
Absolutely no complaints regarding the joint			40
Indefinite complaint (feeling strange, fatigue), no pain			35
No pain during walks (however there may be pain at the start of the walk or after walki	ng long distar	ices)	30
No spontaneous pain. Pain during walks disappears after short rests.			20
Spontaneous pain is sometimes present. Pain present during walks but is relieved after r	est.		10
Continuous or spontaneous pain or nocturnal pain.			0
IV. Activities of daily living (ADL)			
Evaluation	easy	difficult	impossible
Knee blankets	4	2	0
Standing work (includes housework) (continues for about 30 minutes).	4	2	0
If rests are required, it is considered "difficult". In cases where exercise			
can only be continued for 5 minutes, it is considered "impossible".			
Squatting and standing (those who require assistance: consider it "difficult")	4	2	0
Climbing up and down stairs (those who require a railing: consider it "difficult")	4	2	0
Getting on and off cars and buses	4	2	0



Figure 1. A: MRI findings before and after KAATSU training. Before training (left), after training (right) B: Dual-energy x-ray absorptiometry (DEXA) scan bone mineral density findings before and after KAATSU training. After training, bone mineral density in the right leg (affected side) was clearly increased, compared with the left leg (healthy side).



Figure 2. Efficacy of KAATSU training in a patient with femoral head avascular necrosis. A: Effect on JOA score. a: JOA score (total, comprehensive), b: Pain, c: Walking, d: ROM (range of motion), B: SF-36v2. Role physical, Body pain, General health, Vitality, Social functioning, Role emotional, Mental health

a case of secondary arthrosis. Surrounding areas included a small high-signal intensity subchondral bone cyst. The other hip joint showed no signs of abnormally high intensity areas. Based on these MRI findings, the patient was diagnosed with Association Research Circulation Osseous (ARCO) stage IV right femoral head avascular necrosis and secondary hip arthrosis.

Before and after training, several evaluations were carried out. Fig. 2A shows the effectiveness of KAATSU training on JOA hip scores. JOA (pain, b) scores were 10 (right), 40 (left); while the JOA (walk, c) scores were 16 (right), 20 (left); and JOA (score, comprehensive, a) was 55 (right), 84 (left). After training, all scores showed marked improvement in the right affected leg compared to the left healthy leg. JOA (ROM (range of motion), d) revealed no signs of improvement on the affected side. In the latter half of training, pain of the hip joint during walking dissipated so that the patient no longer required a cane to walk. In addition, there was a clear improvement in the patient's walking style.

Fig. 2B shows the effect on SF-36v2 before and

after KAATSU training. Role physical, body pain, general health, vitality, social functioning, role emotional, and mental health were evaluated. Physical function, role physical, body pain, general health, vitality, social functioning all improved after KAATSU training

Figure 3 shows the effects of KAATSU training on lower limb muscle strength (extension and flexion). Muscle strength during extension and flexion was markedly improved in the right leg on the affected side, compared to the healthy left leg.

Fig. 1B shows the efficacy of using KAATSU training on bone mineral density measured by DEXA. There was a clear increase in right affected leg bone mineral density from 0.798 to 0.836. On the other hand, the left healthy leg went from 0.857 to 0.868 with no clear signs of improvement. Furthermore, MRI findings after KAATSU training shown in Fig. 1A (right) showed a tendency for the right femoral head necrosis to improve.



Figure 3. Effect of KAATSU training on lower limb muscle strength (knee extension, knee flexion) measured by Cybex.

5. Discussion

We treated a patient with femoral head avascular necrosis for 3 months with KAATSU training and noted the following improvements. 1) After KAATSU training, the JOA score of the hip joint and the JOA pain score both improved markedly. 2) Together with an apparent improvement in gait, femoral muscle strength improvement was noted while bone mineral density of the lower limbs had clearly increased in the affected side. 3) MRIs showed a tendency for improvement of the right femoral head necrotic site.

In this patient, SF–36v2 was evaluated. Of the life functions, physical function, role physical, general health, vitality, social functioning, and walking ability based on the Japan Orthopedic Association hip score (JOA hip score) (Takatori et al., 2010) had all improved. Furthermore, muscle strength on the affected side had increased, proving that muscle strength improvement with KAATSU training can lead to improved walking ability and have a major impact on enhancing QOL. This meant that in the latter half of the training, the patient became able to walk without a cane. In addition, the affected limb could support weight without use of a cane, and it is believed that there was a clear increase in bone mineral density on the affected side.

The mechanism for this effect of KAATSU training includes a characteristic of KAATSU training (Takarada et al., 2000; Sato et al., 2007) which is that exercise under restricted muscle blood flow conditions leads to increased muscle strength and muscle growth after only a short period of low stress exercise. In this case, we observed a clear increase in muscle strength with only 3 months of KAATSU training. KAATSU training is just the right rehabilitation method for patients with various diseases and our aging society (Nakajima, 2010; Abe et al., 2010; Ozaki et al., 2011; Nakajima et al., 2011; Yasuda et al., 2014). Loading included nonweight bearing, elastic band (Yasuda et al., 2015), a dumbbell, walking (Abe et al., 2006), and ergometer (Abe et al., 2010) loads. In this way, KAATSU training is an extremely useful rehabilitation method for use in patients in whom excessive stress would be inappropriate. In our case, we added squats and calf raises to the KAATSU walking schedule, carrying out various forms of exercise in a way that the femoral head would not have to bear any weight. As a result, patient ADLs improved markedly. However, the ROM did not improve. Therefore, in the future we believe a longer investigation is warranted.

Furthermore, after KAATSU training, pain scores based on the SF-36v2 and JOA hip scores showed clear signs of improvement. With improvement of her pain, the patient no longer required a cane and we believe her quality of life has been greatly improved because she regained the ability to walk. The mechanism responsible for improving pain in KAATSU training is still unknown, but increased muscle strength, muscle growth and alleviation of the weight-bearing on the hip joints are believed to play a role. On the other hand, KAATSU training leads to a decrease in intramuscular partial O2 pressure and hypoxemia during exercise under restricted blood flow. Reactive hyperemia develops and vascular endothelial growth factor (VEGF), and nitric oxide (NO) production is enhanced (Takano et al., 2005; Horiuchi and Okita, 2012) in response to vascular refilling after the belts are released, leading to improvements in endothelial function and blood flow. We propose these improvements in blood flow helped to heal the necrosis in the femoral head. Animal models of femoral head avascular necrosis caused by steroids have actually shown that as blood flow into the femoral head decreases, both VEGF protein and mRNA decrease (Wang et al., 2010). However, the improvement of MRIs in the present study for 3 months was minor so the effects should be further confirmed in a long-term study. In addition,

investigations into elucidating the mechanism behind pain relief with KAATSU training are warranted.

Conservative treatment for femoral head avascular necrosis should be instituted carefully to avoid crushing the femoral head, and there are currently no effective rehabilitation methods other than symptomatic treatment such as using a cane or walker to reduce placing weight on the affected hip joint. From the present study, KAATSU training may be a useful rehabilitation method to treat femoral head avascular necrosis.

Summary

We reported our experience with a patient who developed femoral head avascular necrosis while on steroid therapy who was successfully treated with KAATSU training. We believe further clinical research will be necessary.

<Nakajima T, Yasuda T, Fukumura K, and Morita T have participated in seminars until September 2014 donated by KAATSU Japan.>

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KAATSU training as a new effective exercise therapy in a case of femoral medial condyle osteonecrosis

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See end of article for authors' affiliations Idiopathic osteonecrosis of the medial condyle of the femur, a relatively common disease among Japanese women 60 years or older, is often progressive and requires surgical treatment. In this paper, we report on a case where KAATSU training was effective for a 71-year-old woman with bone necrosis in the left femoral medial condyle revealed by magnetic resonance imaging (MRI). At the first visit, the patient was suffering severe pain, and unable to walk without a cane. After 1 to 2 months of KAATSU training, her pain was mitigated and in about 3 months she was able to walk without a cane. In 6 months she could go upstairs and downstairs using the handrails and about 2 to 3 months later, she no longer needed to use the handrails. MRI revealed marked shrinkage (to 10 mm x 15 mm) of the necrotized region as a result of bone tissue remodeling. From these results, KAATSU training seems to be useful as a new method of rehabilitation for patients with idiopathic osteonecrosis of the medial condyle of the femur.

Introduction

Osteonecrosis (ON) of the medial condyle of the femur may develop as a secondary condition associated with the prolonged use of steroids. However, idiopathic ON of the medial condyle of the femur is relatively common among women 60 years or older. In the early stages, the disease is difficult to distinguish from knee osteoarthritis by radiography and sometimes it is accompanied by severe pain. The patients may be followed up after conservative treatment. In many cases, however, surgical treatments such as arthroplasty are unavoidable because progression cannot be suppressed by conservative treatment alone^{1,2)}.

KAATSU training^{3,4)} is expected to be effective for increasing muscle volume and muscle power, even with low loads by training under blood flow restriction. KAATSU training represents a method of rehabilitation suitable for patients with various diseases and this aged society⁵⁻⁹⁾. The exercises include load-free exercises, elastic bands¹⁰⁾, and free weights, as well as walking¹¹⁾ and ergometers¹²⁾. As such, KAATSU training is attracting much attention as a method of rehabilitation which avoids high physical loads. We recently reported a case in which KAATSU training was effective in a patient with steroid-induced osteonecrosis of the femoral head¹³⁾. In this paper, we report a patient with idiopathic ON of the medial condyle of the femur who achieved remarkable improvement of the disease by KAATSU training.

1. Case review

Patient: A 71-year-old woman (157 cm in height, weighing 70 kg)

Diagnosis: Idiopathic osteonecrosis of the medial condyle of the femur

Chief complaint: Left medial knee pain on walking

Family history of illness: Non-contributory.

History of present illness:

In 2014, the patient presented with left medial knee pain at a nearby orthopedic clinic. She was diagnosed with knee osteoarthritis, and underwent ambulatory treatment for several months. Since her symptoms did not remit, and the pain worsened, she visited another medical institution in November 2014. The diagnosis of osteonecrosis of the medial portion of the medial condyle of the left femur was established. In January 2015, MRI findings suggested further exacerbation. KAATSU training was started in March 2015. One year 3 months later, in June 2016, the outcome was good with marked shrinkage of the necrotized region and improved activities of daily living.

2. KAATSU training protocol

KAATSU training was performed using the KAATSU MASTER equipment (KAATSU Japan Co., Ltd.). Taking into account the fact that the patient was an elderly woman, the wearing pressure was set at low levels (20-30 SKU). In March to April 2015, at the start of training, KAATSU training was performed for the leg on the dis-

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CR; November 19, 2014



MRI; November 19, 2014

Figure 1. Bone x-ray and MRI images taken before and after KAATSU training.



CR; January 21, 2015



MRI; February 18, 2015



CR; June 22, 2016 CR



MRI; June 22, 2016

eased side (left) only. Drills which were performed exclusively on the diseased side of the lower half of the body included the following: 1) compression and decompression, 2) three point set: Toe curls, ankle dorsiflexion, and ankle plantar flexion. 3) leg extensions, and 4) leg curls. In April to May 2015, the patient became accustomed to unilateral leg pressurization and switched to bilateral pressurization. In addition to the unilateral program, quarter squats were added. Bilateral sessions for the lower half of the body included the following: 1) compression and decompression, 2) quarter squats, 3) diseased-side leg extensions, 4) diseased-side leg curls, and 5) bilateral pressurized walking (KAATSU walking). In June 2015, a set of sessions for the upper half of the body was newly included in the training program. Bilateral sessions for the upper half of the body consisted of the following: 1) compression and decompression, 2) three point set: Toe curls, ankle dorsiflexion,

and ankle plantar flexion, 3) stiff shoulder course, and 4) dumbbell curls (1 kg). Bilateral sessions for the lower half of the body included: 1) compression and decompression, 2) quarter squats, 3) high-knee drills, 4) diseased-side leg extensions, 5) diseased-side leg curls, and 6) bilateral pressurized walking (KAATSU walking). Equipment pressure was increased step by step with gradual adaptation, and it was finally set at 320 SKU for unilateral application, and 280 SKU for bilateral application.

3. Course of symptoms

At the first visit, the patient was suffering severe pain, and was unable to walk without a cane. After 1 to 2 months of KAATSU training, her pain was remarkably mitigated and in about 3 months she was able to walk without a cane. In 6 months she was able to go upstairs and downstairs using handrails and about 2 to 3 months later, she no longer needed the use of handrails.

4. Bone X-ray and MRI findings (see Figure 1)

November 19, 2014

CR showed a mild regression of bone cortex, suspected of being bone necrosis in the load-exerted portion of the left femoral medial condyle; MRI revealed a 10 mm x 40 mm necrotized bone region at the same site.

February 18, 2015

The necrotized region was found to have expanded.

June 22, 2016

MRI revealed marked shrinkage of the necrotized region (to 10 mm x 15 mm) as a result of bone tissue remodeling, with mild regression persisting on the load-exerted surface of the medial condyle at the necrotized bone.

Discussion

Idiopathic femoral osteonecrosis develops mainly in the medial condyle of the femur, the anatomical site on which much of human body weight is exerted. Therefore, patients may undergo conservative treatments, including symptom relief by the reduction of stress on the affected portion with the use of, for example, a cane, and enhancement of lower limb function by training of the quadriceps femoris muscle. In many cases, however, surgical treatments such as arthroplasty are unavoidable because the progression cannot be suppressed by conservative treatment alone. Surgical treatments for idiopathic osteonecrosis of the medial condyle of femur include unilateral knee arthroplasty (UKA), total knee arthroplasty (TKA), and opening wedge high tibial osteotomy (OWHTO)¹⁴⁾. In particular, OWHTO sometimes leads to bone regeneration via drilling following curettage of the necrotized portion.

In the present case of osteonecrosis of the medial condyle of femur, KAATSU training was found to be effective; it produced bone regeneration, without surgical maneuvers. The patient achieved marked mitigation of pain in 1 to 2 months after the start of KAATSU training. Although the mechanisms for this pain mitigation by KAATSU training remains unknown, muscle strengthening and building by KAATSU training^{3,4)} might lessen the physical load on the patient's knees. KAATSU training may be described as a method of rehabilitation suitable for patients with various diseases and for this aged society⁵⁻⁹. The available means of applying training burdens include load-free exercises, elastic bands¹⁰, dumbbells, walking¹¹, and ergometers¹²; KAATSU training is considered quite a useful method of rehabilitation for patients who do not wish to undergo heavy physical loads. In the present case, the patient undertook pressurized walking, squatting, self-weighted leg extensions, leg curls, and other exercises in a way such that the femoral head would not bear a high load.

On the other hand, it remains unclear as to the mecha-

nism of bone regeneration through KAATSU training without surgical treatment. In addition to its effects on muscle strengthening and building, and the endocrine system, including growth hormones¹⁵⁾, KAATSU training may increase the production of vascular endothelial growth factor (VEGF), nitric oxide (NO), and other substances by decreasing muscular oxygen tension and hypoxia in the muscles during exercises, and by inducing vascular reactive hyperemia following belt release^{16,17)}, and hence it improves endothelium function and blood circulation, which in turn may lead to the amelioration of the necrosis.

In conclusion, KAATSU training is potentially highly useful as a new method of rehabilitation for patients with idiopathic osteonecrosis of the medial condyle of the femur. Further investigation is expected to elucidate the mechanism of the onset of the effects of this non-invasive method with slight physical burden.

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CASE REPORT

Effect of KAATSU training on thigh muscle size and safety for a patient with knee meniscectomy over 3 years

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[Objective] It is well known that knee meniscectomy is one of the major knee surgeries, which induces thigh muscle atrophy. However, it is unclear whether thigh muscle size after knee meniscectomy can be improved with KAATSU training. We examined effect of KAATSU training on thigh muscle size and safety for a patient with knee meniscectomy.

[Methods] The patient was a 57-year-old woman (standing height 159 cm and body weight 52 kg). The KAATSU training composed of 7 types of resistance exercise and one type of cycling exercise was provided for a total of 125 sessions over approximately 3 years. Transverse scans were carried out for mid-thigh length. Thigh muscle cross-sectional area (CSA) in affected-leg and un-affected-leg was measured by the CT scan before, 63 weeks, and 152 weeks after the training. **[Results]** Thigh muscle CSA was highly increased for affected-leg, and the attained level was exact-

ly similar for both legs after the 152 weeks training period.

[Conclusion] The long-term KAATSU exercises were a highly safe and effective training method for a patient with knee meniscectomy.

Key words: knee meniscectomy, muscle hypertrophy, thigh muscle, long duration, rehabilitation.

Introduction

It is well known that knee meniscectomy is one of the major knee surgeries, which induces thigh muscle atrophy (Akima and Furukawa, 2005; Ericsson et al., 2006). In general, traditional high-intensity exercise training ($\geq 70\%$ 1-repetition maximum: 1RM) improves skeletal muscle morphology and function (ACSM, 2009), but it appears that this method is not practical and may even be dangerous in patients with knee meniscectomy. On the other hand, KAATSU training (≤ 30% 1RM) can produce muscle hypertrophy and does not decrease vascular function (Ozaki et al., 2013; Yasuda et al., 2015; Yasuda et al., 2016). Additionally, recent studies (Nakajima et al., 2015; Hiraizumi et al., 2016) reported that KAATSU training is quite useful as a rehabilitation method in disorder of bone (femoral head avascular necrosis, femoral medial condyle osteonecrosis). However, it is unclear whether thigh muscle size after knee meniscectomy can be improved with KAATSU training.

Thus, the purpose of this study was to examine effect of KAATSU training on thigh muscle size and safety for a

patient with knee meniscectomy.

1. Case review

Patient: A 57-year-old woman (standing height 159 cm, body weight 52 kg), a physical education teacher Diagnosis: knee meniscectomy Chief complaint: Left knee joint pain on walking.

Family history of illness: No notable findings.

History of present illness & course of symptoms: On 15 October, 2014, she received meniscectomy implementation of the left knee under arthroscopy, and visited Okamoto-Ishii Hospital for receiving rehabilitation. At the first visit, the patient was suffering from strong leg swelling, heat sensation, and severe pain for left knee and was unable to walk without a cane. The KAATSU training started from October 30. At the start of rehabilitation, after performing pressurization / depressurization, exercises of isometric contraction without joint movement were performed. After that, she received icing. The pain gradually decreased, and she could raise the stairs with crutches. After 1 month of KAATSU training, her pain was remark-

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Unilateral knee extension



Bilateral calf raise



Go up and down with one step

Figure 2. KAATSU training protocols for each session.



Unilateral knee flexion



Figure 1. Pictures show 7 types of KAATSU resistance training.

ably mitigated and she was able to walk without cane. On January, 2015, the pain was abolished. After 9 months, she was able to go upstairs and downstairs, and began to run a little without pain. She noticed the increase of muscle power and mass. After that, she also began to climb the stairs without problems, and could run through the hallway, and jump rope in physical education class. At present, she is doing mountain climbing at her elementary school excursions. On September 2017, she has received KAAT-SU training for approximately three years. Any side effects have not been occurred during the long-term training.

The principles of the World Medical Association Declaration of Helsinki and the American College of Sports Medicine Guidelines for Use of Human Subjects were adopted in this stady. The study was approved by the Ethics Committee, and informed assent consent was obtained from the patient.

2. KAATSU training protocol

During the KAATSU training sessions, a patient wore a specially designed pneumatic cuff (50 mm width, KAAT-SU Master, KAATSU Japan Co., Ltd., Tokyo, Japan) around the most proximal portion of both thighs. KAAT-SU training was provided for a total of 125 sessions over approximately 3 years (from October 2014 to September 2017, 152 weeks). Basically, the cuffs were set at 40 SKU

Exercise	Menu	Intensity	
Unilatoral knop ortonsion	6 sets	Lower leg weight	
Offilateral knee extension	(30, 30, 20, 20, 10, 10 reps)	+ 1.5 kg weight	
Bilatoral calf raise	6 sets	Body weight	
Dilateral call faise	(30, 30, 20, 20, 10, 10 reps)	+ 3.0 kg weight	
Go up and down	6 sets	Body weight	
with one step	(30, 30, 20, 20, 10, 10 reps)	+ 1.5 kg weight	
Unilatoral know flowion	6 sets	Lower leg weight	
	(30, 30, 20, 20, 10, 10 reps)	+ 1.5 kg weight	
Hip adduction	6 set	Lower leg weight	
The adduction	(30, 30, 20, 20, 10, 10 reps)	Lower leg weight	
Hip ortonsion	3 sets	Lower body weight	
i np extension	(30, 20, 10)	Lower body weight	
Uin Amian	3 sets	I arrea ha du maiaht	
	(30, 20, 10)	Lower body weight	
Bicycle ergometer	30 min	65-70 watt	

and air pressure was inflated to 200 SKU. The training protocol (7 types of resistance exercise and one type of cycling exercise) was shown as Figure 1 and 2. Eight exercises over 60 min were performed each session.

3. Computed tomographic (CT) finding

Subjects rested quietly in the body coil in a supine position with their legs extended and relaxed. Transverse scans were carried out for mid-thigh length (from the top edge



Figure 3. Thigh muscle cross-sectional area (CSA) in affected-leg and unaffected-leg before, 63 weeks, and 152 weeks after training.

of the great trochanter to the lateral condyle of femur). From the cross-sectional image, outlines of the thigh muscles were traced, and digitized by using a personal computer (MacBook, Apple, Tokyo, Japan) for analysis using image analysis software (Image J 1.5, NIH, USA), and muscle CSA was calculated. The CT scan was measured at October 9, 2014, December 24, 2015, and September 9, 2017. Thigh muscle CSA was highly increased for affect-ed-leg, and the attained level was exactly similar for both legs at September 9, 2017 (Figure 3).

Discussion

The improvement of intrinsic morphology for the thigh muscles is important in exercise training for patients with knee injury and knee surgery. To the best of our knowledge, this is the first study to investigate the effect of KAATSU training on muscle strength and function for a patient with knee meniscectomy. The primary finding of this study was that KAATSU training remarkably increased the thigh muscle CSA for affected-leg, which was comparable with the unaffected-leg after the long-term period for 152 weeks.

Our findings show that KAATSU training after 63 weeks produces a hypertrophic potential of 0.33% per session for unaffected-leg, which is similar to that observed following KAATSU training after 12 weeks using weight machines (0.33% per session, Yasuda et al. 2014). This suggests that this training protocol is enough method for improving thigh muscle size for unaffected-leg over a long period. Additionally, the increase in thigh muscle CSA is approximately 2-fold higher in for affected-leg (0.61% per

session) compared to unaffected-leg after the 63 weeks training period. Taken together, KAATSU training using body weight can induce large improvement in thigh muscle size for a patient with knee meniscectomy over the long period.

In the present study, heart rate and ratings of perceived exertion during 7 types of KAATSU resistance exercises (90-129 BPM and 11-16, respectively) and KAATSU cycling exercise (\sim 127 BPM and \sim 11, respectively) were not high level (data not shown). These results are similar to that reported in previous KAATSU training studies for healthy subjects (Yasuda et al., 2014; Kim et al., 2016; Yasuda et al., 2016). Thus, it appears that these KAATSU exercises were a highly safe and effective training method for a patient with knee meniscectomy.

In conclusion, KAATSU training is potentially highly useful as a new method of rehabilitation for a patient with knee meniscectomy.

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Combination of KAATSU training[®] and BCAA intake for a patient after aortic valve replacement surgery: A case study

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Abstract

We report a case in which a favorable course was obtained by using KAATSU training[®] and BCAA intake early after aortic valve replacement surgery. The patient was a 43-year-old male with low cardiac function who underwent aortic valve replacement surgery. Heart failure and hypotension persisted while waiting for surgery. He tended to line on a bed all day, so that disuse muscular atrophy advanced. Therefore, in addition to the postoperative rehabilitation program, KAATSU training and BCAA intake were performed postoperatively and continued in outpatient rehabilitation. The KAATSU training was performed using knee extension twice or three times a week. For BCAA intake (2.5 g), one pack of jelly containing BCAA (Reha-Time Jelly, Clinico Co., Ltd.) was taken within 30 minutes after the training. About 3 months later, the thigh circumference (+ 7.3 cm), the maximum voluntary isometric contraction of knee extension (+ 20 kgf), the quadriceps muscle thickness (+ 1 cm) evaluated by a B-mode ultrasound, the muscle mass of the lower limb (+ 1 kg), and a marked increase in thigh muscle cross-sectional area as measured by CT scan were observed. No deterioration of circulatory hemodynamics and side effects were observed during the course. In conclusion, the combined use of KAATSU training and BCAA intake early after cardiac operation seems to be a safe and effective way to obtain muscle hypertrophy and muscle strengthening, but further studies are needed to clarify it.

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E-mail: nakat@dokkyomed. ac.jp Running head: KAATSU training and BCAA

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Key word: KAATSU training, cardiac surgery, muscle hypertrophy, muscle strength, BCAA

Introduction

Rehabilitation for patients with heart disease is based on aerobic exercise to improve cardiopulmonary function and resistance training for increasing muscle strength and mass. However, excessive exercise load may increase the risk of arrhythmia and heart failure exacerbation¹⁾. Therefore, aerobic exercise based on exercise prescription under anaerobic threshold (AT) is usually performed. For a kind of aerobic exercise, an ergometer or a treadmill is used, and its strength is determined by cardiopulmonary exercise testing (CPX). On the other hand, resistance exercise more than 67% of 1 repetition maximum (1 RM) is required for improving muscle strength and mass²⁾. But, it is recommended to start from a load of 50-60% in the lower limbs, taking safety into consideration^{3,4)}. For that reason, the improvement of muscle strength and an increase in muscle mass are not observed in several cases.

KAATSU training is a method that inventor Dr. Yoshiaki Sato repeatedly have conducted research for over 47 years. It is a novel exercise under the conditions with the restricted muscle blood flow, by binding the proximal portion of lower or upper extremities with a specially-designed belt. This training has been reported to induce muscle hypertrophy and strengthen muscle in athletes and healthy subjects, by using a short-term low-intensity exercise⁵⁻⁷⁾. Until now, several clinical studies using KAATSU training have been also reported to promote muscle hypertrophy in patients with cardiovascular diseases including ischemic heart disease^{7,8)}.

In addition to resistance training, the effect of ingesting

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a beverage containing branched-chain amino acid (BCAA) has been attracted attention. BCAA is considered to induce an increase of the muscle mass by enhancing the synthesis of muscle protein⁹⁾ and preventing the degradation of muscle protein¹⁰⁾.

Here, we report a case with low cardiac function in which a marked improvement of muscle strength and mass were obtained by using KAATSU training[®] and BCAA intake early after aortic valve replacement surgery.

Materials and method 1. Subjects

The patient was a 43-year-old male (173.0 cm, 66.3 kg, BMI 22.2 kg / m^2). He was urgently introduced to our hospital with suspected heart failure from a nearby doctor. He had no medical history, and coronary risk factors including dyslipidemia, hyperuricemia, and hypertension. At the time of admission, plasma brain natriuretic peptide (BNP) level was 2566 pg / ml. The cardiac ultrasound examination showed thinner heart wall, and prominent left ventricular enlargement (left ventricular (LV) end-diastolic diameter (LVDd) 72.1 mm, left ventricular end-systolic diameter (LVDs) 57.8 mm, left ventricular end-diastolic interventricular septum (IVS) thickness (IVST) 7.5 mm, left ventricular end-diastolic posterior wall thickness (LVPWT) 9.2 mm, left atrial diameter (LAD) 47 mm). The left ventricular ejection fraction (LVEF) was 34%. He was diagnosed as severe aortic regurgitation, and firstly received medication treatment. Cardiac rehabilitation started from the 7th disease day. However, the circulatory hemodynamics was unstable, and the exacerbation of heart failure was observed on the 16th disease day. Symptoms of hypotension also persisted and he became lying bed, so systemic marked waste muscle atrophy was recognized. Postoperative rehabilitation was resumed on the 42th day (Postoperative day 4: POD 4) after aortic valve replacement by thoracotomy on the 38th disease day. Since he was young, muscle recovery was desired for social reintegration. For that reason, KAATSU training and BCAA intake were introduced from the 49th disease day (POD 11), and outpatient rehabilitation continued even after discharge.

This study has been approved by the ethics committee of Dokkyo Medical University Hospital

2. Method

In the preoperative evaluation, only body composition analysis (see below) was carried out in consideration of hypotension and risk. On the 44th disease day (POD 6), the thigh circumference, the maximum voluntary isometric contraction (MVC) of knee extension, body composition analysis, quadriceps muscle thickness measured by a B-mode ultrasound and thigh muscle cross-sectional area was measured by CT scan. The KAATSU training started on the 49th disease day (POD 11) and reassessment was performed on the 66th disease day before discharge (POD 28). After discharge from the hospital, he received outpatient rehabilitation at a frequency of twice or three times a week and clinical evaluation was performed approximately every month.

The apparatus named KAATSU NANO (KAATSU JA-PAN Co., Ltd) was used for KAATSU training and a pressure cuff was attached to the base of both thighs. The set pressure was gradually increased to a proper pressure (100) to 200 SKU) at a mounting pressure of 15 ~ 25 SKU. Under the restriction of muscle blood flow, knee extension exercise (OG wellness company, GX-320) was performed. At the first time, the knee extension was done from a set of load of 20-30 % of 1 RM × 1 time of 20 repetitions. Number of times and set number increased stepwise, and finally 30 repetitions 3 sets (time between sets 30 seconds) was carried out. The frequency of KAATSU training was performed twice or three times a week. The KAATSU training was conducted under the guide of a medical doctor who was qualified as a KAATSU instructor. The blood pressure measurement, electrocardiogram monitoring, and subjective strength measurements (Borg scale) were performed according to the rehabilitation guideline for cardiovascular diseases¹¹⁾. The BCAA (Reha-Time Jelly, Clinico Co., Ltd., Japan) was applied within 30 minutes after the KAATSU training. The protein content was 10 g / 120 g, and the total BCAA content was 2.5 g / 120 g with 800 I.U. (20 µg) vitamin D / 120 g. The BCAA composition (per 120 g of a bag) was isoleucine 0.63 nmol / ml (0.6 g / 120 g), leucine 1.44 nmol / ml (1.4 g / 120 g), and valine 0.47 nmol / ml (0.5g / 120 g).

3. Clinical Evaluation

The thigh circumference was measured at the midpoint of the thigh length at the supine position. Hand Held Dynamometer (ANIMA Corporation, μ Tas F-1) was used for measuring MVC of knee extension. In measurements, limb position was a sitting posture with the trunk in the vertical position. The hip and the knee joint were bent 90 degrees and the upper limbs were assembled with the front chest. The position of the sensor was the distal part of the lower leg, and the fixing belt was attached so as to be perpendicular to the direction in which the force was applied. The measurement was performed twice on each side and the maximum value was adopted as MVC of knee extension.

The quadriceps muscle thickness was measured at the midpoint of the thigh length using an ultrasonic tomography apparatus (GE Healthcare Japan, Ltd., LOGIQ e Premium) as previously described¹². It was measured at supine position, and the measurement was performed twice at each side of the thigh, and the average value was adopted.

Body composition analysis was measured in the supine position using a body composition analyzer (Inbody Japan, Body Composition Analyzer Inbody S10) as shown previously¹². The muscle mass of left and right leg was evaluated.

The femoral muscle cross-sectional area was obtained by

reconstructing the range from the upper edge of the greater trochanter to the upper edge of the patella with a slit of 1.5 mm with a 320-row CT apparatus (Canon Inc., Aquilion ONE). It was measured using Ziostation software. The image data was opened by 3D analysis. The cine was created by re-saving the cine and enlarging the right thigh with the midpoint from the upper edge of the greater trochanter to the upper edge of the patella as the measurement point of the axial image. The rescued right femoral region image was opened with the protocol of body fat measurement. The CT value of the subcutaneous fat of thigh part was 50 ~150 Hounsfield Unit (HU), and the CT value of the visceral fat of 0 ~ 80 HU was changed to muscle CT value and measured. Bones were excluded, and the outer periphery of the thigh muscles was finely adjusted to measure the cross sectional area of the thigh muscle.

For nutritional evaluation, CONUT (controlling nutritional status) modified method¹³⁾ using three items, serum albumin (Alb), peripheral total lymphocyte count (TLC) and hemoglobin (Hb), was used. In the measurement of serum BCAA concentration, blood was collected in control without taking breakfast (control) and 60 and 120 minutes after one bottle of jelly containing BCAA (2.5 g, Reha -ime Jelly, Clinico Co. Ltd., Japan).

4. Result

After the KAATSU training plus BCAA intake, the thigh circumference, the MVC of knee extension, quadriceps muscle thickness, and lower limb muscle mass gradually increased (Table 1). At 80 days after starting the training, the thigh circumference was + 7.3 cm, and the MVC of knee extension was about + 20 kgf for both left and right, compared with the control level before the training. The quadriceps muscle thickness increased about + 1 cm, and the lower limb muscle mass increased about + 1 kg in both left and right (Table 1). Also, in the femoral CT image after 100 days of the training, there was a marked increase in femoral muscle cross-sectional area of 56.9 cm² on the right and 55.9 cm² on the left, compared with the control level (Table 1, Figure 1).

Table 2 shows changes in body weight and the nutrition states before and after KAATSU training combined with

Table 1. Changes of each evaluation before and after KAATSU training combined with BCAA intake

Days after KAATSU training	Before	7 days later	17 days later	29 days later	52 days later	80 days later	100 days later
	44th disease day	56th disease day	66th disease day	78th disease day	101th disease day	129th disease day	149th disease day
Femoral circumference (cm)	38.2	39.0	39.5	39.8	44.3	45.5	
Right knee extension muscle force (kgf)	40.4	38.5	38.9	39.8	53.7	64.0	
left knee extension muscle force (kgf)		31.7	34.0	37.7	44.5	53.8	
Quadriceps muscle thickness (cm)	2.1	2.4	2.4	2.6	2.8	3.1	
Muscle mass of right lower limb (kg)	6.7	7.2	7.4	7.7	7.8	7.9	
Muscle mass of left lower limb (kg)	6.7	7.1	7.3	7.7	7.7	7.9	
Femoral muscle cross-sectional area (left) (cm ²)	61.6						117.5
Femoral muscle cross-sectional area (right) (cm ²)	62.2						119.1



Figure 1. Comparison of femoral CT scan images before (A) and 100 days after KAATSU training (B) combined with BCAA intake

Table 2.	Changes in	body weight and	nutrition	before and	after KAATS	U training	combined	with	BCAA	intake
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Days after KAATSU training	Before	7 days later	17 days later	29 days later	52 days later	80 days later
	44th disease day	56th disease day	66th disease day	78th disease day	101th disease day	129th disease day
Body weight (kg)	58.7	59.0	59.4	61.0	63.5	65.9
Modified CONUT score (point)	3	4	1	0		
Alb (g/dl)	3.3	3.1	3.5	3.9		4.3
TLC (µL)	2101.6	1387.5	1929.6	1795.5	2037.6	
Hb (mg/dl)	10.9	10.4	11.6	13.7	13.9	14.8

Alb: Serum albumin, TLC: Peripheral total lymphocyte count, Hb: Hemoglobin

Table 3. Changes in blood BCAA concentration before and after BCAA (2.5 g) intake

Amino acids	Valine	Isoleucine	leucine	Total BCAA
Reference value (nmol/ml)	147.8~307.0	43.0~112.8	76.6~171.3	265.8~579.1
Before	236.9	86.5	146.9	470.3
60 minutes	333.7	187.8	329.8	851.3
120 minutes	296.3	116.6	231.4	644.3

BCAA: Branched Chain Amino Acids

BCAA intake. The modified CONUT score showed 3 points at the start of the training, and decreased to 0 point after 29 days of the training. Eighty days after the training, he recovered to almost the weight before the operation (Table 2).

Table 3 shows the changes of serum BCAA concentration before and 120 and 180 minutes after the BCAA intake. The serum concentration of BCAA showed a remarkable increase in numerical value at 60 minutes after BCAA intake, and maintained a high level at 120 minutes, compared to the control level (Table 3).

5. Discussion

This case was a patient with low cardiac function and prominent left ventricular enlargement. However, there was no appearance of any arrhythmia and side effects during the training even early after the cardiac operation. Furthermore, by performing the combined KAATSU training and BCAA administration immediately after the training, the marked muscle strength improvement and muscle hypertrophy could be obtained.

Rehabilitation intervenes during the perioperative period. Resistance training is frequently necessary to improve the reduction of muscular strength and muscle mass due to surgical invasion and waste syndrome due to decreased activity. However, lower limb resistance training for patients after cardiac surgery begins with low load exercise at the level of daily living behavior in the early postoperative period. Subsequently, the resistance exercise with load is recommended to start 5 weeks after surgery¹⁴. For that reason, the improvement of muscle strength and an increase in muscle mass are not recognized in several cases. In addition, in this case, the preoperative bedtime period due to exacerbation of heart failure and hypotension was long, and the severe muscle weakness and atrophy devel-

oped. However, the KAATSU training can be conducted from a low load. The ordinary resistance training mobilizes from small slow muscle fibers of the movement unit according to the principle of size. As the exercise intensity rises, large fast muscle fibers of the exercise units can be mobilized. Therefore, at a low intensity of 20-30% of 1 RM, fast muscle fibers are hardly mobilized and it is difficult to recognize muscle hypertrophy and muscle strength improvement. On the other hand, in KAATSU training, hypoxia caused by restricted muscle blood flow promotes recruitment of fast muscle fibers as well as muscle activity of slow muscle fibers^{5,15)}. Therefore, it can expect muscle hypertrophy and muscle strengthening similar to normal high-intensity training ^{5-7, 15}, even by using low-intensity load. In fact, we have already reported that KAATSU training was a useful method for improving muscle strengthening and muscle mass in patients with ischemic heart disease⁸⁾. In this study, we performed the KAATSU training in a young cardiac surgery patient with low cardiac function early after aortic valve replacement surgery. Since a low load at an intensity of 20-30% of 1 RM can be used during the KAATSU training, we started the training early after the operation. In addition, in terms of nutrition, resistance training under malnutrition is predominant in protein degradation, making it difficult to increase muscle mass¹⁷⁾. Before the KAATSU training, nutritional status was bad in this case with blood albumin of 3.3 g / dl and CONUT score 3 points. Therefore, BCAA administration was concomitantly used for the training. As a result, the marked increase in muscle strengthening and muscle hypertrophy could be observed after the intervention. During the training, the nutritional state also improved, and the CONUT score decreased to 0 point after 29 days of the training. From these observations, in addition to the effects of KATTSU training, it is most likely that protein synthesis has become dominant by ingesting BCAA immediately after exercise. Protein synthesis is considered to increase rapidly after 1 to 2 hours after exercise¹⁸. Looking at the trend of BCAA in this case, serum BCAA concentration was about twice higher than the control level before taking BCAA. Therefore, it is likely that considering the resistance training and intake time of BCAA was effective for efficient muscle strengthening and muscle hypertrophy in this patient.

In this study, the KAATSU training was conducted under a medical doctor who was qualified as a KAATSU instructor. And, according to the rehabilitation guidelines for cardiovascular disease¹¹, blood pressure measurement, electrocardiogram monitoring, and subjective strength measurements (Borg scale) were carried out. No side effects as previously described¹⁶ were observed during the training. The most common side effects in KAATSU training are petechia as described previously¹⁶. This patient received warfarin therapy, but no petechia had occurred during the training.

6. Conclusion

By using KAATSU training together with BCAA intake, a marked improvement in muscle strength and muscle hypertrophy was obtained in a patient with low cardiac function early after aortic valve replacement surgery. The combined use of KAATSU training and BCAA intake early after cardiac operation seems to be a safe and effective way to obtain muscle hypertrophy and muscle strength increase, but further studies are needed to clarify it.

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Effect of knee extension exercise with KAATSU on forehead cutaneous blood flow in healthy young and middle-aged women

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Dynamic exercise induces changes in the redistribution of whole-body organ-tissue blood circulation, including cutaneous blood circulation. We hypothesized that limb exercise combined with the restriction of muscular blood flow (KAATSU) may influence cutaneous blood flow redistribution. To examine this hypothesis, forehead (supraorbital) cutaneous blood flow was compared in women performing exercises with and without KAATSU. Ten young and middle-aged female subjects in the supine position performed three sets of 15 repetitions of unloaded unilateral knee extension exercises (30-s rest between sets). Blood flow was calculated from blood velocity and red blood cell mass (blood flow = velocity * mass) determined by laser blood flowmetry. While exercise without KAATSU did not induce alterations in velocity and mass (hence, no alterations in blood flow) throughout the entire exercise series, exercise with KAATSU induced increases (P<0.05) in blood flow owing to increases in velocity. These increases were not eliminated during the rest periods between exercise sets. Heart rate (HR) increased (P<0.05) with the second and third sets of exercises with KAATSU compared with HR before exercise initiation, and was higher than the HR resulting from a corresponding set of exercises without KAATSU. There were no changes in blood lactate and hematocrit in both types of exercises. Norepinephrine increased (P<0.05) at the completion of the exercise sets. These results suggest that forehead cutaneous blood circulation was increased by unloaded KAATSU leg exercise.

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Key words: cutaneous blood flow, laser blood flowmetry, knee extension exercise, norepinephrine

INTRODUCTION

Whole-body circulation during physical exercise is highly organized. Exercise-induced activation of sympathetic nerve activity leads to increased heart rate (HR), cardiac output (Q), and stroke volume (SV). At the same time, dynamic exercise induces changes in the redistribution of whole-body blood circulation; blood flow into working muscles to meet oxygen demand precedes flow into inactive regions. It has been suggested that cutaneous circulation is controlled by sympathetic nerve activity, which regulates vasoconstriction and the active vasodilation tone system (Kellogg et al, 1991, Kellogg, 2006, Johnson et al 1986).

Exercise combined with the restriction of muscular blood flow (KAATSU) is a distinctive type of exercise training that results in obvious hypertrophy and increased muscle strength. KAATSU is extremely low intensity and is characterized by its short training duration (Abe et al, 2005, 2006, Takarada et al, 2000) One of the most striking characteristics of this training method is the restriction of blood flow in the exercising limbs using a restriction cuff belt during exercise, which leads to alteration in circulation dynamics. It has been proposed that exercise with KAATSU may lead to acidic and anaerobic intramuscular states, while restriction-induced elevation in metabolites, such as lactate and growth hormone, might stimulate hypertrophy (Takano, et al, 2005b).

KAATSU has been suggested to induce alterations in hemodynamic parameters, such as Q and SV, as well as changes in HR and blood pressure (BP), both at rest and during exercise. Iida et al (2005) demonstrated that application of a KAATSU belt to both legs during rest restricted venous blood flow and caused venous blood pooling in the legs distal to the belt, resulting in pressure-dependent reduction of arterial blood flow. Consequently, decreases in Q and SV, a slight increase in HR, and no change in BP were demonstrated. In exhaustive knee extension exercise with KAATSU, HR and BP as well as Q have been demonstrated to increase; on the other hand, SV decreased (Takano et al, 2005a). These studies suggest that these parameters play a role in controlling whole-body circulation in response to KAATSU with and without exercise.

In the aforementioned studies (Iida et al, 2005,

Takano et al, 2005b), blood pooling in the exercising limbs has been clearly demonstrated. On the other hand, it is unknown how circulation in other parts of the body responds to KAATSU in the exercising limbs. In the present study, we investigated the response of cutaneous circulation in the forehead (suparorbital region) to knee extension exercise combined with KAATSU-induced blood flow restriction.

METHODS

Subjects

Ten healthy female aged 23 to 47 years (mean age, 34.5 (SD 10.0) years) volunteered to participate in the study (Table 1). All subjects led active lives, with 8 of 10 participating in KAATSU training for the last 2 years. All subjects were informed of the procedures, risks, and benefits of the study, and signed an informed consent document before participation. The study was approved by the Ethics Committee for Human Experiments, The University of Tokyo.

Exercise protocol

The subjects assumed a supine position and performed three sets of knee extension exercises following a warm-up period, with a 30-s rest period between sets (Figure 1). In each set, subjects performed 15 repetitions of left knee extension exercises at 12 repetitions per minute, taking approximately 75 s to perform the entire set. The subjects performed this three-set series of exercises twice - for the first time without KAATSU, and for the second time with KAATSU. The KAATSU belt (Kaatsu-Master, Sato Sports Plaza, Tokyo, Japan) was placed around the most proximal portion of the subject's left leg. Before starting the exercise with the KAATSU belt, the air pressure in the belt, ranging from an initial 140 mm Hg to the a final pressure of 200 mm Hg, was repeatedly held for 30 s and then released for 10 s. The final restriction pressure of 200 mm Hg was selected for its ability to serve as an

Table 1.	Descriptive	characteristics	of	subjects
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n	10
Age, y	34.5 (10.0)
Standing height, cm	161.1 (5.8)
Body mass, kg	51.3 (4.3)
BMI, kg/m ²	19.8 (1.3)
Midthigh girth, cm	46.9 (3.3)

Values are means (SD); BMI, body mass index.

occlusive stimulus, as described previously (Abe et al, 2006). Before starting to exercise with the final restriction pressure, subjects rested for 1 min. The restriction of muscular blood flow was maintained throughout the second exercise set, including the 30-s rest periods. The belt pressure was released immediately upon completion of the session.

Cutaneous blood flow measurement.

Cutaneous blood flow in the forehead (supraorbital region) was measured using laser blood flowmetry (Omega Wave, Tokyo, Japan), multiplying blood velocity (the velocity of red blood cells) by mass (the number of red blood cells passing through a given surface area in a specific unit time). The laser probe was placed in the middle of the region, and the circulation at ~1 mm in depth was measured (Figure 2.). Facial skin thickness varies from 0.4 to 1.4 mm (epidermis + dermis) depending on the region; the laser beam is able to penetrate to the dermis. Blood flow measurement was performed at the beginning of the rest period (with and without KAATSU) and lasted through the entire exercise series, both with and without KAATSU. The data obtained for the last 30 s were used for analysis.

Blood sampling and biochemical analysis

Venous blood samples were obtained four times from nine subjects in a supine position before and immediately after exercise both with and without KAATSU. All blood samples were processed to plasma before storage at -20°C. Blood lactate (LA)

Exercise without Kaatsu



Exercise with Kaatsu





Rest: rest at final pressure restriction, **†** : blood sampling

Figure 1. Experimental protocol.



Figure 2. Structure of face skin and measurement of cutaneous blood flow. Laser beam captures blood flow in capillary blood vessels or arterioles.

concentrations were determined using a portable analyzer (Lactate Pro, Arkray, Kyoto Primary Science, Kyoto, Japan), and hematocrit was measured in duplicate by microcentrifugation. Plasma norepinephrine (NE) concentration was measured at SRL, Inc. (Tokyo, Japan). HR during exercise was measured using a finger probe (Onyx, Nonin Medical, MN, USA). The data obtained from the last 30 s were used for analysis.

Statistical analysis

Results are expressed as mean \pm standard deviation (SD) for all values. The effects of exercise with KAATSU compared with exercise without KAATSU

on changes in cutaneous blood flow and in blood parameters over time (pre- and postexercise) were tested by a two-factor ANOVA for repeated measurement. Further analysis used Student's paired *t* test, if interaction, KAATSU x group was significant. Statistical significance was set at P<0.05.

RESULTS

Forehead cutaneous blood flow

Figure 3 shows changes in mass, velocity, and blood flow. Exercise without KAATSU did not show any changes in mass, velocity, and blood flow for all three measurements. On the other hand, exercise with KAATSU increased (*P*<0.05) the velocity and blood flow in all exercise sets compared with the velocity and blood flow measurements made before the start of exercise. Significantly, these KAATSU-induced increases in both velocity and blood flow were not eliminated following the 30-s rest period between sets.

Heat rate and blood parameters

HR increased significantly (*P*<0.05) at the second and third exercise sets with KAATSU compare with pre-exercise HR without KAATSU (Figure 4). Also, HR is significantly higher than HR measurements made during the corresponding exercise sets without KAATSU.

Table 2 shows changes in plasma NE concentration. NE increased (P<0.05) from 0.21 (SD 0.08) ng/mL at rest to 0.30 (SD 0.15) ng/mL at the end of exercise



Figure 3. Effect of exercise with blood flow restriction (Kaatsu) on cutaneous circulation in the forehead. The samples are expressed relative to Con (rest without Kaatsu). Panel A: Velocity, Panel B: Blood flow, *: significantly different from Con, P<0.05. (*): tends to be different from Con, 0.05 < P < 0.10



Figure 4. Effect of exercise with blood flow restriction (Kaatsu) on heart rate. *: *P*<0.05

with KAATSU. There were no changes in either blood LA or hematocrit values (Table 2).

DISCUSSION

The main findings of the present study are as follows: 1) Unloaded knee extension exercise with KAATSU significantly stimulated cutaneous circulation in peripheral regions; an elevation in blood flow mainly due to increased velocity was observed. Further, this increase was maintained during rest periods between exercising. 2) Exercise with KAATSU increased HR and circulating NE. 3) No alterations were found in plasma LA and hematocrit values in exercise both with and without KAATSU.

In the present study, subjects performed free extraload knee extension exercises. In spite of its extremely low intensity, exercise with KAATSU resulted in changes in cutaneus circulation, with an elevation in NE secretion, suggesting that exercise with KAATSU stimulates sympathetic nerve activity and cutaneous circulating dynamics.

Although a significant elevation in velocity and blood flow in response to exercise with KAATSU was observed in almost all subjects, in some subjects the increase was very slight, as illustrated in Figure 5. Subjects who did not show a clear elevation also showed a sluggish increase in NE concentration (<0.04 ng/mL). This suggests that even if exercise with KAATSU is performed in the same way by all subjects, there might be differences among subjects in their sensitivity to these stimuli. In other words, in subjects who showed clear increases in velocity and blood flow, those increases might relate to NE elevation.

In addition to increasing cutaneous circulation dynamics, KAATSU exercise had a potent effect during the rest periods. The exercise-induced increase in velocity and blood flow was maintained during rest periods (rest 1 and rest 2), and there were no significant differences in these parameters during

Table 2. Blood norepinephrine and lactate concentration, and hematocrit in response to low-intensity knee extension exercise with and without KAATSU.

	Control-	Exercise	Kaatsu-l	Exercise
	Pre	Post	Pre	Post
n	9	9	9	9
NE (pg/mL)	0.23 (0.16)	0.19 (0.09)	0.21 (0.08)	0.30 (0.15)*
Hematocrit (%)	39.3 (2.4)	38.3 (2.3)	38.4 (3.0)	39.3 (2.7)
Lactate (mM)	0.82 (0.04)	0.81 (0.03)	0.82 (0.04)	0.81 (0.03)

NE, norepinephrine

* Significantly higher than preexercise in the same exercise style (P < 0.05).



Figure 5. Typical pattern of cutaneous blood flow in response to exercise with blood flow restriction (Kaatsu). Increases in velocity and blood flow in response to exercise with Kaatsu were more obvious in subject A than subject B.

exercise and at rest. In addition, during the rest period before the start of the first exercise set with KAATSU, velocity was already showing a tendency to increase (Figure 3). The main factor behind the increase in velocity and blood flow is currently unknown. If the impact of KAATSU on cutaneous circulation dynamics precedes the impact of exercise, the effect of the extremely low exercise intensity might be obscured by the effect of the blood flow restriction itself.

The KAATSU-induced increase in venous blood pooling has been demonstrated in both legs distal to the KAATSU belt during rest (Iida et al, 2005). This restriction resulted in decreases in Q and SV, a slight increase in HR, and no change in BP. On the other hand, during exhaustive leg extension exercises in a seated position, an increase in Q, HR, and BP and a decrease in SV have been demonstrated (Takano et al, 2005a). Both studies suggest that KAATSU induces a decrease in SV owing to decreased venous return during both rest and exercise. Further, as Takano et al (2005a) elucidated, an increase in Q during exercise is controlled mainly by an increase in HR. In the present study, a slight but significant increase in HR, possibly induced by elevation of NE, was found in exercise with KAATSU. Thus, the increase in HR might be a response to decreased SV to satisfy blood flow for increasing Q demand. However, measurement of Q, SV, and BP was not done in the present study, so precise interaction of these parameters in response to KAATSU is still uncertain. Additionally, postural effect on the hemodynamic system during exercise with KAATSU also should be considered when evaluating the interaction of these parameters because unlike in previous studies, exercise in the present study was performed in the supine position. In this position, the reduced hydrostatic pressure causes a shifting of blood volume toward the heart, thereby increasing SV and Q. Leyk et al (1994) have demonstrated that in the supine position, Q increased further in response to right or moderate cycle exercise, and this increase was mainly mediated by an increase in SV but not HR. In the present study, a shifting of blood volume toward the heart might also have occurred, and thus Q might increase too. However, because of KAATSU, HR might increase instead of SV as a compensatory response to increased Q. It is therefore necessary to understand that in interpreting the response of the hemodynamic system to exercise with KAATSU, the increase in HR in the present study might be influenced by both postural effect and an exerciseinduced NE increase.

The mechanism that induced an increase in blood flow via an increase in velocity in response to exercise with KAATSU is unidentified. In a previous paper (Takano et al, 2005a), BP has been demonstrated to increase in response to exercise with KAATSU. In the present study, if an increase in BP occurs in the same way during exercise with KAATSU, peripheral vascular resistance is expected to increase. Also, it is logically assumed that the pooling of blood in one part of the body leads to a shortage of blood flow through the rest of the body, even though blood continues to circulate. Therefore, it seems unlikely that cutaneous blood flow in peripheral regions would increase via acceleration of velocity in response to exercise with KAATSU. A possible explanation of accelerated velocity might be found in changes in vessel diameter. If a signal leading to vasodilation is activated independently from BP modulation, peripheral vascular resistance would decrease and blood flow might increase, with a possible combined increase in velocity. NE has also

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been suggested to be related to both vasoconstriction and vasodilation regulation (Kellogg, 2006). Elucidation of the vasodynamic system in conjunction with BP alteration in response to exercise with and without KAATSU is required.

There was a wide range in the ages (23-47 years) of the subjects in the present study. Since all subjects had an active life with no symptoms demonstrating decreased circulatory function due to aging, such as hypertension or arteriosclerosis, it seems that the effect of age on the hemodynamic system in response to exercise with KAATSU may be minor. However, if KAATSU is to be applied to people of different ages or people with different health conditions, further examination to clarify the effect of KAATSU on whole-body hemodynamics is required.

In conclusion, unloaded knee extension exercise with KAATSU increased forehead cutaneous blood flow. HR increased owing to elevation of NE in response to exercise with KAATSU, possibly influenced by modulation of cutaneous circulation dynamics.

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Increases in Thigh Muscle Volume and Strength by Walk Training with Leg Blood Flow Reduction in Older Participants

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We examined the effects of walk training combined with leg blood flow reduction (BFR) on muscle hypertrophy as well as on peak oxygen uptake (VO_{2peak}) in older individuals. Both the BFR walk training (BFR-Walk, n = 10, age; 64 ± 1 years, body mass index [BMI]; $22.5 \pm 0.9 \text{ kg/m}^2$) and control walk training (CON-Walk, n = 8, age; 68 ± 1 years, BMI; $23.2 \pm 1.0 \text{ kg/m}^2$) groups performed 20 minutes of treadmill walking at an exercise intensity of 45% of heart rate reserve, 4 days per week, for 10 weeks. The BFR-Walk group wore pressure belts (160–200 mm Hg) on both legs during training. After the training, magnetic resonance imaging–measured thigh muscle cross-sectional area (3.1%, p < .01) and muscle volume (3.7%, p < .01) as well as maximal isometric (5.9%, p < .05) and isokinetic (up to 22%, p < .01) strength increased in the BFR-Walk group, but not in the CON-Walk group. Estimated VO_{2peak} during a bicycle graded exercise test increased (p < .05) and correlated with oxygen pulse in both groups. In conclusion, BFR walk training improves both muscle volume and strength in older women.

Key Words: Walking—Aerobic capacity—Muscle hypertrophy—Occlusion.

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An age-related decline of cardiovascular fitness (eg, maximal oxygen uptake $[VO_{2max}]$) has been attributed in part to changes in body composition, particularly a loss of skeletal muscle mass, and referred to as "senile sarcopenia" (1). Sarcopenia leads to an increased risk of developing osteoporosis, insulin resistance, type 2 diabetes, and obesity (2), as well as reduced levels of daily activity and physical function. Additionally, it has been reported that a low agerelated VO_{2max} level is a risk factor for both cardiovascular disease and all-causes mortality in middle-aged and elderly populations (3). Based on existing evidence, cardiovascular fitness and muscular strength must be maintained in an optimal range to minimize the potential risks of age-related diseases.

To improve muscular strength and cardiovascular fitness in middle-aged and elderly populations, several societies (4,5) have published guidelines that recommend combining training intensity, volume, and frequency to optimize muscle hypertrophy and strength gains as well as improve VO_{2max} . In general, the magnitude of the acquired training adaptation is proportional to the training stimulus, depending on the individual's training experience and/or initial physical fitness level. For instance, a training intensity of more than 65% of one repetition maximum (1-RM) is

considered the minimum intensity required to achieve muscle hypertrophy and strength gains (6). On the other hand, normally, the magnitude of change in VO_{2max} induced followed a training increases as exercise intensity is raised above 50% VO_{2max}. The minimum stimulus necessary to evoke change is more than 40% or 50% of VO_{2max} . In fact, a few studies (7,8) have reported an increase in VO_{2max} after training at intensity as low as 45% of VO_{2max}. The guidelines also recommend a training frequency of 3-5 days per week for aerobic training and 2-3 days per week for resistance training (4). Because the typical duration of these training sessions is approximately 60 minutes, including warm-up and cool down, about 300-480 minutes (5-8 hours) per week would be needed to complete the program. However, the vigorous training intensity and/or high training frequency might constitute major hindrance, preventing middle-aged and elderly populations from participating in the training programs.

Muscular blood flow reduction (BFR) during resistance training has been shown to elicit muscle hypertrophy and strength gains similar to those elicited by traditional highintensity resistance training (HI-RT), but with much lower exercise intensities (9,10). An intensity as low as that associated with walking, when combined with BFR, can lead to significant improvements in knee joint strength and leg muscle size (11,12). Improvements in leg muscle size and aerobic capacity using a single exercise of BFR walk training may warrant the use of this training method in the broader population, including the frail and elderly population. During exercise with BFR, the decline in venous return to the heart from blood flow-restricted limb muscles results in a decreased stroke volume (SV) and an increased heart rate (HR) while maintaining cardiac output (13). Consequently, the increased HR at the same systolic blood pressure during exercise with BFR may produce high mechanical stress on the heart, as indicated by a greater ratepressure product (14). In addition, the increases in muscle activation (15) and oxygen uptake (11) observed during BFR exercise may be the result of an increased arterial and mixed venous blood oxygen (a-v) O₂ difference because cardiac output during exercise with and without BFR is the same (13). The increase in the $(a-v) O_2$ difference may stimulate adaptations in the mitochondria, the myoglobin content of muscles, and/or muscle capillarization. We hypothesized that the potential benefits of BFR walk training could include not only an anabolic response by the muscular system but also improvements in the cardiovascular system. However, there are few published studies documenting concurrent improvements in VO_{2max} and muscle hypertrophy using a single exercise training for older participants (16,17). Thus, the purpose of this study was to investigate the effects of BFR walk training on muscle size and function as well as aerobic capacity in the older women.

Methods

Participants

A total of 18 sedentary women, aged 57-73 years, volunteered to participate in the present study. The participants were recruited through printed advertisements and by word of mouth and had not participated in a regular exercise program for at least the previous 3 years. All participants were free of overt chronic disease as assessed by medical history, physical examination, and complete blood chemistry and hematologic evaluation. Candidates who had smoked in the previous 4 years or were taking medications or female hormone supplements were excluded. All participants were informed of the methods, procedures, and risks, and signed an informed consent document before participating in the study. The study was conducted according to the Declaration of Helsinki and was approved by the Ethics Committee for Human Experiments of the University of Tokyo, Japan. Participants were subsequently divided into either BFR walk training group (BFR-Walk, n = 10) or a walk training group without BFR (CON-Walk, n = 8) in random order but balanced the participants to match anthropometric variables between groups.

Training Protocol

The participants in both the BFR-Walk and CON-Walk groups performed 20 minutes of treadmill walking at a predetermined exercise intensity of 45% of heart rate reserve (HRR). This training was performed under the close supervision of those with technical knowledge in BFR training. One week before the start of the training study, walking speed and grade were adjusted for each participant during a submaximal walking test, and the exercise load condition of each participant was determined and remained constant throughout the training period. The mean treadmill speed and grade were 4.5 ± 0.0 km/h and 1.6 ± 0.4 degrees in the BFR-Walk group and 4.4 ± 0.1 km/h and 1.5 ± 0.5 degrees in the CON-Walk group. Age-predicted maximum heart rate (HRmax; 220 - age) was used to determine the HRR for each participant. Training sessions were conducted 4 days per week for 10 weeks. During all training sessions, HR was recorded at the 5th minute, 10th minute, and 15th minute for both the CON-Walk and BFR-Walk groups. Ratings of perceived exertion were also recorded every 5 minutes during the session.

Blood Flow Restriction and Its Safety

Participants in the BFR-Walk group wore elastic cuffs (5 cm wide) (Kaatsu-Master system; Sato Sports Plaza, Tokyo, Japan) on the most proximal portion of each leg during the training sessions. Before the training sessions, the participants were seated on a chair, and the upper thigh-mounted cuff was inflated at 120 mm Hg (the approximate systolic blood pressure at heart level for each participant) for 30 seconds, and then the pressure was released. The air pressure was increased by 20 mm Hg, held for 30 seconds, and then released for 10 seconds before the next occlusive stimulation was performed. This process was repeated until a final occlusion pressure for each training day was reached. On the first day of training, the final cuff air pressure was 140 mm Hg. As participants adapted to the occlusive stimulus during the early phase of the training, the air pressure was increased by 10 mm Hg each week until a final cuff pressure of as much as 200 mm Hg was reached. Because of significant muscle fatigue experienced by participants during the training sessions, only five participants got to 160-180 mm Hg. The air pressure of 140-200 mm Hg was selected for the BFR stimulus based on a review of the data in elderly participants (12). Blood flow to the leg muscles was reduced during each training session in the BFR-Walk group, and the cuff air pressure was released immediately upon completion of the session.

To ascertain the safety of BFR walk training in these older women, seven participants in the BFR-Walk group performed a treadmill walk test with and without BFR on two separate days, with an interval of more than 2 days between the two tests. The exercise protocol was the same as the predetermined individual training protocol (45% HRR). We confirmed that BFR walk exercise has no impact on blood clotting as assessed by changes in fibrin D-dimer (before BFR walk training, 0.17 ± 0.02 g/mL; immediately after BFR walk training, 0.55 ± 0.44 g/mL; 15 minutes after BFR walk training, 0.20 ± 0.05 g/mL) and fibrin degradation products. These were in accordance with the results of a previous study in young men (18). In addition, previous studies have reported that unlike complete blood flow occlusion and reperfusion, moderate restriction of blood flow while performing low-intensity exercise does not affect the production of reactive oxygen species, as assessed by plasma lipid peroxide (19), blood glutathione status, and plasma protein carbonyls (20). These findings together support the notion that BFR walk training does not pose any immediate health concerns in older individuals.

Muscle Cross-sectional Area and Muscle Volume

Magnetic resonance images were prepared using a General Electric Yokogawa Signa 0.2-T scanner (GE Yokogawa, Tokyo, Japan). A T1-weighted, spin-echo, axial plane sequence was performed with a 520-millisecond repetition time and a 20-millisecond echo time. To avoid an influence of fluid shifts within the muscle, magnetic resonance imaging (MRI) procedure was performed around the same time before and 3 days after the final exercise. Prior to all scans, the participants rested quietly in the magnet bore in a supine position, with their legs extended. The great trochanter was used as origin point and continuous transverse images with 1.0-cm slice thickness (0-cm interslice gap) were obtained from the great trochanter to the lateral condyle of the femur for each participant. All MRI data were transferred to a personal computer for analysis using specially designed image analysis software (Tomo Vision, Montreal, Canada). For each slice, skeletal muscle tissue cross-sectional area (CSA) was digitized, and the muscle tissue volume (cm^3) per slice was calculated by multiplying muscle tissue area (cm²) by slice thickness (cm). Muscle volume of the leg muscle was defined as the sum of the slices of muscle. An average value for the right side of the body was used. We had previously determined that the coefficient of variation of this measurement was less than 1% (21).

Maximum Isometric and Isokinetic Strength

Maximum voluntary isokinetic strength of the knee extensors and flexors was determined using a Biodex System 3 dynamometer (Biodex Medical Systems, Shirley, NY). Participants were carefully familiarized with the testing procedures of voluntary force production for the thigh muscles during several submaximal and maximal performances about 1 week before testing. Each participants was seated on a chair with the hip joint angle positioned at 85°. The center of rotation of the knee joint was visually aligned with the axis of the dynamometer's lever arm, and the ankle of the right leg was firmly attached to the lever arm with a strap. Several warm-up contractions were performed before testing. Participants were then instructed to perform maximal isometric knee extension at a fixed knee joint angle of 75° followed by maximal isokinetic knee extensions and flexions, from 0° to 90°, at 30° and 180° per second. A knee joint angle of 0° corresponded to full extension of the knee. Whole muscle–specific tension was also calculated by dividing maximum isometric knee extension strength by mid thigh muscle CSA.

Estimation of Peak Oxygen Uptake

Oxygen uptake (VO₂) was measured during a bicycle graded exercise test (GXT) using an automated breath-bybreath mass spectrometry system (Aeromonitor AE-300S; Minato Medical Science, Tokyo, Japan). For this test, the pedaling rate was maintained constant at 60 revolutions per minute. The load was initially set at 40 W and increased by 5 W every minute until the participants reached approximately 80% of their age-predicted HR_{max}. Each participant's electrocardiograph was monitored throughout and used to measure HR at intervals of 60 seconds. Peak oxygen uptake (VO_{2peak}) was estimated by fitting the age-predicted maximum HR value into the linear regression equation computed from the individual VO₂ and HR value during GXT. Oxygen pulse (O₂ pulse) was calculated by dividing VO₂ by HR at each submaximal exercise load.

Functional Ability Tests

Two tests were used to assess functional abilities for each participant before and after the training program. The Up & Go test measured the time it took for participants to stand up from a chair without the use of their arms, walk 2.4 m, turn around, walk back to the chair, and return to a seated position. The second functional test, the chair-stand test, required participants to stand up from a seated position, as many times as possible, within 30 seconds (22).

Statistical Analyses

Results are expressed as means and standard error for all variables. Statistical analyses were performed by a two-way analysis of variance (ANOVA) with repeated measures [Group (BFR-Walk and CON-Walk) × Time (pre- and posttesting)]. Post hoc testing was performed using a paired *t* test when appropriate. All baseline differences and percent changes between the BFR-Walk and CON-Walk groups were evaluated with one-way ANOVA. Statistical significance was set at p < .05.

RESULTS

Before training, there were no significant differences between the two groups for age and anthropometric variables

	BFR-Walk						
	Pre	Post	%	Pre	Post	%	Time Effect
Anthropometric variables							
Age, y	64 (1)			68 (1)			
Standing height, m	1.54 (0.02)			0.52 (0.02)			
Body mass, kg	53.5 (1.4)	53.1 (1.3)	-0.8	53.4 (2.8)	52.8 (2.1)	-1.2	p < .01
BMI, kg/m ²	22.5 (0.9)	22.4 (0.8)	-0.8	23.2 (1.0)	22.9 (1.0)	-1.2	p < .01
Midthigh girth, cm	47.1 (0.8)	47.5 (0.8)	0.8	48.3 (1.3)	48.2 (1.3)	-0.3	-
Lower leg girth, cm	33.8 (0.3)	34.3 (0.4)*	1.4^{+}	33.7 (1.0)	33.8 (0.9)	0.2	p < .05
Muscle CSA, cm ²							-
Midthigh	96.4 (3.5)	99.3 (2.9)*,‡	3.1‡	93.9 (4.8)	93.9 (4.5)	0.1	
Midquadriceps	43.2 (1.7)	44.5 (1.5)*,‡	3.0‡	43.6 (1.8)	42.9 (1.9)	-1.6	
Muscle volume, cm ³							
Thigh	1335 (44)	1384 (40)*	3.7‡	1329 (63)	1327 (64)	-0.1	<i>p</i> < .01
Quadriceps	612 (23)	628 (22)*	2.7‡	620 (28)	616 (28)	-0.6	<i>p</i> < .05

Table 1. Changes in Anthropometric Variables, Skeletal Muscle Size, and Volume After 10 Weeks of BFR-Walk or CON-Walk Training

Notes: Data are given as mean (±SE). BFR-Walk = BFR walk training; BMI = body mass index; CON-Walk = control walk training; CSA = cross-sectional area. *p < .01, Pre versus Post; $^{\dagger}p < .05$, $^{\ddagger}p < .01$, BFR-Walk versus CON-Walk.

(Table 1). After the training program, lower leg girth was increased (p < .01) in the BFR-Walk group, and there were significant (p < .01) time effects in body mass and BMI (Table 1).

During training sessions, mean HR and estimated exercise intensity for CON-Walk participants were 104 ± 5 beats per minute and $44 \pm 2\%$ of HRR, respectively. In the BFR-Walk participants, they were 122 ± 7 beats per minute and $62 \pm 9\%$ of HRR, respectively. The ratings of perceived exertion were slightly higher (p < .05) in the BFR-Walk group than in the CON-Walk group at the 5th minute (11.5 ± 0.3 and 10.4 ± 0.3 , respectively), 10th minute (11.7 ± 0.2 and 10.6 ± 0.3 , respectively), and 15th minute (12.0 ± 0.2 and 10.9 ± 0.3 , respectively) of the walk sessions.

After training, the CSA of the thigh and quadriceps muscle (3.1% and 3.0%, respectively) and muscle volume (3.7% and 2.7%, respectively) were increased (p < .01) in the BFR-Walk group, but not in the CON-Walk group (Table 1). Maximal isokinetic knee extension (up to 8%, p < .01) and flexion (up to 22%, p < .01) strength were also increased in the BFR-Walk group but not in the CON-Walk group (Table 2). There were significant (p < .05) time effect in isometric knee extension. There were no significant changes for whole muscle–specific tension in both training groups (BFR-Walk group: 2.0%; CON-Walk group: 1.1%).

There were significant (p < .05) time effects in both absolute and relative VO_{2peak} (Table 3). There was also significant (p < .05) time effect in O₂ pulse at 75% HR_{max} during the submaximal exercise test (Table 3). There was a positively correlation (p < .05) between changes in O₂ pulse and absolute VO_{2peak} in both the BFR-Walk (r = .67) and CON-Walk (r = .77) groups.

The Up & Go test improved (p < .01) in only the BFR-Walk group (10.7%). On the other hand, there was significant (p < .05) time effect in the chair-stand test although percent change tended (p = .14) to be higher in BFR-Walk group (BFR-Walk: 20.5%; CON-Walk; 7.8%). In the BFR-Walk group, the change in the Up & Go test results tended

Table 2. Changes in Maximum Isometric and Isokinetic Knee Extension and Flexion Torque and Functional Performance After 10 Weeks of BFR-Walk or CON-Walk Training

				0			
		BFR-Walk			CON-Walk		
	Pre	Post	%	Pre	Post	%	Time Effect
Knee extension torque (nm)							
Isometric	120 (5)	127 (6)	5.9	120 (8)	122 (10)	1.6	p < .05
30° per second	103 (5)	112 (5)*	8.4 [§]	98 (6)	98 (6)	0	p < .05
180° per second	66 (3)	68 (3)	2.9 [‡]	65 (3)	64 (4)	-1.9	_
Knee flexion torque (Nm)							
30° per second	48 (2)	56 (2)*,§	16.1 [§]	44 (4)	42 (4)	-3.2	p < .05
180° per second	31 (2)	38 (2)*,§	22.3§	30 (2)	29 (2)	-4.2	p < .01
Functional performance							
Up & Go test, s	5.0 (0.2)	4.5 (0.1)*	-10.7^{\ddagger}	4.9 (0.2)	4.9 (0.2)	-0.1	p < .05
Chair stand test, times	23 (1)	27 (1)	20.5	24 (2)	26 (2)	7.8	p < .05

Notes: Data are given as mean (±SE). BFR-Walk = BFR walk training; CON-Walk = control walk training.

*p < .01, Pre versus Post; p < .05, p < .01, BFR-Walk versus CON-Walk.

	BFR-Walk				CON-Walk			
	Pre	Post	%	Pre	Post	%	Time Effect	
Estimated VO _{2peak}								
L/min	1.34 (0.08)	1.46 (0.10)	9.0	1.40 (0.10)	1.55 (0.11)	10.2	p < .05	
mL/kg/min	25.0 (1.3)	27.5 (1.6)	9.7	26.3 (1.1)	29.1 (1.4)	10.9	p < .05	
O ₂ pulse								
mL	6.7 (0.5)	7.5 (0.7)	11.7	7.1 (0.4)	8.0 (0.5)	12.4	p < .01	

Table 3. Changes in Estimated VO_{2peak} After 10 Weeks of BFR-Walk or CON-Walk Training

Notes: Data are given as mean (±SE). BFR-Walk = BFR walk training; CON-Walk = control walk training.

to correlate with the change in knee flexion strength (r = .59, p = .09), and the change in the chair-stand test results also tended to correlate with the change in knee extension strength (r = .61, p = .08).

DISCUSSION

This study indicated that BFR-Walk training, a single mode of exercise training, performed at relatively low exercise intensity can elicit improvements in muscle volume and strength in older women. Additionally, aerobic capacity improved in both groups although its value was estimated. Previously, concurrent improvements in muscular strength and VO_{2max} by single exercise training have been achieved after high-intensity, long-duration, aerobic-type, single exercise training (23,24); yet, none of the studies demonstrated muscular hypertrophy. This suggests that the increased muscular strength was due mainly to neural adaptations. Thus, high-intensity, long-duration, single-mode, aerobic-type exercise training rarely produces significant muscle hypertrophy. It has been reported in earlier studies that maximal knee extension strength was reduced and the thigh muscle CSA was unchanged after 4 weeks of cycle training under conditions of local leg ischemia, although VO_{2max} increased (25,26). Unlike previous studies, our findings show that BFR walk training is an innovative method for improving both muscle volume and aerobic capacity in older women. Furthermore, the reported rate of perceived exertion (on a scale of 6 to 20) during walk training sessions was low in both training groups although there was a significant difference between the BFR-Walk (11.5-12.0) and the CON-Walk (10.4–10.9) groups.

In the present study, thigh muscle CSA/volume and isokinetic knee joint strength increased by 3%–4% and 3%– 22%, respectively, after 10 weeks of BFR walk training in older women. Takarada and colleagues (9) reported that 16 weeks of BFR resistance training at a relatively low intensity (30%–50% 1-RM) increased upper arm muscle CSA (17%–20%) and isokinetic elbow joint strength (18%) in older women. Additionally, some studies have demonstrated that 8–12 weeks of HI-RT leads to increases in thigh muscle CSA (7%–11%) and isometric and isokinetic knee joint strength (10%–19%) in the elderly participants (27,28). The magnitude of increases in muscle size and strength in the present study are lower than those reported in some HI-RT studies (27,28) and in a BFR resistance training study (9). The differences in exercise intensity and volume or BFR stimulus might have caused the variability in the training-induced muscle hypertrophy and strength gain.

Our previous human studies have demonstrated that 20% 1-RM intensity knee extension exercise with BFR increased vastus lateralis (VL) muscle protein synthesis (40%-50% at 3 hours postexercise) through the mammalian target of rapamycin (mTOR) signaling pathway in young (29) and old (30) men, although the rate of muscle protein breakdown was not measured. These anabolic responses may contribute significantly to BFR walk training-induced muscle hypertrophy and strength gain. On the other hand, the same laboratory using the same technique reported that 70% 1-RM intensity knee extension exercise without BFR increased VL muscle protein synthesis (48% at 2 hours postexercise) and through the mTOR pathway in young men (31). Those results indicated that increases in postexercise muscle protein synthesis are probably similar between high-intensity resistance exercise and low-intensity BFR resistance exercise.

Our results showed that aerobic capacity improved in both groups although its value was estimated. Several studies have reported that HI-RT (32,33) resulted in essentially little or no effect on aerobic capacity in aging adult populations. Conversely, aerobic exercise training is thought to stimulate improvements in aerobic capacity (VO_{2max} increased by 10%-20% after 8-12 weeks of training) in the elderly participants (34,35). Previously, we reported no significant improvement in estimated VO_{2peak} after 6 weeks of slow (67 m/min) BFR walk training in the elderly participants (12). In that study, the average exercise intensity during training sessions was about 45% of HRR (average HR = 104 beats per minute). In general, the minimal stimulus necessary to evoke change is as high as 50% of VO_{2max} (7). In the present study, exercise intensity and duration were set at 45% of HRR and 20 minutes in the BFR-Walk group. During the training sessions, however, the exercise intensity was 62% HRR on average because HR increased due to decrease in SV by leg BFR without changes in cardiac output (36). Therefore, the exercise intensity in the BFR-Walk group may be within the effective exercise intensity for improvements in VO_{2peak}.

The BFR walk training-induced increase in VO2peak may be due to improvements in central cardiovascular and/or peripheral metabolic adaptations. The VO2max is the product of cardiac output and arterial and mixed venous blood oxygen (a-v O₂) difference at maximal exercise intensity. However, until now, there are few studies investigating the cardiovascular hemodynamic and muscle metabolic responses to BFR exercise training. In the present study, change in VO_{2peak} estimated was correlated with change in O₂ pulse in both the BFR-Walk and CON-Walk groups. Also, Sundberg (25) found an increase in VO_{2max} by utilizing supine onelegged cycle training with 50 mm Hg chamber pressure (reduced leg blood flow by 16%) for 4 weeks (four sessions per week). In that study, oxidative muscle enzyme and capillary density were increased in the ischemically trained leg, but cardiovascular adaptations to the ischemic exercise training were not found. A recent study reported that increases in VO_{2max} and submaximal exercise SV were observed after 2 weeks of twice-daily, 6-days per week BFR walk training, whereas resting SV remain unchanged (37). Therefore, it seems that the increase in VO_{2peak} from BFR walk training may be due to adaptations in muscle oxidative capacity (a-v O₂ difference) as well as in SV. Spina (38) suggested that for older women, the increase in VO_{2peak} is solely the result of an improved a-v O2 difference at maximal exercise, as there was no evidence of SV adaptation. In addition, the increase in lower body muscle mass may be associated with improvements in VO_{2max} in the BFR training group.

Previous cross-sectional studies (39,40) showed that Up & Go and chair-stand test results are correlated with knee extension strength in the elderly population. Our results conform to the cross-sectional studies showing that Up & Go and chair-stand performance was improved by BFR-Walk training and tended to correlate with knee joint strength. The improvements of functional fitness in the present study may be mainly due to increases in strength as measured by significant increases in maximal isometric and isokinetic knee joint torques.

In summary, low-intensity walk training with leg BFR concurrently improved thigh muscle size and knee joint strength in older women. Also, functional fitness was improved by this training, which may be mainly due to increases in strength. Further research is needed to determine the mechanism of concurrent improvement in muscular and cardiovascular adaptations by BFR walk training.

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OXFORD

Original Article

Effects of Low-Load, Elastic Band Resistance Training Combined With Blood Flow Restriction on Muscle Size and Arterial Stiffness in Older Adults

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Abstract

We examined the effect of low-load, elastic band resistance training with blood flow restriction (BFR) on muscle size and arterial stiffness in older adults. Healthy older adults (aged 61–85 years) were divided into BFR training (BFR-T, n = 9) or non-BFR training (CON-T, n = 8) groups. Both groups performed low-load arm curl and triceps down exercises (four sets, total 75 repetitions for each) using an elastic band, 2 d/wk for 12 weeks. The BFR-T group wore inflated pneumatic elastic cuffs (120–270 mm Hg) on both arms during training. Magnetic resonance imaging-measured muscle cross-sectional area of the upper arm, maximum voluntary isometric contraction of the elbow flexors and extensors, cardio-ankle vascular index testing, and ankle-brachial pressure index were measured before and 3–5 days after the final training session. Muscle cross-sectional area of the elbow flexors (17.6%) and extensors (17.4%) increased, as did elbow flexion and elbow extension maximum voluntary isometric contraction (7.8% and 16.1%, respectively) improved (p < .05) in the BFR-T group, but not in the CON-T group. In cardio-ankle vascular index and ankle-brachial pressure index testing, there were no changes between pre-and post-results in either group. In conclusion, elastic band BFR-T improves muscle cross-sectional area as well as maximal muscle strength but does not negatively affect arterial stiffness in older adults.

Key Words: Electromyography—Muscle hypertrophy—Sarcopenia—Strength—Vascular occlusion

Age-related skeletal muscle loss (sarcopenia) inhibits mobility and increases the risk of falls, fractures, disability, and heart disease (1,2). High-load resistance training (HL-T) can induce muscle

hypertrophy and improve insulin resistance and type-2 diabetes in the elderly (3,4), suggesting that HL-T prevents and even improves sarcopenia in the elderly (5). However, the use of heavy weights with weight machines/free weights required for muscle adaptation with traditional resistance exercise may not be practical and may even be dangerous when carried out without proper supervision. Thus, the effectiveness of alternative exercise methods should be investigated.

Elastic bands/tubing have been used widely in rehabilitative medicine and in health enhancement for resistance training (6,7). A previous study reported that a home-based resistance training program for older adults using elastic bands could serve as a practical and effective means of improving muscle strength (8). Elastic bands are also portable and are less expensive and easier to use than weight machines and/or free weights. Elastic band resistance training has thus been shown to be a feasible alternative to conventional training with free weights or machines (9,10). However, as elastic resistance training is commonly performed using low-to-moderate resistance level, this training typically has little or no effect on muscle hypertrophy (8,9).

In the past decade, several studies have reported that muscle hypertrophy can be produced with low-load resistance training (20%–30% one-repetition maximum [1RM]) combined with blood flow restriction (BFR-T), termed "kaatsu training," regardless of age (11,12). The mechanism by which BFR potentiates the training effect of low-load resistance training remains obscure but appears to be related, in part, to an increase in muscle activation (11,13–15). Recently, Yasuda and colleagues (16) revealed that muscle activation increased progressively during BFR-T when exercises were performed at low-resistance levels using an elastic band as well as when using free weights. BFR-T using elastic bands for resistance may thus be an effective home-based resistance training program for promoting both muscle hypertrophy as well as strength.

In general, reductions in arterial compliance or increases in arterial stiffness reduce the arterial buffering function of the pulsation of blood pressure and blood flow, which contribute to elevations in systolic blood pressure, left ventricular hypertrophy, coronary ischemic disease, and reductions in arterial baroreflex sensitivity (17,18). This means that prevention and treatment for decreased arterial compliance or stiffness is also important. However, HL-T has been reported to induce a 20%-30% reduction in arterial compliance in young and older adults (19,20), indicating that the use of heavy loads during resistance training has a potentially deleterious effect regarding arterial stiffness in older adults. On the other hand, previous studies have reported that BFR-T using low loads could improve or maintain arterial compliance or stiffness in young and older adults (21-23). We hypothesized that BFR-T using elastic bands may be a useful method for promoting muscle hypertrophy with a low risk of increased arterial stiffness in older adults. Thus, the purpose of this study was to examine the effect of low-load elastic band training with BFR on muscle size and arterial stiffness in older adults.

Materials and Methods

Participants

Seventeen men and women (aged 61–85 years) volunteered to participate in the study and were selected according to the exclusion criteria (blood pressure >160/100 mm Hg, body mass index >30 kg/m², history of anemia, cerebrovascular disease, myocardial infarction, and arthroscopic joint surgery) used to define "medically stable" older participants for exercise studies proposed by Greig and colleagues (24). In addition, volunteers who suffered from a chronic disease such as severe hypertension (>180/110 mm Hg), orthopedic disorders, deep venous thrombosis, peripheral vascular disease, or cognitive dysfunction were excluded from the study. None of the participants had participated in resistance-type training for a minimum of 6 months prior to the study. All participants were free of overt chronic disease as assessed by medical history, physical examination, and complete chemistry and hematologic evaluation. Participants were divided into either the BFR training (BFR-T) group (2 men and 7 women: n = 9; age [mean $\pm SD$]: 71.8 ± 6.2 years) or the non-BFR training (CON-T) group (1 man and 7 women: n = 8; age: 68.0 ± 5.1 years). All participants were informed of the risks associated with involvement in the study and signed an informed consent document before participation. The principles of the World Medical Association Declaration of Helsinki and the American College of Sports Medicine Guidelines for Use of Human Subjects were adopted in this study. The study was approved by the Ethics Committee of the University of Tokyo.

Training Protocol

BFR-T and CON-T groups performed bilateral arm curl and triceps press down exercise training 2 d/wk for 12 weeks. Both exercise groups used the "Heavy (Green)" band for men and "Thin (Yellow)" band for women (Hygenic Corporation, Akron, OH). This training was performed under the close supervision of those with technical knowledge in BFR training. One week before the start of the training study, both groups performed practice sessions for the maximum isometric strength (maximum voluntary isometric contraction [MVC]) test. In addition, BFR-T participants were familiarized with the BFR stimulus. Three or four days before training, MVC was determined. Training volume was 75 repetitions (30, 15, 15, and 15 repetitions, with 30-second rests between sets) for both exercises (90-second rests between exercises). This protocol is typical of submaximal BFR studies (12,16,22,23,25,26). Once the pneumatic cuffs were inflated, they remained so for the two exercises, including rest periods between sets and exercises. During arm curl exercise, subjects were comfortably seated on a chair (16). Elbow joint range of motion during the exercise was approximately 20-145° (0° being full extension). During triceps press down exercise, subjects were comfortably seated on a rowing chair with the body supported in the vertical position (16). Elbow joint range of motion during the exercise was approximately 140-5° (0° being full extension). Subjects were instructed not to let the band snap them back to the start position but to consciously control the return movement such that it would take twice as long as the stretching movement. The repetition duration was 2.4 seconds (1.2-second concentric and 1.2-second eccentric exercise cycle) for both exercises. The total length of the two exercises per day was 9.5 minutes for both groups.

Blood Flow Restriction

During the training sessions, BFR-T subjects wore a specially designed pneumatic cuffs (30-mm width, KAATSU Master, Sato Sports Plaza, Tokyo, Japan) around the most proximal portion of the both arms. On the first day of training, the cuffs were set at 30 mm Hg and air pressure gradually inflated to 120 mm Hg (Day 1). The air pressure was increased by 10–20 mm Hg at each subsequent training session until a pressure of approximately 270 mm Hg was reached if each subject could perform at high levels of pressure intensity. The restriction pressure was selected in accordance with previous study (16). The mean pressure intensity throughout the period of training was 196 \pm 18 mm Hg (180–270 mm Hg at 24th training session). Immediately after the two exercises, the pressure cuff was

quickly removed. The amount of time under BFR was approximately 11 minutes.

Measurements Schedule

Subject testing took place before the start of the study (pre) and 3-7 days after (post) the 12-week training period. The order of measurements were magnetic resonance imaging (MRI), venous blood samples, arterial function (flow-mediated dilatation [FMD], cardio-ankle vascular index [CAVI] testing, and ankle-brachial pressure index [ABI]) tests, and MVC measurements. Figure 1 shows the testing schedule for each of the measurements taken during the 12-week experimental period. All subjects were right handed except for two women (one for BFR-T and one for CON-T). All data were obtained from the right side of the body. Considering the schedule of examiners, all subjects and variable technologists, the MRI (10:00 and 15:00 hours), venous blood samples and arterial function tests (after 6-7 hours' fast, 10:00 and 15:00 hours), and MVC (10:00 and 15:00 hours) measurements were obtained in 2 days (few days interval). The subjects were instructed to refrain from ingesting alcohol and caffeine for 24-hour prior to pre- and post-training measurements.

MRI-Measured Muscle Cross-Sectional Area

Muscle cross-sectional area (CSA) was obtained using a MRI scanner (0.2-T Open MRI, Hitachi, Tokyo, Japan). A T-1 weighted, spin-echo, axial plane sequence was performed with a 500-millisecond repetition time and a 23-millisecond echo time. Subjects rested quietly in the magnet bore in a supine position, with their arms extended along their trunk. After transverse angle was set at perpendicular to the humerus in the sagittal plane, continuous transverse images (from the lateral epicondyle of the humerus to the acromial process of the scapula) with 10-mm slice thickness were obtained from the both upper arms of the body along the humerus. Although the difference in 5° of transverse angle is equal to the difference in approximately 0.4% in muscle CSA (cylindrical model), the differences between pre and post for all subjects were within 5°. All MRI data were transferred to a personal computer for analysis using specially designed image analysis software (sliceOmatic, Tomovision Inc., Quebec, Canada). Skeletal muscle tissue CSA was measured for elbow flexors (biceps brachii and brachialis) at 6 cm above the elbow joint and for elbow extensors (triceps brachii) at 16 cm above the elbow joint. The coefficient variation of this measurement was less than 1.0% (16).

Maximum Isometric Strength Measurement

MVC of the elbow flexors and elbow extensors was measured twice by a dynamometer (Taiyo Kogyo Co., Tokyo, Japan). Each subject was comfortably seated on an adjustable chair, with the arm positioned on a stable table at chest level with the elbow bent at an angle of 90° (0° at full extension). The upper arm was maintained in the horizontal plane (at 90°), while the wrist was fixed at the end of the dynamometer lever arm in a position of supination for elbow flexion and in a position halfway between supination and pronation for elbow extension. Both forces were measured with a transducer while the subject performed two trials separated by a 60-second rest interval (90-second rests between elbow flexion and elbow extension). If MVC torque for the first two MVCs varied by more than 5%, up to two additional MVCs were performed with 60-second rest between trials (14,15). Subjects were instructed to perform an MVC as quickly as possible during a period of about 2 seconds. The recorded value for the MVC was taken as the highest and most stable approximately 1 second of the 2-second contraction. The highest MVC value was used for data analysis. The coefficient variation for this measurement from test to retest was 1.3%. The intraclass correlation coefficient of the measurements was 0.97.

Arterial Function Tests

FMD, CAVI, and ABI (arterial function) measurements were conducted in the supine position. The participants were instructed to fast 6-7 hours before testing and refrain from ingesting alcohol or caffeine for at least 12 hours prior to testing. After the participants were asked to rest in the lying position in a quiet, dark, air-conditioned room (25-26°C) for 5 minutes, a standard cuff (UNEXCUFF, no. 5) was positioned around the right arm, 2–3 cm distal the antecubital fossa and their systolic and diastolic blood pressures were assessed using oscillometric methods (UA-767PC, A&D Co., Ltd., Tokyo, Japan). Then, after the participants had rested again for at least 15 minutes in a supine position in the same room, endothelium-dependent FMD of the brachial artery was measured using an established noninvasive method (27). After basal measurements were obtained, arterial occlusion was created by inflating a cuff placed around the forearm with suprasystolic compression (50 mm Hg above systolic blood pressure) of the right forearm for 5 minutes. After 5 minutes of inflation, the cuff was deflated producing a brief high-flow state resulting in arterial dilation due to increased shear stress. The diastolic diameter of the brachial artery was

	Pre													Post
		1 wk	2 wk	3 wk	4 wk	5 wk	6 wk	7 wk	8 wk	9 wk	10 wk	11 wk	12 wk	
Muscle CSA	\uparrow													\uparrow
MVC	\uparrow													\uparrow
Arterial function	\uparrow													\uparrow
Blood samples	\uparrow													\uparrow
EMG													\uparrow	
MTH		\uparrow			\uparrow			\uparrow			\uparrow			
Heart rate		$\uparrow \uparrow$												
RPE		$\uparrow \uparrow$												

Training period

Figure 1. Experimental timeline. CSA = cross-sectional area; MTH = muscle thickness; MVC = maximum voluntary isometric contraction; RPE = ratings of perceived exertion.

determined semiautomatically using an instrument equipped with software for monitoring the brachial artery diameter (UNEX EF, Unex Co. Ltd., Nagoya, Japan). Percentage of FMD was calculated as reported previously (28,29). Then, after 20- to 30-minute rest, CAVI and ABI were measured noninvasively using a VS-1500 system (Fukuda Denshi Co., Ltd, Tokyo, Japan). Subjects were placed supine. Electrocardiogram and heart sound were monitored. CAVI is automatically calculated using the formula; a $\{2\rho/\Delta P \times \ln(P_s/P_d) PWV^2\} + b$ (*a* and *b*, constants; ρ , blood density; *P*, difference in systolic and diastolic pressure; P_s , systolic pressure wave velocity; P_a , diastolic pressure; PWV, heart-ankle pulse wave velocity). ABI is the ratio of the systolic blood pressure in the ankle to the systolic blood pressure in the right arm (30).

Blood Sampling and Biochemical Analyses

Venous blood samples were obtained from the antecubital vein and measured for fibrin/fibrinogen degradation products (FDP), d-dimer, and creatine kinase (CK). The plasma concentrations of these samples were measured at a commercial laboratory (SRL Inc., Tokyo, Japan) by following latex immunoassay for FDP and d-dimer and spectrophotometry for NADPH formed by a hexokinase and D-glucose-6phosphate-dehydrogenase-coupled enzymic system for CK.

Measurements of Acute Responses to Training Session

Electromyography

The skin was shaved, abraded with a skin preparation gel (Skinpure, Nihon Kohden, Japan), and cleaned with alcohol wipes. During the experiment, skin impedance was less than 2 k Ω . The ground electrode was positioned on the lateral epicondyle. Bipolar (2-cm centerto-center) surface electromyography (EMG) electrodes (Ag/AgCl; Vitrode F; Nihon Kohden, Tokyo, Japan) were placed over the muscle belly (mid-portion) along the longitudinal axis of the biceps brachii and triceps brachii of the right upper arm (14,15). EMG signals were recorded and collected on a personal computer (MacBook Pro 10,1; Apple, Japan) for subsequent analysis. All EMG signals were digitized at a sampling rate of 1,024 Hz with a bandwidth of 0 Hz to 500kHz (AB 6216; Nihon Kohden). To determine integrated EMG (iEMG), signals were fully rectified and integrated (Lab Chart 7 software, AD Instruments, Japan). During the experimental session, surface EMG was recorded continuously and each iEMG value was divided into groups of five successive repetitions. The average for each group of five repetitions was represented as a single data point for statistical analysis (14,16,25). To determine the iEMG ratio of agonist muscles, iEMGs during each exercise was normalized to Pre, which was iEMG without BFR before the first set of training session. EMG measurement was performed during the 23rd training session (see Figure 1). The coefficient variation for this measurement from test to retest was 5.7%.

Relative exercise loading

To determine the relative exercise loading of performing the arm curl and triceps press down exercises, the iEMGs for the elastic band exercises (three to five repetitions) were compared to the iEMGs during MVC of the elbow flexors and elbow extensors. This measurement was completed on the same day before 23rd training session.

Ultrasound-measured muscle thickness

Since muscle thickness (MTH) using B-mode ultrasound (Acuson Sequoia 512; Siemens, Tokyo, Japan) has the advantage of evaluating acute change in muscle cell swelling following exercises (25),

MTH of the elbow flexors and elbow extensors was measured at 10 cm above the elbow joint and at mid-upper arm of the right upper arm, respectively. Briefly, the measurements were carried out while the subjects stood with their elbows extended and relaxed. The upper arm length (from the lateral epicondyle of the humerus to the acromial process of the scapula) was measured, and the 10 cm above the elbow joint for elbow flexors and 50% distance from elbow joint for elbow extensors were marked on the subject's skin with a pen. A 10.0 MHz scanning head (5.5 cm length probe) was placed on the skin perpendicular to the tissue interface. The scanning head was coated with a water-soluble transmission gel to provide acoustic contact without depressing the dermal surface. The subcutaneous adipose tissue-muscle interface and the muscle-bone interface were identified from the ultrasonic image. The perpendicular distance from the adipose tissue-muscle interface to the musclebone interface was taken as MTH. Ink markers on the elbow flexors were used to ensure similar positioning over repeated MTH measurement. The coefficient variation of this measurement from test to retest was 1.4%. The intraclass correlation coefficients of the measurements were 0.94 and 0.96, respectively (25). The same investigator (T.Y.) made all the ultrasound measurements. The MTH of the elbow flexors and elbow extensors was recorded before and immediately after the exercise bout. This measurement was completed at every 3 weeks (1st, 4th, 7th, and 10th week) of training period, and the average of 4 times for "before" or "immediately after" was represented as a single data point for statistical analysis, respectively.

Heart rate

During all training sessions, heart rate was recorded at baseline (pre) and immediately after the last set of each exercise (post) (Model 9560, Onyx II; Nonin Medical Inc.).

Ratings of perceived exertion

During all training sessions, ratings of perceived exertion based on a numerical scale of 6–20 were collected to assess subjective feelings of physical effort (ie, exertion). Ratings of perceived exertion data were recorded immediately after the last set of each exercise (31).

Statistical analyses

Results are mean \pm *SD*. The data were tested for normality using Shapiro–Wilk test. Because all variables were normally distributed, parametric statistical analyses were performed. Statistical analysis was performed by a two-way analysis of variance with repeated measures group by time. When significant main effects and/or interaction were observed, post hoc testing was performed using the Tukey technique. Percent changes from pre were also compared between groups using Tukey's test. Statistical significance was set at p <.05. The sample size was estimated from a priori power analysis (32) to detect differences (power of 0.80, an α of 0.05, two-tailed, and an effect size of 1.7) in elbow flexors muscle CSA for the interventions planned by reference to the result of previous BFR study (25). Consequently, it was determined that a minimum of seven BFR training and seven control subjects were required to test both the main and interaction effects.

Results

Before training, there were no significant differences between the two groups for age (p = .193) and anthropometric variables (standing height, p = .315; body weight, p = .821; body mass index, p = .157) (Table 1). A significant group by time interaction was not

observed for body weight (p = .520) and body mass index (p = .469) in either group following the training.

Acute Effect of BFR-T and CON-T

A significant group by time interaction was observed for iEMG (p < .001 for both muscles) and MTH (p = .013 for elbow flexors and p = .016 for elbow extensors muscles). During the exercise session, iEMG ratio for agonist muscle increased progressively in the BFR-T group for arm curl exercise from first to last set (from p = .023 to p < .001) and for press down exercise from the third to last set (from p = .041 to p < .001) and BFR-T was greater than CON-T at last set (p = .001) for arm curl exercise (Figure 2). Immediately after the exercise session, mean MTH was greater with the BFR-T compared to the CON-T at anterior (p = .021, 14.2% vs 7.0%, respectively) and posterior (p = .024, 8.5% vs 3.3%, respectively) 50% of upper arm (Figure 3).

There were no differences in elastic band elongation between BFR-T and CON-T groups for arm curl (p = 1.000, flexed position:

11.3 ±4.1 cm vs 11.4 ±1.5 cm and p = .113, extended position: 68.5 ±4.2 cm vs 63.4 ±6.1 cm) and press down (p = .998, flexed position: 16.0 ±4.8 cm vs 16.4 ±4.3 cm and p = .151, extended position: 74.8 ±6.0 cm vs 69.5 ± 3.2 cm) exercises. There were no differences in relative exercise load (p = .649 for arm curl and p = .935 for press down exercises, respectively), range of motion (p = .412 for arm curl and p = .082 for press down, respectively) between both groups. However, ratings of perceived exertion were higher in the BFR-RT group than in the CON group (p = .004 for arm curl and p = .001for press down exercises, respectively) (Table 2).

Chronic Effect of BFR-T and CON-T

A significant group by time interaction was observed for CSA (p < .0001 for elbow flexors and p = .013 for elbow extensors muscles, respectively) and MVC (p = .008 for elbow flexion and p = .013 for elbow extension). Elbow flexors and elbow extensors muscle CSA (17.6% and 17.4%, respectively) were increased (p < .0001

Table 1.	Changes i	in Anthropome	tric Variables	After 12 Week	of Training Period
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	BFR-T			CONT-T			
	Pre	Post	%	Pre	Post	%	
Anthropometric variables							
Age (y)	72(6)			68 (5)			
Standing height, m	1.60 (0.11)			1.55 (0.07)			
Body mass, kg	51.7 (11.4)	51.6 (11.4)	-0.3	52.8 (8.0)	52.3 (8.3)	-0.9	
BMI, kg/m ²	20.1 (2.2)	20.1 (2.4)	-0.3	22.0 (3.0)	21.7 (2.8)	-0.9	
Upper arm girth, cm	26.6 (2.0)	27.1 (1.8)*	2.1^{+}	27.7 (2.6)	27.5 (2.4)	-0.7	

Notes: Data are given as mean (\pm SD). BFR-T = BFR resistance training; BMI = body mass index; CON-T = non-BFR resistance training; upper arm girth, at 50% distal between the lateral epicondyle of the humerus and the acromial process of the scapula.

 $p^* = .08$, pre vs post.

 $^{\dagger}p < .05$, BFR-T vs CON-T.



Figure 2. iEMG ratio of biceps brachii and triceps brachii muscles during arm curl (a) and triceps press down (b) for each set performed. Average for each five repetitions was represented as a single data point. Values are means and *SD*. **Different from first five repetitions, p < .01. *Different from first five repetitions, p < .01.



Figure 3. Muscle thickness (MTH) of the elbow flexors (a) and the elbow extensors (b) before and immediately after exercise session. Values are means and *SD*. **Different from before, p < .01. *Different from before, p < .05.

Jable 2. Relative Exercise Intensity, Range of Motion, Heart Rate, and Ratings Perceived Exertio	n During Two Exercise	eived Exertion During Two E
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	BFR-T		CON-T	
	Mean (± <i>SD</i>)	Range	Mean (±SD)	Range
Relative exercise intensity, %MV	/C			
Arm curl	26.7 (11.1)	13.8-44.9	28.8 (6.7)	17.8-40.4
Press down	30.8 (12.3)	17.2-53.3	30.3 (7.9)	21.7-45.1
Range of motion, degree				
Arm curl	124 (14)	93-140	118 (14)	95-137
Press down	130 (5)	123-139	136 (9)	122-146
Heart rate, bpm				
Arm curl (total)	88 (15)	61–140	75 (7)	56-100
120–150 mm Hg	84 (13)	63–118	_	_
160–190 mm Hg	85 (13)	66–125	_	_
200–230 mm Hg	88 (17)	61–140	_	_
240–270 mm Hg	99 (18)	69–132	_	_
Press down (total)	89 (15)	64–141	77 (9)	51-102
120–150 mm Hg	83 (11)	68–113	_	_
160–190 mm Hg	86 (14)	67–132	_	_
200–230 mm Hg	89 (17)	64–141	_	_
240–270 mm Hg	98 (17)	73–132	_	_
Ratings of perceived exertion				
Arm curl (total)	14.1 (2.1)*	9–19	11.5 (0.7)	9-15
120–150 mm Hg	15.5 (2.1)	12–19	_	_
160–190 mm Hg	14.0 (2.5)	9–19	_	_
200–230 mm Hg	13.8 (1.9)	9–17	_	_
240–270 mm Hg	13.7 (2.0)	10-17	_	_
Press down (total)	14.0 (1.8)*	11-17	11.2 (0.5)	8-14
120–150 mm Hg	14.9 (2.4)	11–19	_	_
160–190 mm Hg	14.2 (2.4)	9–19	_	_
200–230 mm Hg	13.7 (1.9)	9–19	—	_
240–270 mm Hg	13.7 (2.1)	11-17	—	—

Notes: BFR-T = BFR resistance training; CON-T = non-BFR resistance training; MVC = maximum voluntary contraction.

**p* < .01, BFR-T vs CON-T.

and p = .0131, respectively) in BFR-T group, but not in the CON group (Figure 4). Elbow flexion and elbow extension MVC (7.8% and 16.1%, respectively) were increased (p = .0082 and p = .0131, respectively) in the BFR-RT group, but not in the CON group (Figure 5).

A significant group by time interaction was not observed for heart rate (p = .671), systolic and diastolic blood pressures (p = .126 and p = .104, respectively), CAVI (p = .150), ABI (p = .485), FMD (p = .116), FDP (p = .128), d-dimer (p = .506) and CK (p = .390) (Table 3).

Discussion

It has previously been observed that BFR-T using weight machines or free weight leads to increased muscle size and maintenance of arterial stiffness in older adults as well as in young adults (22,23). However, to date, no studies have discussed the muscle size and arterial stiffness following BFR-T using elastic bands. Our findings show that low-load, elastic band resistance training (arm curl and press down) with BFR can lead to a significant increase in muscle CSA (elbow flexors and elbow extensors) as well as maximal contractile



Figure 4. Muscle cross-sectional area (CSA) in the elbow flexors (a) and the elbow extensors (b) pre- and post-training period. Values are means and SD. **Different from before, p < .01.



Figure 5. Maximum isometric strength (MVC) of the elbow flexors (a) and the elbow extensors (b) pre- and post- training period. Values are means and SD. **Different from pretraining, p < .01.

adults.

Table 3. Changes in Arterial Function, Coagulation System, and Muscle Damage After 12 Week of Training Period

	BFR-T		CON-T		
	Pre	Post	Pre	Post	
Heart rate, bpm	68 (19)	67 (10)	63 (11)	60 (6)	
Systolic BP, mm Hg	135 (16)	134 (20)	127 (17)	120 (9)	
Diastolic BP, mm Hg	81 (9)	82 (9)	80 (11)	77 (8)	
CAVI, m/s	8.9 (1.2)	9.2 (1.1)	8.5 (0.7)	8.2 (0.8)	
ABI, unit	1.17 (0.07)	1.14 (0.09)	1.10 (0.10)	1.10 (0.09)	
FMD, %	3.6 (2.1)	4.3 (3.1)	4.3 (1.6)	3.5 (2.0)	
FDP, µg/dL	2.9 (0.9)	3.7 (1.1)	2.9 (0.4)	3.0 (0.8)	
d-Dimer, µg/dL	0.2 (0.1)	0.3 (0.1)	0.2 (0.1)	0.3 (0.2)	
CK, IU/L	186 (247)	112 (44)	91 (52)	88 (38)	

Notes: Data are given as mean (±SD). BFR-T = BFR resistance training; CON-T = non-BFR resistance training; BP = blood pressure; CAVI, cardioankle vascular index; ABI, ankle-brachial pressure index; FMD, flow-mediated dilation; FDP, fibrin/fibrinogen degradation products; CK, creatine kinase.

strength in older adults. In addition, we observed no changes in hemodynamic parameters (heart rate and blood pressure), arterial stiffness (CAVI), vascular endothelial function (FMD), coagulation factors (FDP and d-dimer), and muscle damage (CK). Thus, elastic band BFR-T can be considered as a useful method for preventing and even improving sarcopenia in old healthy adults.

In this study, muscle size did not change in the CON-T group, suggesting that low-load elastic band training without BFR is not effective at causing significant muscle hypertrophy. On the other hand, BFR-T (<30% MVC) produced a hypertrophic potential of 0.73% per session (17.6% increase in elbow flexor muscle CSA over 24 training sessions), which is greater than that observed following traditional high-load resistance training at 80% 1RM or BFR-T using free weights at 30%-50% 1RM (0.47% or 0.59% per session, respectively) in elderly adults (11). Additionally, the observed gains in elbow flexion MVC strength (0.33% per session) was equally effective as high-load resistance training or low-load BFR training using free weights (0.39% or 0.32% per session, respectively) (11) for improving arm muscle strength in older adults. Therefore, our data suggest that low-load elastic band training combined with BFR can provide an effective hypertrophic stimulus for muscles in older There are some trigger mechanisms underlying the BFR-T induced

muscle hypertrophy. In high-load training, myogenic stem cells most likely play an important role to enhance the activity of muscle protein synthesis (33). Recently, Nielsen and coworkers (34) revealed that BFR-T (23 training sessions, 20% 1RM using machine) leads to marked proliferation of myogenic stem cells, resulting in an increase in myonuclei in skeletal muscle, which is accompanied by marked myofiber hypertrophy. Therefore, there is a high possibility that myogenic stem cell-derived myonuclei provide an improved capacity for myofibrillar gene transcription, which is likely to contribute to an enhanced activity of muscle protein synthesis for BFR-T using elastic bands as well as weight machines and/or free weights.

Previous studies reported that BFR-T-induced muscle cell swelling may contribute significantly to the anabolic benefits of BFR (25,35). With respect to acute MTH responses, BFR-T exercise resulted in greater acute MTH compared with CON-T exercise. Additionally, we confirmed that low-load arm curl exercise with BFR results in a decreased plasma volume of venous blood for active muscles (ref. 36; Yasuda et al., unpublished observations). Therefore, increased muscle cell swelling may be observed following BFR-T exercise. It is known that acute cell swelling, due to osmotic water moving into the cell, simulates anabolic processes, through both an increase in protein synthesis and a decrease in proteolysis (37,38). Thus, the BFR-T exercise-induced enhancement of muscle protein metabolism may serve as the basis for the observed increase in muscle size. In addition, muscle activation during low-load elastic band exercise increased progressively only when BFR-T exercise was performed (Figure 2). In previously reported BFR studies, the greater muscle activation during low-load BFR resistance exercise was hypothesized to occur as a compensation for a deficit in force development. Such force development occurs secondary to changes in energy supply which are caused by decreased oxygen availability to the muscle and an accumulation of metabolites (36). Taken together, it appears that rapid equilibration of osmotic gradients created from the intracellular accumulation of metabolites may be higher with BFR-T compared with CON-T, and thus BFR-T may cause greater muscle cell swelling.

Previous studies have demonstrated that elastic band resistance training is well tolerated, as indicated by non-exacerbation of chronic disease conditions and lack of training-induced injury (7,8). Home-based resistance training using elastic bands has therefore been used widely for older adults and for patients with a lower level of activity (7-9). Compared with most previous BFR-T studies (11,25,26), in the present study, the pressure cuff intensity for upper arms (180-270 mm Hg) was higher when BFR-T was combined with the use of free weights. However, ratings of perceived exertion were not higher in BFR-T group for arm curls and triceps press down $(14 \pm 2 \text{ for both exercises})$ when compared to ratings in a previous study involving exercise training on resistance machines $(14 \pm 2 \text{ and }$ 15 ± 2 , respectively) in older adults (23). In addition, hemodynamic parameters (heart rate and blood pressure), arterial stiffness (CAVI), coagulation factors (FDP and d-dimer), and muscle damage (CK) were not changed in this study as well as in a previous study (23). Together, all of these findings suggest that BFR-T using elastic bands is a relatively safe training method. However, it should be noted that the possibility of side effects cannot be denied when subjects perform such training until near exhaustion or particularly to complete exhaustion (39,40).

The present study has some limitations. First, it should be noted that our sample size was small. In this study, the sample size was estimated by reference to the result of one BFR study (25), which had a large effect size for elbow muscle CSA in BFR studies. However, some conditions (sex, age, type of exercise, duration etc.) were different in between two BFR studies, and consequently the effect size for muscle CSA in this study was lower than that in the previous study (0.7 vs 1.7, respectively). Thus, future studies using more robust experimental design with a large sample size should be taken to verify the finding in this study. Second, since cuff pressure intensity for arms was higher than that reported in the previous studies (14,15,25,26,36), BFR-T required paying great attention to the side effects. Third, there were large individual differences in the relative

exercise intensity, because it is difficult to standardize the resistance offered by the resistance bands between individuals. Fourth, we measured CAVI, which reflects changes in both central and peripheral muscle arterial compliance, although previous studies measured central and/or peripheral arterial compliance (19–22). Hence, more work is needed to understand the relationship between elastic band BFR-T and muscle size and/or arterial function in older adults.

In conclusion, low-load arm curl and triceps press down training combined with BFR using elastic bands for resistance elicited marked gains in upper arm muscle CSA and strength and did not negatively affect arterial stiffness in older healthy adults. Thus, our results demonstrate that low-load, elastic band resistance training with BFR would be beneficial in the development of safe and effective methods of promoting muscle hypertrophy in older adults.

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ORIGINAL ARTICLE

Hemodynamic and neurohumoral responses to the restriction of femoral blood flow by KAATSU in healthy subjects

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Abstract The application of an orthostatic stress such as lower body negative pressure (LBNP) has been proposed to minimize the effects of weightlessness on the cardiovascular system and subsequently to reduce the cardiovascular deconditioning. The KAATSU training is a novel method to induce muscle strength and hypertrophy with blood pooling in capacitance vessels by restricting venous return. Here, we studied the hemodynamic, autonomic nervous and hormonal responses to the restriction of femoral blood flow by KAATSU in healthy male subjects, using the ultrasonography and impedance cardiography. The pres-

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K. Hirose Department of Rehabilitation Medicine, The University of Tokyo, Tokyo, Japan surization on both thighs induced pooling of blood into the legs with pressure-dependent reduction of femoral arterial blood flow. The application of 200 mmHg KAATSU significantly decreased left ventricular diastolic dimension (LVDd), cardiac output (CO) and diameter of inferior vena cava (IVC). Similarly, 200 mmHg KAATSU also decreased stroke volume (SV), which was almost equal to the value in standing. Heart rate (HR) and total peripheral resistance (TPR) increased in a similar manner to standing with slight change of mean blood pressure (mBP). Highfrequency power (HF_{RR}) decreased during both 200 mmHg KAATSU and standing, while low-frequency/high-frequency power (LF_{RR}/HF_{RR}) increased significantly. During KAATSU and standing, the concentration of noradrenaline (NA) and vasopressin (ADH) and plasma renin activity (PRA) increased. These results indicate that KAATSU in supine subjects reproduces the effects of standing on HR, SV, TPR, etc., thus stimulating an orthostatic stimulus. And, KAATSU training appears to be a useful method for potential countermeasure like LBNP against orthostatic intolerance after spaceflight.

Keywords KAATSU training · Hemodynamics · Autonomic function · Spaceflight · Cardiovascular deconditioning

Introduction

During spaceflights, several serious adaptive changes in cardiovascular function occur and consequently crew's health and safety are endangered. When gravitational hydrostatic gradients are abolished, there is a shift of intravascular fluid from the capacitance vessels of the legs and lower body centrally toward the head. Then, elevation of capillary blood pressure and increased capillary perfusion pressure in tissues of the head cause facial, intracranial edema and headache, which probably distresses astronauts or space travelers. After short- and long-duration spaceflights, very nearly all crewmembers experience orthostatic hypotension and reduced upright exercise capacity, which may be attributed in part to microgravity-induced hypovolemia, decreased baroreflex responsiveness, decreased skeletal muscle tone and increased venous compliance. On return to Earth, orthostatic intolerance is the most serious symptom of cardiovascular deconditioning; in addition, significantly reduced exercise capacities and increased resting heart rate are also observed (Blomqvist et al. 1994; Buckley et al. 1996). Accordingly, effective countermeasures during spaceflight are critical in order to maintain the cardiovascular system, as well as the musculoskeletal structure and function and ensure the well-being and safety of crewmembers during and after return to Earth. A passive countermeasure called "bracelets", which are specially designed elastic thigh cuffs developed in Moscow, has been reported to reduce cephalic edema and make the adaptation to zero gravity (0 G) more comfortable (Arbeille et al. 1995, 1998; Lindgren et al. 1998, Millet et al. 2000). This equipment contributes to reduce the edema and the venous stasis in the cephalic region by pooling blood into the vascular and extravascular compartment of the legs. Consequently, it can compensate partially for the cardiovascular changes induced by exposure to 0 G, thus reducing the cardiovascular deconditioning induced by microgravity (Herault et al. 2000). Thus, it would seem that the most effective countermeasure regimen to prevent cardiovascular deconditioning would be a gravitation-like stress combined with exercise.

Lower body negative pressure (LBNP) induces the retention of blood flow in lower extremities, and causes subsequent hemodynamic changes including autonomic nervous activities (Stevens and Lamb 1965; Bonde-Petersen et al. 1984; Tomaselli et al. 1987; Lathers and Charles 1993). Until now, when combined with intensive exercise, it has been known to be a useful method to prevent such orthostatic intolerance after spaceflight, probably through its effect as orthostatic stimulus (Güell et al. 1990, 1992; Lee et al. 1997; Watenpaugh et al. 2000; Lucini et al. 2004). KAATSU training is a novel method for muscle training combined with exercise. Under the conditions of restricted muscle blood flow, even short-term, low-intensity exercise such as resistance training and walking can induce muscle strength and increase muscle mass (Takarada et al. 2000a, b, c; Abe et al. 2004). Since KAATSU induces the venous pooling of blood into legs and inhibition of venous return (Takano et al. 2005), it appears to be an effective method to promote a state of blood pooling in the capillaries within the limb musculature and apply an orthostatic stress like LBNP. However, the detailed hemodynamic and neurohumoral responses to KAATSU by itself have not been investigated in details.

The purpose of the present study was to investigate the hemodynamic and neurohumonal responses to the restriction of femoral blood flow by KAATSU in supine subjects, and compare with those induced by standing. Here, we provide some evidence that KAATSU can be an effective method for applying an orthostatic stress like LBNP.

Methods

Subjects

Two protocols were used to investigate the effects of KAATSU (see below) on the femoral blood flow, and hemodynamic, autonomic nervous and hormonal parameters in healthy subjects. Both protocols were approved by the ethics committee of the University of Tokyo, and summarized in Fig. 1. All subjects were non-trained volunteers, and informed consent was obtained prior to the study. None of the subjects had any diseases and took any medications. In the first study (protocol 1), nine normal healthy adult males, aged 31.6 \pm 1.1 (26–36; mean height 1.73 \pm 0.02 m; mean body mass 67.8 ± 3.0 kg), participated to investigate the effects of KAATSU on the femoral arterial blood flow, and echocardiographic parameters including the diameter of inferior vena cava (IVC). In the second study, the hemodynamic, autonomic and hormonal responses induced by KAATSU were investigated on seven males, aged 29.4 ± 2.0 (26-41), using impedance cardiography. Mean of subject's stature was 1.74 ± 0.02 m, and mean body mass was 73.6 ± 1.6 kg.



Fig. 1 Experimental design

Reduction of femoral muscle blood flow by KAATSU

The method for inducing the reduction of muscle blood flow as shown in Fig. 2 is similar to that previously reported by Takarada et al. (2000a, b, c). Specially designed KAATSU[®] belts (60 mm in width and 605 mm in length, Fig. 2A) or KAATSU mini belts (33 mm in width and 880 mm in length, Fig. 2B) were applied to the proximal ends of the subject's thighs to restrict venous blood flow and cause pooling of blood in capacitance vessels distal to the cuff, and to restrict arterial blood flow. The cuff pressure was automatically controlled by KAATSU apparatus (Fig. 2A, B).

Experimental protocol number 1

Measurement of femoral arterial blood flow

The blood flow of superficial femoral artery was calculated from the cross-sectional area (CSA) of the artery and velocity time integral (VTI) using Aplio80 (Toshiba, Tokyo, Japan). The site recorded was ~5 cm distal to the KAATSU[®] belt (Fig. 2A). First, superficial femoral artery was identified in the two-dimensional mode, and CSA was measured at the end-systolic period. Then, in the pulse-Doppler method, VTI, calculated as the integral under the velocity curve, was measured. Adjustment of the angle for the measurement was within 60°. Blood flow per minute was obtained by multiplying CSA by VTI and heart rate. The blood flow was acquired in supine position before (pre) and during the application of KAATSU (approximately 5 min after the application of KAATSU), and 10 min after releasing the pressure. The KAATSU[®] belts

Fig. 2 A KAATSU Master apparatus and KAATSU[®] belt. B KAATSU Mini apparatus and mini belt. C Supine, skin color became dark-red as cutaneous blood volume in the legs is increased by pressurization in supine subjects (*a* before pressurization, *b* during pressurization) were used in this study, and KAATSU pressure was set at 0, 100, 150, 200 and 250 mmHg.

Measurement of cardiac size, CO and diameter of IVC

Transthoracic echocardiography was performed using Aplio80. The left ventricular end-systolic dimension (LVDd; mm) and left ventricular end-systolic dimension (LVDs; mm) were measured on the M-mode recording in the parasternal long-axis view with the pulsed wave Doppler sample volume positioned just below the aortic valve, and the aortic velocity time integral (VTI_{Ao}; cm) was calculated. The diameter of left ventricular outflow tract (D; cm) was measeured with two-dimensional echocardiography. Crosssectional area (CSA_{Ao}; cm²) of flow was calculated as $\pi (D/2)^2$ based on a two-dimensional echo diameter (D) measurement. Then, CO is calculated as CSA_{Ao} multiplied by the VTIAo and HR. The maximal diameter of IVC was measured from the subcostal approach. The effects of KAATSU on the echocardiographic parameters including the diameter of IVC (mm) were evaluated. The parameters were measured before (pre), during the pressurization of both thighs by KAATSU[®] belts (approximately 5 min after the application of KAA-TSU), and 10 min after the release of the pressure. The KAATSU pressure was set at 50 and 200 mmHg.

Experimental protocol number 2

Experimental studies

This experimental design is shown in Fig. 1. An indwelling heparin-lock catheter was inserted into the superficial



antebrachial vein of left arm. After a 30-min rest in a supine position, control blood samples were collected. Then, after taking rest measurements of hemodynamic parameters in this position for 3 min by using an impedance method (see below), the pressure was applied to both thighs by means of another specially designed belt, named "KAATSU mini" in Japanese (Fig. 2B, C). During KAATSU, the hemodynamic parameters were continuously monitored. Then, after 15 min of KAATSU (50 mmHg), the pressure was released and the hemodynamic parameters were measured again during a 5-10-min recovery period. Blood samples were obtained before, 1 and 10 min recovery period. All blood samples were processed to serum or plasma before storage at -20°C until analysis. After more than an hour of recovery time, a similar study was done at a KAATSU pressure of 100-250 mmHg. Finally, after a 30-min rest time, the hemodynamic parameters were taken at rest and during standing (5 min). The blood samples were also taken at rest and 5 min after standing.

Measurement of hemodynamic parameters

To evaluate hemodynamic parameters, the Task Force Monitor (CNSystmes Medizintechnik, Graz, Austria) (Gratze et al. 1998; Fortin et al. 1998), which includes surface electrocardigrams (ECG), impedance cardiography (ICG), beat-to-beat blood pressure by the vascular unloading technique (Penaz 1973) and oscillometric blood pressure were sampled at 1,000 Hz each. The data were obtained on a beat by beat basis and were then used to calculate online all hemodynamic parameters, which included heart rate (HR; bpm), mean blood pressure (mBP; mmHg), stroke volume (SV; ml), cardiac output (CO; l/min) and total peripheral resistance (TPR; dyne s cm⁻⁵). TPR was calculated in relative units as mBP/CO, and the calculation of CO and TPR was as follows.

$$\label{eq:compared} \begin{split} \text{CO} &= \text{SV} \ \times \ \text{HR} \\ \text{TPR} &= \text{mBP} \ \times \ 80/\text{CO}. \end{split}$$

Histograms of RR intervals were computed and pseudodigitized at ten samples per second. Auto-regressive modeling (Burg method) was used to construct frequency domain spectrograms of the heart rate variability (HRV) (Bailey et al. 1994; Burklow et al. 1999). Parameters extracted from the variability spectra were low-frequency power (LF_{RR}, 0.03–0.15 Hz) and high-frequency power (HF_{RR}, 0.16–0.50 Hz), normalized to total power over the range from 0.01 to 0.50 Hz. LF_{RR}/HF_{RR} has been demonstrated to measure changes in sympathetic activity (Malliani et al. 1991). Measurement of hemoglobin, noradrenaline, plasma renin activity and vasopressin

Blood samples for measurement of blood hemoglobin (2 ml) and hormone determination (7 ml) were collected in pre-heparinized syringes. For hemoglobin, blood was drawn into test tubes containing EDTA-2Na. For hormone determination, blood was drawn into test tubes containing 10.5 mg of EDTA-2Na. All samples were kept in ice-cold water and centrifuged (3,000 rpm) for 10 min and the plasma stored at -20° C until the assays were performed.

Blood hemoglobin (Hb, g/100 ml) estimation was made by the cyanomethemoglobin method (Coulter hemogrobinometer). The hematocrit (Hct, %) was measured by microhematocrit using ultra centrifugation. Plasma concentrations of noradrenaline (NA) were measured using high performance liquid chromatography (HPLC) method. The lower limit of detection of the assay was 6 pg/ml. Plasma renin activity (PRA) was measured by the radioimmunoassay (RIA) method with a lower detection limit of 0.1 ng/ml per h. Vasopressin (ADH) was also determined by the RIA method. The lower limit of detection of the assay was 0.2 pg/ml. These variables were measured at commercially available laboratories (SRL Inc., Tokyo, Japan).

The change of blood volume (BV) (%) and plasma volume (PV) (%) was derived from the following equation;

$$\begin{split} BV_B/BV_A &= Hb_A/Hb_B\\ \% \Delta PV &= 100 \times (Hb_B/Hb_A)\\ &\times ((1 - Hct_A \times 10^{-2})(Hct_B \times 10^{-2})) - 100 \end{split}$$

where A is the value at rest (pre), and B is the value at 1 and 10 min recovery period after KAATSU.

Data analysis

All values are expressed as means \pm SEM. Comparison of time courses of parameters was analyzed by one-way ANOVA for repeated measures. When differences were indicated, a Bonferroni/Dunnett's comparison was used to determine significance. Differences were considered significant if *P* value was less than 0.05.

Results

Reduction of femoral arterial blood flow by KAATSU

Figures 3 and 4a show the effects of KAATSU on blood flow of the superficial femoral artery. Figure 3 shows a representative data recording of femoral arterial blood flow without (pre) and with KAATSU in supine subjects. Under the conditions with KAATSU (100 mmHg), the diameter of the femoral vein (described by blue arrow) was remarkably increased [Figs. 3Aa (pre) and Ba (100 mmHg)] and femoral arterial blood flow (described by red arrow) was decreased by 56.5% (Fig. 3Ab, Bb). The changes in femoral arterial blood flow against the cuff pressure are depicted in Fig. 4a. Application of 100 mmHg KAATSU decreased femoral arterial blood flow from 298.3 ± 41.8 ml/min (pre) to 129.8 ± 25.6 ml/min (100 mmHg, n = 9, P < 0.01). Arterial blood flow decreased with increasing levels of the applied-pressure. The arterial blood flow decreased to 63.8 ± 21.7 ml/min (200 mmHg, n = 9, P < 0.01), and 34.8 ± 16.2 ml/min (250 mmHg, n = 9, P < 0.01). Immediately after releasing the pressure, femoral arterial blood flow recovered (data not shown). These results indicate that the application of KAATSU to both thighs in supine subjects restricts venous blood flow and causes venous pooling in the thighs distal to the cuff with the pressure-dependent reduction of arterial blood flow.

Hemodynamic responses to the restriction of femoral blood flow by KAATSU

Figure 4 shows the effects of KAATSU on the diameter of IVC, cardiac size and CO measured by echocardiography, when the subjects were supine. We measured the diameter of IVC as a quantitative marker of venous return. Application of 200 mmHg KAATSU reduced the diameter of IVC from 16.2 \pm 1.0 to 14.0 \pm 1.0 mm (Fig. 4b, n = 9, P < 0.01). Simultaneously, left ventricular end-diastolic dimension (LVDd) was reduced from 48.1 \pm 0.9 to 44.4 \pm 0.9 mm (n = 9, P < 0.01, Fig. 4c). Left ventricular end-systolic dimension (LVDs) was also decreased, but not significantly (Fig. 4c). In addition, cardiac output (CO) was



Fig. 4 Effects of KAATSU on blood flow of superficial femoral artery, diameter of inferior vena cava, cardiac size and cardiac output. **a** Changes of blood flow of superficial femoral artery. The blood flow of right superficial femoral artery was obtained before (0 mmHg) and during the application of various degrees of KAATSU (100–250 mmHg), when the subjects were supine. **b**, **c** and **d** The parameters (**b** diameter of IVC, **c** LVDd, LVDs, **d** CO) are shown in control (pre) and during a set pressure of 50 mmHg, and a cuff pressure of 200 mmHg, and after the release of KAATSU (post), when the subjects were supine. All values are means ± SEM. obtained from nine subjects. **P* < 0.05, ***P* < 0.01 vs. control (pre)

decreased from 5.64 ± 0.50 to 4.73 ± 0.41 l/min (n = 9, P < 0.01, Fig. 4d). Thus, KAATSU in supine subjects appears to induce the pooling of blood in the thighs,

Fig. 3 The ultrasonographic findings of femoral blood flow. The typical data are indicated before (pre) and during the application of KAATSU (100 mmHg), when the subjects were supine. Figure 2 Aa and **B***a* indicate the data recording of longitudinal section of superficial femoral artery (red arrow) and femoral vein (blue arrow). Figure 1Ab and Bb show the data recording of blood flow velocity of superficial femoral artery. Note that KAATSU in supine subjects markedly induces pooling of venous blood with restriction of arterial blood flow



resulting in inhibiting venous return, and reducing cardiac preload and CO.

Figures 5 and 6 show a typical time course response of SV and HR determined by ICG during standing, and during the application of pressure on both thighs in supine subjects. Table 1 shows the hemodynamic changes including SV and HR following standing and 2 KAATSU conditions (50 and 200 mmHg). As shown in Fig. 5a, immediately after beginning of standing, SV rapidly decreased (from 109.4 \pm 0.2 ml to 69.0 \pm 0.3). Also pressurization of 200 mmHg gradually decreased SV (62.1 \pm 0.3 ml at 200 mmHg). SV at 200 mmHg KAATSU (Fig. 5c, Table 1) was much lower than that at 50 mmHg (Fig. 5b, Table 1) and at standing position. The decrease in SV continued during the application of KAATSU, when the subjects were supine. After the release of pressure, SV rapidly returned to the pre test level within several minutes.

After an orthostatic stress (standing), HR promptly increased with decreasing SV (Fig. 6a). Pressurization of

A

ml



Fig. 5 Effects of orthostatic stress (standing) and KAATSU in stroke volume (SV). The time courses of SV before and during standing (a) or the pressurization of both legs by KAATSU [50 (b) and 200 mmHg (c)] and after the release of KAATSU, when the subjects were supine. Note that during pressurization of both thighs by KAATSU, SV gradually decreased. After the release of KAATSU, SV returned to a control level immediately

50 mmHg (Fig. 6b) in supine subjects did not affect HR. On the other hand, 200 mmHg KAATSU increased HR gradually from 68.1 ± 0.6 bpm to 77.2 ± 0.2 . After the release of pressure, HR returned to the pre test level (64.7 ± 0.6 bpm) within several minutes. MBP increased significantly during standing (from 90.60 ± 3.69 mmHg to 102.41 ± 6.55 , n = 7, P < 0.05), and 200 mmHg increased MBP only slightly and non-significantly (from 87.70 ± 4.81 mmHg to 90.93 ± 3.52 at 50 mmHg, n = 7, n.s., and from 89.93 ± 2.75 to 91.22 ± 3.97 at 200 mmHg, n = 7, P=n.s.).

Cardiac output tended to decrease during standing (from 5.27 ± 0.32 l/min at supine rest to 4.83 ± 0.14). The pressurization of KAATSU (50 and 200 mmHg) in supine subjects decreased CO significantly as shown in Table 1 (from 5.46 ± 0.35 l/min to 4.58 ± 0.26 at 50 mmHg,



Fig. 6 Effects of orthostatic stress (standing) and KAATSU on heart rate (HR). The time courses of HR before and during standing (a) or the pressurization on both legs by KAATSU [50 mmHg (b) and 200 mmHg (c)] and after the release of KAATSU, when the subjects were supine. Note that HR increased during orthostatic stress (standing). When this volunteer changed position from supine rest to standing (shown by moving), HR rapidly increased, and then decreased, and reached to a steady-state level. HR also increased during the pressurization of both thighs at 200 mmHg in supine subjects, but not 50 mmHg. Immediately after the release of KAATSU, the increased HR returned to a control level

n = 7, P < 0.01, from 5.35 ± 0.33 to 4.05 ± 0.09 at 200 mmHg, n = 7, P < 0.01). TPR increased during both standing and pressurization. Standing increased TPR from 1,351.91 \pm 63.48 dyne s cm⁻⁵ to 1,665.93 \pm 119.95 (n = 7, P < 0.05). The pressurization of 50 and 200 mmHg in subjects also increased TPR from 1,272.77 \pm 97.42 dyne s cm⁻⁵ to 1,567.16 \pm 105.54 at 50 mmHg (n = 7, P < 0.01) and from 1,329.37 \pm 68.96 to 1,758.25 \pm 83.76 at 200 mmHg (n = 7, P < 0.01).

Autonomic nervous responses to the restriction of femoral blood flow by KAATSU

Figure 7a depicts the changes in the power spectra of heart rate variability (HRV) in control (pre), during 200 mmHg KAATSU, after the release of KAATSU, when the subjects were supine, and subsequently during standing. As shown in Fig. 7b and c, the HF_{RR}-component, which is a marker of parasympathetic activity, was reduced by both 50 and 200 mmHg KAATSU in supine subjects (Fig. 7b). On the other hand, LF_{RR}/HF_{RR} component was increased by both 50 and 200 mmHg as shown in Fig. 6c. HF_{RR} markedly decreased from $33.75 \pm 5.38\%$ to 23.94 ± 3.34 (n = 7, P < 0.01) and LF_{RR}/HF_{RR} increased from 2.81 ± 0.89 before KAATSU to 4.12 ± 0.79 (n = 7, n.s.) at 200 mmHg KAATSU. Hormonal responses to the restriction of femoral blood flow by KAATSU

Figure 8 depicts hormonal changes induced by KAATSU and standing. NA, a well-known neurotransmitter released from sympathetic nerve, increased under the pressurization. About 50 mmHg of KAATSU in supine subjects did not change significantly (from 197.00 ± 23.30 to $198.71 \pm$ 12.36 pg/ml, n = 7, n.s.), but 200 mmHg increased significantly (from 224.57 ± 29.98 to 333.14 ± 23.51 pg/ml, n = 7, P < 0.01). The standing also raised NA from 207.29 ± 31.90 to 401.14 ± 63.35 pg/ml (n = 7, P < 0.01). PRA was raised by both 200 mmHg of KAATSU in supine subjects and standing. 50 mmHg of pressurization slightly increased PRA from 1.04 ± 0.25 to 1.19 ± 0.28 ng/ml per h (n = 7, n.s.), and 200 mmHg of KAATSU had more effect on PRA (from 1.09 \pm 0.23 to 1.66 \pm 0.38 ng/ml per h, n = 7, P < 0.01). In addition, ADH was also increased during 5-min standing and during the application of KAATSU at 200 mmHg in supine subjects (from 1.07 ± 0.06 to $1.31 \pm 0.08 \text{ pg/ml}, n = 7, P < 0.05).$

Figure 8d and Table 2 show the percent changes of blood volume (BV) and plasma volume (PV) induced by KAATSU, when the subjects were supine. These values tended be decreased by KAATSU, but these changes were not significant except for 50 mmHg (n = 7).

Table 1 Hemodynamic parameters during standing, and KAATSU in supine position

	HR (bpm)	sBP (mmHg)	mBP (mmHg)	dBP (mmHg)	SV (ml)	CO (l/min)	TPR (dyne s cm^{-5})
Pre	58.14 ± 2.01	119.55 ± 5.19	90.60 ± 3.69	74.48 ± 3.12	90.92 ± 4.62	5.27 ± 0.32	$1,351.91 \pm 63.48$
Standing	$74.12 \pm 3.65^{**}$	$130.38 \pm 8.52*$	$102.41 \pm 6.55^*$	$87.79 \pm 6.22^{**}$	$65.81 \pm 2.01^{**}$	4.83 ± 0.14	$1,665.93 \pm 119.95^*$
Pre	60.60 ± 3.93	116.68 ± 5.89	87.70 ± 4.81	73.81 ± 4.01	91.51 ± 6.26	5.46 ± 0.35	$1,272.77 \pm 97.42$
50 mmHg	58.73 ± 2.55	117.93 ± 4.82	90.93 ± 3.52	76.83 ± 3.21	$78.85 \pm 5.16^{**}$	$4.58 \pm 0.26^{**}$	1,567.16 ± 105.54**
Post	57.75 ± 2.20	117.39 ± 4.63	89.86 ± 3.46	74.91 ± 3.21	90.07 ± 4.72	5.19 ± 0.35	$1,370.49 \pm 91.00$
Pre	58.15 ± 1.59	116.66 ± 3.95	87.82 ± 2.69	74.47 ± 2.25	91.97 ± 4.35	5.33 ± 0.27	$1,298.06 \pm 75.14$
100 mmHg	59.73 ± 2.81	118.42 ± 5.20	89.56 ± 3.65	75.41 ± 2.06	$75.83 \pm 5.21*$	$4.45 \pm 0.19^{*}$	$1,568.65 \pm 65.74*$
Post	58.91 ± 2.27	121.14 ± 5.42	92.02 ± 4.16	76.74 ± 3.16	87.80 ± 3.65	5.14 ± 0.25	$1,418.74 \pm 91.31$
Pre	60.19 ± 5.07	113.17 ± 5.01	84.37 ± 3.59	70.57 ± 3.09	83.40 ± 6.68	4.83 ± 0.22	$1,362.48 \pm 66.47$
150 mmHg	$64.24 \pm 6.00^{*}$	119.01 ± 5.95	90.62 ± 4.31	77.11 ± 3.81*	$65.61 \pm 4.94^{**}$	$4.05 \pm 0.10^{*}$	$1,746.12 \pm 87.36^{**}$
Post	60.37 ± 4.62	115.40 ± 4.19	88.11 ± 3.56	73.27 ± 3.44	80.76 ± 6.20	4.71 ± 0.19	$1,461.73 \pm 76.28$
Pre	59.16 ± 2.32	118.33 ± 4.33	89.93 ± 2.75	77.30 ± 2.47	90.97 ± 4.79	5.35 ± 0.33	$1,329.37 \pm 68.96$
200 mmHg	$65.67 \pm 2.87^{**}$	119.00 ± 4.60	91.22 ± 3.97	80.13 ± 3.18	$62.71 \pm 3.04^{**}$	$4.05 \pm 0.09^{*}$	$1,758.25 \pm 83.76^{**}$
Post	59.44 ± 2.85	118.57 ± 5.65	90.18 ± 4.76	77.00 ± 3.46	84.09 ± 4.99	4.93 ± 0.30	$1,457.82 \pm 87.87$
Pre	60.49 ± 4.99	114.06 ± 3.52	86.14 ± 2.53	72.76 ± 2.37	85.30 ± 6.56	4.97 ± 0.23	$1,352.33 \pm 42.75$
250 mmHg	$71.39 \pm 5.37^{**}$	$119.79 \pm 4.49^*$	$93.03 \pm 3.00^*$	$79.02 \pm 2.45^*$	$55.82 \pm 3.91^{**}$	$3.87 \pm 0.14^*$	1,881.21 ± 96.38**
Post	60.37 ± 5.11	$121.13 \pm 4.35^*$	$91.72 \pm 2.50^*$	$75.87 \pm 2.04*$	83.04 ± 6.80	4.79 ± 0.20	$1,495.34 \pm 45.55$

*P < 0.05, **P < 0.01 versus Pre



Fig. 7 a The time courses of heart rate variability (HRV) during KAATSU in a supine subject. Note that during KAATSU (200 mmHg), LF_{RR} increased, compared with the control, while HF_{RR} decreased, when the subject was supine. **b**, **c** Effects of orthostatic stress (standing) and KAATSU, when the subjects were supine, on the heart rate variability (HRV). **b** Effects of orthostatic stress (standing) and KAATSU, when the subjects were supine, on HF_{RR}/LF_{RR}. **c** Effects of orthostatic stress (standing) and KAATSU, when the subjects were supine, on HF_{RR}/LF_{RR}. **c** Effects of orthostatic stress (standing) and KAATSU, when the subjects were supine, on HF_{RR}. All values are means ± SEM. obtained from seven subjects. **P* < 0.05, ***P* < 0.01 vs. control (pre)

Discussion

We studied the effects of KAATSU on the hemodynamic, autonomic nervous and hormonal systems. Application of KAATSU on both thighs in supine subjects was revealed to induce the hemodynamic, hormonal and autonomic alterations that were similar to standing.

During an orthostatic stress (i.e., standing), part of the blood and plasma volume can pool in the capillary bed of the legs. Subsequently, SV decreased to about 72.4% of the control. Application of KAATSU on both thighs in supine subjects by a specially designed KAATSU belt also decreased SV by pooling blood into the vascular and extracellular compartment of the legs, which depends on the KAATSU pressure. The increasing cutaneous blood volume in the legs could be observed by the dark-red coloration of the skin in the lower body. At 200 mmHg, SV decreased to 68.9% of the control. The addition of KAA-TSU on both thighs in supine subjects gradually decreased SV during the application of KAATSU. As shown in Fig. 4a, KAATSU suppressed the arterial blood flow to the thighs in a pressure-dependent manner. However, even at 250 mmHg of KAATSU, arterial blood flow was not completely blocked, which may partly contribute to the safety of KAATSU training wildly used for muscle training. Immediately after the release of KAATSU, the depressed SV promptly returned to the control level.

In the hemodynamic response to orthostatic stress, initially SV is decreased, but the decrease in SV is successfully compensated for by an increased HR and TPR via baroreceptor control of circulation. Similarly, SV was decreased, and TPR was increased in cases of KAATSU even at a set pressure of 50 mmHg. Mean BP and HR did not change significantly. It suggests that the primary mechanism responsible for sympatho-excitation during the low pressure is primarily due to the cardiopulmonary baroreceptors, which are located in the atria and ventricles of the heart and in the pulmonary artery and veins and are responsive to changes in central venous pressure (Furlan et al. 2001; Brown et al. 2003). On the other hand, application of 200 mmHg induced a much larger decrease in SV, with the increase in HR and TRP. Thus, during high pressure such as 200 mmHg, both arterial baroreceptors and cardiopulmonary baroreceptors were also unloaded. Regarding autonomic nervous response, HF_{RR}/HF_{RR} , a marker of sympathetic activity, and the serum concentration of NA, a well-known neurotransmitter released from sympathetic nerve, increased, which depends on the pressure of KAATSU. On the other hand, HF_{RR} , a marker of parasympathetic activity, decreased. Overall, it seems likely that at low levels of pressure, the reduced venous return induces a cardiac-unloading, resulting in an inhibition of cardiogenic sympathetic excitatory mechanisms and in an increased arterial baroreflex gain, while at high level, the arterial baroreceptor-unloading is the dominant phenomenon leading to sympathetic excitation. During an orthostatic stress (standing), part of the blood and plasma volume can pool in the legs owing to the shift to the capacitance vessels. The subsequent decrease in the plasma volume and renal blood flow stimulates the secretion of

Fig. 8 Effects of orthostatic stress (standing) and KAATSU on the concentration of noradrenaline (NA, a), plasma rennin activity (PRA, b), vasopressin (ADH, c) and blood volume (BV, d). The blood was obtained before (supine rest), after 5 min standing and 15 min after the pressurization (50 and 200 mmHg), and 10 min after the release of KAATSU, when the subjects were supine. All values are means ± SEM obtained from seven subjects. *P < 0.05, **P < 0.01 vs. control (pre)



Table 2Hb, Hct, percentchanges in plasma volume (PV)and blood volume (BV) duringstanding, and KAATSU insupine position

	Hb (mg/dl)	Hct (%)	BV, %Δ	ΡV, %Δ
Pre	14.5 ± 0.2	44.8 ± 0.5	_	_
50 mmHg	$14.7 \pm 0.2^*$	$45.4 \pm 0.6^{*}$	$-2.49 \pm 0.88^{*}$	$-4.29 \pm 1.36^{*}$
Post	$14.7 \pm 0.3^*$	$45.5 \pm 0.7*$	$-2.35 \pm 1.25^*$	$-4.13 \pm 2.08*$
Pre	14.4 ± 0.3	44.3 ± 0.8	_	_
100 mmHg	14.5 ± 0.4	44.5 ± 1.0	-1.92 ± 1.45	-2.78 ± 2.48
Post	14.4 ± 0.3	44.6 ± 0.8	-1.31 ± 1.86	-1.91 ± 2.96
Pre	14.4 ± 0.2	43.9 ± 0.4	-	_
150 mmHg	14.5 ± 0.3	44.2 ± 0.7	-2.44 ± 2.00	-4.02 ± 3.16
Post	14.2 ± 0.2	43.5 ± 0.5	-0.97 ± 1.47	-1.53 ± 2.38
Pre	14.6 ± 0.3	44.8 ± 0.8	-	_
200 mmHg	14.8 ± 0.2	45.5 ± 0.7	-2.71 ± 1.58	-5.69 ± 2.29
Post	14.7 ± 0.3	45.2 ± 0.8	-1.73 ± 1.73	-4.10 ± 2.99
Pre	14.5 ± 0.3	44.4 ± 0.7	-	-
250 mmHg	14.6 ± 0.3	44.4 ± 0.8	-1.62 ± 1.14	-2.17 ± 1.81
Post	14.6 ± 1.3	44.9 ± 0.9	-1.75 ± 1.41	-3.17 ± 2.33
Pre	14.7 ± 0.3	45.7 ± 0.8	_	_
Standing	15.3 ± 0.3	47.2 ± 0.9	-2.66 ± 2.22	-5.03 ± 4.18

*P < 0.05, **P < 0.01 versus Pre

PRA and ADH. The secretion of PRA and ADH were also increased during KAATSU, which also depends on the degree of the pressure. However, the blood and plasma volume did not change significantly, suggesting that only early adaptation to KAATSU was studied in the present study. Thus, it is likely that the application of KAATSU on both thighs in supine subjects simulates systemic cardiovascular, autonomic nervous and hormonal effects of orthostatic in 1 G (standing). In spaceflight, without regular exposure to gravity (G) forces, the cardiovascular functions are compromised. After short- and long-duration spaceflights, very nearly all crewmembers experience orthostatic hypotension and reduced upright exercise capacity named cardiovascular deconditioning and severe muscle atrophy (Blomqvist et al. 1994; Buckley et al. 1996; Fritsch-Yelle et al. 1996; Meck et al. 2001). The cardiovascular deconditioning may be attributed in part to microgravity-induced hypovolemia,

decreased baroreflex responsiveness, decreases skeletal muscle tone and increased venous compliance. In addition, muscle atrophy produces greater compliance of lower limbs and a predisposition to orthostatic intolerance. To maintain the structure and function of musculoskeletal and cardiovascular systems during spaceflight, and to ensure well-being and safety of crew members during spaceflight and after return to Earth, effective countermeasures during spaceflight are critical (Convertino and Sandler 1995; Nicogossian et al. 1995). A passive countermeasure called a "bracelet" developed in Moscow has been used to reduce this cephalic edema and make the adaptation to 0 G more comfortable (Arbeille et al. 1995; Herault et al. 2000). The bracelets are fixed at the upper part of each thigh and applying pressure of approximately 20-30 mmHg (where 1 mmHg = 133.3 N/m^2) on the skin. The bracelets reduce the venous return by trapping a significant amount of fluid into the lower-limb vascular and interstitial space, subsequently followed by the fluid shift (Lindgren et al. 1998). It has been reported that such apparatus does not prevent the cardiovascular deconditioning induced by microgravity (Herault et al. 2000). However, it would be interesting to know whether the KAATSU with low pressure (20-50 mmHg) can prevent facial edema and make the adaptation to 0 G (Arbeille et al. 1995; Lindgren et al. 1998; Herault et al. 2000).

Currently, astronauts practice 2-3 h of intensive exercise using treadmill, ergometer and resistance machines. These time-consuming countermeasures only cannot completely prevent them from cardiovascular deconditioning. Therefore, alternate countermeasure strategies that are more effective and efficient are necessary. Now, it is likely that the most effective countermeasure regimen would be a gravitation-like stress combined with exercise. A human centrifuge is a possible candidate, but the centrifuge apparatus is relatively expensive and it is technically laborious to accommodate the apparatus on a space-craft. In addition, it may be difficult to minimize cetrifugationinduced Coriolis effects on the vestibular system and the consequent motion sickness associated with onboard centrifuge. LBNP has been known to induce the retention of blood flow in lower extremities, and induce subsequent hemodynamic changes such as decreased SV and CO and increased TPR (Stevens and Lamb 1965; Bonde-Petersen et al. 1984; Güell et al. 1990, 1992; Melchior et al. 1994; Murthy et al. 1994; Lee et al. 1997; Watenpaugh et al. 2000), and produce sympathetic activation and vagal withdrawal (Franke et al. 2000). Therefore, at present, it is likely that exercise against the suction force produced by LBNP may provide a low mass and low-cost alternative procedure to stress the cardiovascular systems (Hargens 1994). However, LBNP is difficult to apply when combined with several exercises, such as resistance exercises,

which need large machines. Furthermore, LBNP without exercise by itself cannot protect crew members from cardiovascular deconditioning. The present study provides evidence that KAATSU simulates orthostatic effects (1 G) on cardiovascular, autonomic nervous and hormonal system like LBNP at bed rest. KAATSU was originally developed as a novel method for muscle strength training and to induce muscle hypertrophy. Under the condition with restriction of muscle blood flow by KAATSU, even a short-term and low-intensity exercise can induce muscle strength and muscle hypertrophy (Takarada et al. 2000a, b, c; 2002a, b; Abe et al. 2004, 2006). Thus, KAATSU training can be easily applied to almost all types of exercises such as treadmill, ergometer, and resistance machines, and it may be able to be used by astronauts in order to protect against both muscle atrophy and cardiovascular deconditioning. Furthermore, we measured the serum concentration of lipid peroxide as a marker of oxidant stress in some of subjects of the present study. The lipid peroxide did not change significantly 1 and 10 min after 15 min of pressurization (150, 200, and 250 mmHg, data not shown). Thus, it is very likely that KAATSU training may be a very promising and safe method to counter symptoms of orthostatic intolerance and muscle atrophy in astronauts. But further studies are needed to clarify these interesting possibilities.

In conclusion, the application of KAATSU on both thighs simulates cardiovascular effects of orthostasis in 1 G. In addition, it is likely that the KAATSU training can be a useful method for potential countermeasure like LBNP against orthostatic intolerance in spaceflight.

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Effects of walking with blood flow restriction on limb venous compliance in elderly subjects

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Venous compliance declines with age and improves with chronic endurance exercise. KAATSU, an exercise combined with blood flow restriction (BFR), is a unique training method for promoting muscle hypertrophy and strength gains by using low-intensity resistance exercises or walking. This method also induces pooling of venous blood in the legs. Therefore, we hypothesized that slow walking with BFR may affect limb venous compliance and examined the influence of 6 weeks of walking with BFR on venous compliance in older women. Sixteen women aged 59-78 years were partially randomized into either a slow walking with BFR group (n = 9, BFR walk group) or a non-exercising control group (n = 7, control group). The BFR walk group performed 20-min treadmill slow walking (67 m min⁻¹), 5 days per week for 6 weeks. Before (pre) and after (post) those 6 weeks, venous properties were assessed using strain gauge venous occlusion plethysmography. After 6 weeks, leg venous compliance increased significantly in the BFR walk group (pre: 0.0518 ± 0.0084 , post: 0.0619 ± 0.0150 ml 100 ml⁻¹ mmHg⁻¹, P<0.05), and maximal venous outflow (MVO) at 80 mmHg also increased significantly after the BFR walk group trained for 6 weeks (pre: 55.3 ± 15.6 , post: 67.1 ± 15.6) 18.9 ml 100 ml⁻¹ min⁻¹, P<0.01), but no significant differences were observed in venous compliance and MVO in the control group. In addition, there was no significant change in arm compliance in the BFR walk group. In conclusion, this study provides the first evidence that 6 weeks of walking exercise with BFR may improve limb venous compliance in untrained elder female subjects.

Introduction

The venous system of the human body can be looked at as a large blood reservoir that contains more than 70% of the total blood volume at rest and plays an important role in maintaining cardiovascular homeostasis. Small changes in peripheral blood volume can greatly affect cardiac filling pressure and, subsequently, cardiac output. Therefore, appropriate regulation of venous blood volume is essential, especially during various physiological conditions such as haemorrhage and orthostatic load (Olsen et al., 2000).

Ageing is one of the most important factors that influence leg venous compliance. According to Monahan et al. (2001), calf venous compliance was about 40% lower in elderly subjects. Olsen & Länne (1998), Fu et al. (2000), Tsutsui et al. (2002) and Hernandez & Franke (2005) also demonstrated similar results.

The mechanisms of this decline in venous compliance through ageing are not yet obvious; however, structural alterations, namely venous wall thickening and an increase in the collagen/elastin ratio in venous walls, which are also observed in arterial walls, may cause an age-related decline in venous compliance (Pareira et al., 1953). On the other hand, functional alterations such as vasomotor tone and nitric oxide synthesis are also thought to cause age-related decline in venous compliance. Several studies have revealed that venous compliance is improved by habitual endurance exercise, and leg venous compliance is decreased in sedentary subjects compared with endurance-trained men (Louisy et al., 1997; Wecht et al., 2000; Monahan et al., 2001; Hernandez & Franke, 2005).

KAATSU, which is an exercise combined with blood flow restriction (BFR), is one of method of trainings that uses a specially designed belt tightened near the joints of the upper © 2011 The Authors arm or leg, applying pressure to the muscle and temporally restricting blood flow. Existing research has revealed that lowintensity KAATSU training could induce muscular hypertrophy and strength gain (Takarada et al., 2002). Abe et al. (2006) reported that the combination of slow walking and muscle BFR induces increases in muscle size and strength despite the minimal level of exercise intensity. Therefore, slow walking with BFR may be a potentially useful method to promote muscle hypertrophy, especially for the elderly. During slow walking with BFR, arterial flow is reduced and venous blood is pooled in the leg vein (Takano et al., 2005; Iida et al., 2007). Consequently, these changes might produce hydrostatic forces in the leg vein.

We hypothesized that this change affects leg venous compliance; therefore, in the present study, we investigated the effect of 6 weeks of walking with BFR on leg venous compliance in older women using non-invasive methods of determining limb venous compliance.

Methods

Subjects

Sixteen elderly women aged 59–78 years participated in this study. Subjects were partially randomized into either a slow walking with BFR group (n = 9) or a non-exercising control group (n = 7). The subjects were recruited by word of mouth. Volunteers were free of orthopaedic disorders. None of the subjects had participated in strength- or resistance-type training for at least 2 years prior to the start of the study. All subjects were informed of the methods, procedures and risks and signed an informed consent document before participation. The study was conducted according to the Declaration of Helsinki and was approved by the Ethics Committee for Human Experiments of the University of Tokyo, Japan.

Training protocol

The training was conducted once a day, 5 days per week for 6 weeks. The subjects walked on a motor-driven treadmill at 67 m min^{-1} for 20 min. The walking speed and duration remained constant throughout the training period. Subjects in the slow walking with BFR group wore pressure belts (Kaatsu-Master, Sato Sports Plaza, Tokyo) on both legs during the training. A non-exercising control group was advised to perform slow walking daily for 20 min 5 days per week during the study. During the study, all subjects were required not to perform any other strength- or resistance-type training or long running for more than 20 min.

Blood flow restriction (BFR)

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Femoral blood flow was impaired using the KAATSU technique, which restricts venous blood flow causing pooling of blood in capacitance vessels distal to the cuff and partial occlusion of arterial blood flow (Takano et al., 2005; Iida et al., 2007; Nakajima et al., 2008). Prior to slow walking with BFR, the subjects were seated on chairs and the belt air pressure was repeatedly set (30 s) and then released (10 s) from an initial pressure of 100 mmHg to a final training pressure of 140 mmHg (Abe et al., 2006). The training pressure was increased by 10 mmHg each day until a final belt pressure of 200 mmHg was reached, although two subjects trained at 180 mmHg for the duration of the study. A restriction pressure of 140–200 mmHg was selected for the restriction stimulus based on a review of the data in young men (Abe et al., 2006). Blood flow to the leg muscles was restricted for a total time of about 23 min (20 min of walking and 3 min of preparation) during each training session for each subject, and the belt pressure was released immediately upon completion of the session.

Venous occlusion plethysmography

Venous occlusion plethysmography with multiple proximal occlusion pressure was used to obtain venous compliance measurements before and immediately after the study. A strain gauge (EC6 plethysmograph, Hokanson, Bellevue, WA, USA) was stretched around the largest girth of the right calf and forearm. Thigh and upper arm pressure cuffs were connected to a rapid cuff inflator (E20 rapid cuff inflator, Hokanson, Bellevue, WA, USA) to ensure rapid and accurate filling and deflating of the cuff. External pressure was applied on the thigh and upper arm through an occlusion cuff, which was attached to an electronically controlled air pump. Pressure levels of 20, 40, 60 and 80 mmHg were delivered consecutively after having reached a quasi-steady state at the previous level. We calculated the following parameters from the plethysmographic recordings (Bleeker et al., 2004). To be concise, venous volume variation (VVV) (in ml 100 ml $^{-1}$) was defined as the maximal relative volume increase in the limb at a certain cuff pressure; VVV at different occlusion pressures represents the pressure-volume curve. Venous compliance (ml 100 ml⁻¹ mmHg⁻¹), the ratio of a change in volume (ΔV) to a concomitant change in the transmural distending pressure (ΔP) : $V_c = \Delta V / \Delta P$ (Rothe, 1983, 1993), was derived from the slope of the pressure-volume curve. Maximal venous outflow (MVO) (in ml 100 ml⁻¹ min⁻¹) was calculated as the slope of the tangent at the curve from 0.5 to 2.0 s after cuff release.

Statistical analyses

All values are presented as means \pm SD. We used an unpaired Student's t-test to assess baseline differences between slow walking with BFR group and control group. We used a paired Student's t-test to examine the effect of the 6-week intervention in both groups. Differences were considered significant if P<0.05.

Results

Table 1 summarizes the anthropometric characteristics and resting cardiovascular variables of all participants in the study. Three subjects in each group had hypertension, and no subjects

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Table 1	Anthropometrical	characteristics	and	resting	cardiovascu	laı
variables.						

	Control group	Blood flow restriction walk group
Age (year)	68·7 ± 2·8	67·4 ± 1·6
Height (cm)	150·1 ± 1·8	149·9 ± 1·2
Mass (kg)	52·7 ± 3·3	51·9 ± 2·3
BMI (kg m ⁻²⁾	23·9 ± 1·0	23·1 ± 0·9
HR (bpm)	73·0 ± 3·6	76·8 ± 3·3
Systolic blood pressure (mmHg)	129·7 ± 8·2	148·2 ± 7·1
Diastolic blood pressure (mmHg)	71·7 ± 2·6	$84.6 \pm 3.8^{**}$
Leg compliance (ml 100 ml ⁻¹ mmHg ⁻¹)	0.0686 ± 0.0160	$0.0518 \pm 0.0084^{*}$

**P<0.01, *P<0.05.

had heart failure or venous disease, such as varicose vein of lower extremity. Although pre intervention data of diastolic pressure differed significantly between the BFR walk group and control group, there were no significant differences between the two groups for age, standing height, body mass and body mass index. None performed regular moderate or vigorous physical activity before the study.

Venous compliance

Preintervention compliance of the control group was higher than that of the slow walking with BFR group (Table 1). After 6 weeks of training, leg compliance in slow walking with BFR group increased significantly (Fig. 1, pre: 0.0518 ± 0.0084 ml $100 \text{ ml}^{-1} \text{ mmHg}^{-1}$, post: 0.0619 ± 0.0150 ml 100 ml⁻¹ mmHg⁻¹, P<0.05) with significant change of leg girth (pre: 33.5 ± 0.8 cm, post: 33.9 ± 0.8 cm, P<0.05). Meanwhile, no significant differences were observed in the leg compliance of the control group (Fig. 1) and in the arm compliance of the BFR group (n = 6, Fig. 2).

Maximal venous outflow

Figure 3 depicts the MVO at each cuff pressure. Maximal venous outflow at 80 mmHg increased significantly after 6-week training in the slow walking with BFR group (pre: $55\cdot3 \pm 15\cdot6$ ml 100 ml⁻¹ min⁻¹, post: $67\cdot1 \pm 18\cdot9$ ml 100 ml⁻¹·min⁻¹, P<0.01), but no significant difference was observed in the control group.

Discussion

The present study provides new findings that 6 weeks of slow walking with BFR in elderly women induce significantly increased limb venous compliance and increased MVO. It is likely that the decline in venous compliance through



Figure 1 Pressure–volume curves for the leg (\bullet : pre 6-week training and \bigcirc : post 6-week training). Venous volume variation (VVV) values are means \pm SD. (a) Control group; (b) slow walking with BFR group. *P<0.05.



Figure 2 Pressure–volume curves for the arm obtained before and after slow walking with BFR for 6 weeks (: pre 6-week training and \bigcirc : post 6-week training). Venous volume variation (VVV) values are means \pm SD for six subjects. There was no significant change in arm venous compliance in contrast to the change of leg venous compliance.

ageing is reversible and that endurance exercise with xrestriction of leg blood flow improves the limb venous compliance.



Figure 3 Effects of walking for 6 weeks on maximum venous flow in the leg of control group (a) and slow walking with BFR group (b). Maximal venous outflow (MVO) values are means \pm SD. There was a significant change in the maximum venous flow at 80 mmHg in the slow walking with BFR group. **P<0.01.

Ageing induces the decline in limb venous compliance (Olsen & Länne, 1998), and this decline prevents the elderly from responding to acute deterioration of cardiovascular homeostasis. Furthermore, reduced venous compliance may be one of the risk factors of varicose veins and deep vein thrombosis. Therefore, we need some way to counter the decline in venous compliance.

Until now, several authors have demonstrated that venous compliance is improved by endurance exercise. Hernandez & Franke (2005) studied the influence of a 6-month endurancetraining programme on calf venous compliance in older men and women and revealed that it tended to be 20-30% greater in the exercise group after 6 months of training compared with control group. Monahan et al. (2001) sought to determine the reciprocal influences of ageing and habitual endurance exercise on calf venous compliance in humans, and they demonstrated that venous compliance declined with age and that calf venous compliance was approximately 70-120% greater in endurancetrained men compared with age-matched sedentary men and approximately 30% greater in older endurance-trained men compared with young sedentary. These data suggest that endurance training is useful for improving age-related decline in venous compliance.

In the present study, we suggested that slow walking with BFR (Abe *et al.*, 2006) can induce significant improvement in limb venous compliance in elderly women over shorter periods compared with simple endurance exercise.

Although the mechanism of this prominent improvement is unclear, we can infer that slow walking with BFR has additional effects over simple endurance exercise. Because subjects wear a belt around their legs during walking with BFR, slow walking with BFR not only increases physical activity more than endurance exercise does but also changes hydrostatic force in the leg through BFR, and these changes may affect venous vascular function synergistically. As previously reported, venous blood outflow and arterial inflow are restricted, and blood pooling in the lower extremities is produced during walking © 2011 The Authors with BFR (Takano et al., 2005; Iida et al., 2007). During slow walking with BFR, venous pooling might induce the alteration in hydrostatic force in the lower extremities, and altered hydrostatic force affects cardiovascular reflex responses and, consequently, venous properties more quickly and more effectively than simple endurance exercise.

According to Bleeker et al. (2004), after 18 days of headdown-tilt bed rest, leg venous compliance is reduced and leg venous outflow resistance is increased. In the report, they concluded that altered gravitational gradients induce alteration in hydrostatic forces and cardiovascular reflex responses. In their study, the deconditioning induced by 18 days of bed rest led to no significant differences in arm venous vascular properties. In the present study, arm venous compliance was not affected by slow walking with BFR. This differential response between legs and arms may support our inference that walking with BFR may affect hydrostatic gradients only in the leg and that this change plays an important role in improving leg venous function.

Muscle compartment, which can compress the veins and resist the expansion of the veins, is thought to be one of the structural factors to influence the venous compliance. Convertino *et al.* (1988, 1989) found that reduced muscle compartment leads the increased leg compliance observed after exposure to simulated microgravity. In the present study, despite the fact that the girth increased significantly after 6 weeks walking with BFR, the venous compliance increased significantly. This suggests that the increase in limb venous compliance was not caused by the increase in muscle mass.

Haemodynamic factors may affect the venous compliance. Although blood pressure was not checked during exercise, some studies revealed that elevation of blood pressure during walking with BFR is lower compared with high-intensity resistance training (Sakamaki et al., 2008), and there was no significant change in blood pressure after 6 weeks in the present study (data not shown). Furthermore, other factors than muscle size and blood pressure may influence the result, but no remarkable changes in anthropometrical characteristics were observed, and

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we could not find the special characteristics of the responder or non-responder.

However, as a result of the small sample size in this study and the significant difference of preintervention data in leg venous compliance between groups, statements must be made carefully and further investigation is required. Furthermore, as we did not check the state of venous valves during compression of the legs in the present study, more detailed investigation in the state of valves, hydrostatic forces and cardiovascular reflex response is required to make the mechanism clear.

In conclusion, 6 weeks of slow walking with BFR in elderly subjects significantly induced increased limb venous compli-

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ance. Also, slow walking with BFR may be a novel method for improving limb venous compliance in elderly women.

Conflicts of interest

Dr Sato Y is the Managing Director of Best Life Co., Ltd, and President of Sato Sports Plaza Co., Ltd. (and a stockholder of both companies) and is the inventor of the KAATSU Training method. He also received U.S. Patent No. 6,149,618 and corresponding patents outside the United States. (all assigned to Best Life Co., Ltd.) and is the inventor of other applications and patents (assigned to Sato Sports Plaza Co., Ltd.) related to KAATSU Training.

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ORIGINAL ARTICLE

Hemodynamic and autonomic nervous responses to the restriction of femoral blood flow by KAATSU

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KAATSU training is a novel method for strength training to induce muscle strength and hypertrophy. The purpose of the present study was to investigate the hemodynamic and autonomic nervous responses to the restriction of femoral blood flow by KAATSU. Ultrasonography, echocardiography and impedance cardiography were performed in ten healthy male volunteers aged 34 ± 1.5 before (pre), during and after (post) pressurization on both legs with KAATSU belts placed around proximal portion of both leas. The parameters measured were as follows; the superficial femoral arterial blood flow, left ventricular end-diastolic/systolic dimension (LVDd/LVDs), cardiac output (CO), stroke volume (SV), diameter of inferior vena cava (IVC), heart rate (HR), mean blood pressure (mBP), total peripheral resistance (TPR) and heart rate variability (HRV). The pressurization on both legs with KAATSU suppressed venous blood flow, and markedly induced pooling of blood into the legs with pressure-dependent reduction of femoral arterial blood flow. The application of 200 mmHg KAATSU decreased femoral arterial blood flow, LVDd, CO, SV and IVC significantly. HR tended to increase, and TPR increased significantly, but mBP did not change significantly. In addition, high frequency (HFrR), a marker of parasympathetic activity, decreased during KAATSU, while LFrr/HFrr, a quantitative marker of sympathetic autonomic nervous activity, increased significantly. These results indicate that the application of KAATSU on both legs induces venous pooling in the legs, and then inhibits venous return. The reduction of venous return causes a decrease of IVC diameter, cardiac size and stroke volume with an increase in TPR and LFRR/HFRR. Thus, the KAATSU training appears to become a useful method for potential countermeasure like lower body negative pressure (LBNP) against orthostatic intolerance for long-term bed rest or space flight as well as strength training to induce muscle strength and hypertrophy.

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Key words: KAATSU training, lower body negative pressure, hemodynamics, cardiac output, autonomic function, power spectral analysis, bed rest, space flight

INTRODUCTION

KAATSU training is a novel method for muscle training, originally developed by Sato (2005). Under the conditions with the restriction of muscle blood flow, even a short-term and low-intensity exercise can induce muscle strength, hypertrophy and increasing muscle mass (Takarada et al, 2000a, 2000b, 2000c; Abe et al., 2005). And, now, KAATSU training has been used to improve muscle mass and strength in patients with cardiovascular and orthopedic diseases as well as healthy subjects and athletes.

Hydrostatic gradients by gravity play an essential role in determining the distribution of pressure and volume within the cardiovascular system. When these gradients are removed, such as during exposure to space flight and bed rest, a central fluid shift occurs, followed by a neurohumorally mediated reduction in blood and plasma volume and normalization of hemodynamic equilibrium in the direction observed in the upright posture. This adaptation is rapid and appears to be complete within the first 24 to 48 h of microgravity exposure (Levine et al., 1997, 2001). When gravitational force is restored, stroke volume (SV) is reduced, and heart rate is increased. Although vascular resistance increases, the degree of change is variable, and the unwanted symptoms of orthostatic intolerance are frequently developed. Lower body negative pressure (LBNP) induces the retention of blood flow in lower extremities, and causes subsequent hemodynamic changes including autonomic nervous activities (Stevens and Lamb, 1965; Bonde-Petersen et al., 1984; Tomaselli et al., 1987; Lathers & Charles., 1993). And, until now, it has been known to be a useful method to prevent such orthostatic intolerance after space flight and bed rest, probably through its effect as orthostatic stimulus (Guell et al., 1992; Lee et al., 1997; Watenpaugh et al., 2000). Recently, we have reported that

"KAATSU" leg resistance exercise causes a significant exercise-induced growth hormone (GH) response even in short-term low-intensity resistance exercise (Takano et al., 2005a, 2005b). KAATSU also decreased cardiac output (CO) and stroke volume (SV), due to the pooling of blood into legs and inhibition of venous return. Thus, KAATSU appears to be an effective method to promote a state of blood pooling in the capillaries within the limb musculature like LBNP. And, when exercise is combined with KAATSU, KAATSU may become a useful method for potential countermeasure against orthostatic intolerance for long-term bed rest or space flight as well as strength training to induce muscle strength and hypertrophy. However, the hemodynamic and autonomic responses to KAATSU by itself have not been investigated in detail.

The purpose of the present study was to investigate the hemodynamic and autonomic nervous responses to the restriction of femoral blood flow by KAATSU. Ultrasonography, echocardiography and impedance cardiography were used to assess the dependent variables.

METHODS Subjects

Ten normal healthy adult males, aged 34 ± 1.5 (28 to 46), participated in this study. All were non-trained volunteers, and informed consent was obtained prior to the study. Mean height was 175 ± 4 cm, and mean weight was 66 ± 4 kg. None of the subjects had any diseases nor took any medications. The study protocol was approved by the ethics committee of the University of Tokyo.

Reduction of femoral muscle blood flow by KAATSU

A method for inducing the reduction of muscle blood flow was similar as previously reported (Takarada et al., 2000a, 2000b; Takano et al., 2005a, 2005b). Pressure was applied at the proximal ends of both thighs by means of specially designed belts (33 mm in width and 880 mm in length) to restrict venous blood flow and cause pooling of blood in capacitance vessels distal to the cuff, and restrict arterial blood flow. The cuff pressure was first set to approximately 40~50 mmHg (mean 45 mmHg), and the cuff pressure used was 100~300 mmHg. To determine the hemodynamic and autonomic responses to KAATSU, 200 mmHg was applied.

Measurement of hemodynamic parameters

To evaluate hemodynamic parameters, we used the Task Force Monitor (CNSystems Medizintechnik, Graz, Austria) (Gratze et al., 1998; Fortin et al., 1998), which includes surface electrocardiograms (ECG), impedance cardiography (ICG), beat-to-beat blood pressure by vascular unloading technique (Penaz, 1973) and oscillometric blood pressure recording performed on the upper arm. The ECG, impedance signal and beat-to-beat blood pressure was sampled with 1000 Hz each. These data were used to calculate online all hemodynamic parameters. The measurements of hemodynamic parameters were heart rate (HR), mean blood pressure (mBP), stroke volume (SV), cardiac output (CO) and total peripheral resistance (TPR). The calculation of CO and TPR was as follows.

CO=SV*HR

TPR=mBP*80/CO

Histograms of RR intervals were computed and pseudo-digitized at 10 samples per second. Autoregressive modeling (Burg method) was used to construct frequency domain spectrograms of the heart rate variability (HRV) (Bailey et al., 1994; Burklow et al., 1999). Parameters extracted from the variability spectra were low-frequency power (LF_{RR}, 0.03 to 0.15 Hz) and high-frequency power (HF_{RR}, 0.16 to 0.50 Hz), normalized to total power over the range from 0.01 to 0.50 Hz. LF_{RR}/HF_{RR} have previously been demonstrated to measure changes in sympathetic activity (Malliani et al., 1991)

Measurement of femoral arterial blood flow

The blood flow of superficial femoral artery was calculated from the cross-sectional area (CSA) of the artery and velocity time integral (VTI) using Aplio80 (Toshiba, Tokyo). The site recorded was ~5 cm distal to the portion of the KAATSU belt. First, superficial femoral artery was identified in the two-dimensional mode, and CSA was measured at the end-systolic period. Then, in the pulse-Doppler method, VTI, calculated as the integral area under the velocity curve, was measured. Adjustment of the angle for the measurement was within 60°. Blood flow per minute was obtained by multiplying CSA by VTI and heart rate. The blood flow was acquired in supine position before (pre) and during the application of KAATSU, and just after releasing the pressure.

Measurement of cardiac size, CO and diameter of inferior vena cava (IVC)

Transthoracic echocardiography was performed using Aplio80. The left ventricular end-diastolic dimension (LVDd) and left ventricular end-systolic dimension (LVDs) were measured on the M-mode recording in the parasternal long-axis view. The left ventricular outflow velocity pattern was recorded from the apical long-axis view with the pulsed wave Doppler sample volume positioned just below the aortic valve, and the aortic velocity time integral (VTIAO) was calculated. The diameter of left ventricular outflow tract (D) was measured with two-



Figure 1. The ultrasonographic findings of femoral blood flow. The typical data are indicated before (pre) and during the application of KAATSU (100 mmHg). Fig. 1Aa and Ba indicate the data recording of longitudinal section of superficial femoral artery (red arrow) and femoral vein (blue arrow). Fig.1Ab and Bb show the data recording of blood flow velocity of superficial femoral artery. Note that KAATSU markedly induces pooling of venous blood with restriction of arterial blood flow.

dimensional echocardiography. Cross-sectional area (CSA_{AO}) of flow was calculated as $\pi \times (D/2)^2$ based on a two-dimensional echo diameter (D) measurement. CO then is calculated as CSA_{AO} multiplied by the VTI_{AO} and HR. The maximal diameter of IVC was measured from the subcostal approach.

Data analysis

All values are expressed as means \pm S.E.M. Comparison of time courses of parameters was analyzed by one-way ANOVA for repeated measures. When differences were indicated, a Bonferroni/ Dunnett's comparison was used to determine significance. Differences were considered significant if P value was less than 0.05.

RESULTS Reduction of femoral arterial blood flow by KAATSU

Figure 1 & 2 show the effects of KAATSU on blood flow of the superficial femoral artery. Fig. 1 shows a representative data recording of femoral arterial blood flow without (pre) and with KAATSU. Under the conditions with KAATSU (100 mmHg), the diameter of the femoral vein (described by blue arrow) was remarkably increased (Fig. 1Aa (pre) & Fig. 1Ba (100 mmHg)) and femoral arterial blood flow (described by red arrow) was decreased by 22 % (Fig. 1Ab & Fig. 1Bb). The changes in femoral arterial blood flow against the cuff pressure are depicted in Fig. 2. Application of 100 mmHg KAATSU decreased femoral arterial blood flow from 354.1 ± 37.2 ml/min



Figure 2. Changes of blood flow of superficial femoral artery. The blood flow of right superficial femoral artery was obtained before (0 mmHg) and during the application of various degrees of KAATSU (100~300 mmHg). The data show means \pm S.E.M. obtained from 10 subjects. *P<0.05 vs control (Pre-KAATSU).

(pre) to 151.0 \pm 22.3 ml/min (100 mmHg, n=10, P<0.01). And, arterial blood flow decreased with increasing levels of the applied-pressure and disappeared at greater than 250 mmHg in most subjects. The arterial blood flow decreased to 36.4 \pm 12.9 ml/min (200 mmHg, n=10, P<0.01), and 10.8 \pm 8.4 ml/min (250 mmHg, n=10, P<0.01). Immediately after releasing the pressure, femoral arterial blood flow recovered (data not shown). These results

indicate that the application of KAATSU to both legs restricts venous blood flow and causes venous pooling in the legs distal to the cuff with the pressuredependent reduction of arterial blood flow.

Hemodynamic responses to the restriction of femoral blood flow by KAATSU

Figure 3 shows the effects of KAATSU on venous return, cardiac size and CO measured by echocardiography. We measured the diameter of IVC as a quantitative marker of venous return. Application of 200 mmHg KAATSU reduced the diameter of IVC from 17.9 \pm 1.3 mm to 14.3 \pm 1.0 mm (Fig. 3C, n=10, P<0.01). Simultaneously, left ventricular end-diastolic dimension (LVDd) was reduced from 47.5 \pm 1.0 mm to 42.9 \pm 1.0 mm (n=10, P<0.01, Fig. 3A). In addition, cardiac output (CO) was decreased from 5.6 \pm 0.2 l/min to 4.1 \pm 0.3 l/min (n=5, P<0.01, Fig. 3D). Left ventricular end-

systolic dimension (LVDs) was also decreased, but not significantly (Fig. 3B). Thus, KAATSU appears to induce the pooling of blood in the legs, resulting in inhibiting venous return, and reducing cardiac preload and CO.

Figure 4 shows the changes of hemodynamic parameters by application of pressure on both legs measured by impedance cardiography. During pressurization of 200 mmHg, HR increased slightly (Fig.4A). Mean blood pressure (mBP) did not change significantly (Fig. 4B). On the other hand, CO and stroke volume (SV) were significantly decreased from 5.8 ± 0.2 ml/min to 4.3 ± 0.2 ml/min (Fig. 4C, n=10, P<0.01) and from 88.3 ± 3.7 ml to 64.5 ± 5.0 ml (Fig. 4D, n=10, P<0.01), respectively. These changes were consistent with the results obtained by echocardiography. In addition, total peripheral resistance (TPR) increased significantly from 1122.6 \pm 71.1 dyne*s/cm⁵ to 1638.7 \pm 140.7 dyne*s/cm5 at



Figure 3. Effects of KAATSU on LVDd, LVDs, the diameter of IVC and CO measured by echocardiography. The parameters (LVDd, LVDs, IVC and CO) are shown in control (pre) and during a set pressure of 45 mmHg, and a cuff pressure of 200 mmHg, and after the release of KAATSU (post). Values are means \pm S.E.M. obtained from 5-10 subjects. *P<0.05, **P<0.01 vs. control (pre)

200 mmHg KAATSU (n=10, P<0.01, Fig. 4E).

Autonomic nervous responses to the restriction of femoral blood flow by KAATSU

Figure 5 depicts an example of the changes in the power spectra of heart rate variability (HRV) in control (pre), during application of KAATSU at a set pressure of 50 mmHg and a cuff pressure of 200 mmHg, and after the release of KAATSU. Application

of pressure on both legs produced changes in HRV, i.e. markers of autonomic modulation (Fig. 6). The HF_{RR} component was reduced from 41.3 \pm 4.9 normalized unit (nu) to 23.4 \pm 4.2 nu (n=10, Fig. 6A). The LF_{RR}/HF_{RR} as a quantitative marker of sympathetic nervous activity was increased significantly (from 1.8 \pm 0.3 ms² to 4.9 \pm 1.0 ms², n=10, P<0.01, Fig. 6B).



Figure 4. Effects of KAASTU on hemodynamic parameters measured by impedance cardiography. The parameters (HR, mBP, CO, SV and TPR) are shown in control (pre) and during a set pressure of 45 mmHg, and a cuff pressure of 200 mmHg, and after the release of KAATSU (post). Values are means \pm S.E.M. obtained from 10 subjects. *P<0.05, **P<0.01 vs. control (pre)



Figure 5. The time courses of changes of heart rate variability (HRV) during KAATSU (50 and 200 mmHg). Note that during KAATSU (50 and 200 mmHg), LF_{RR} increased, compared with the control, while HF_{RR} decreased.

DISCUSSION

The major findings of the present study were as follows: (1) Application of KAATSU on both legs caused the pooling of venous blood with the pressure-dependent reduction of femoral arterial blood flow; (2) The pooling of venous blood in the legs by KAATSU reduced venous return with a significant decrease in cardiac size and CO, and a compensated increase of TPR; and (3) Application of KAATSU on both legs also affects autonomic nervous activities, where an increase in the sympathetic nervous activity was observed. Thus, KAATSU appears to be an effective method to induce venous pooling in the legs like lower body negative pressure (LBNP). KAATSU training also appears to be a unique method as a potential countermeasure against orthostatic intolerance for long-term bed rest or space flight as well as strength training to induce muscle strength and hypertrophy.

KAATSU training is a novel method for muscle training to strength muscle and induce muscle hypertrophy and increase muscle mass. Under the conditions of restricted muscle blood flow, even a short-term and low-intensity exercise can induce muscle strength, hypertrophy and increasing muscle mass (Takarada et al., 2000a, 2000b, 2000c; Takarada et al., 2002; Abe et al., 2005). Up to now, several mechanisms underlying the effects of KAATSU training have been proposed. First, under the conditions with restriction of muscle blood flow by KAATSU, a large number of fast-twitch muscle fibers are recruited in a hypoxic condition, resulting in muscle hypertrophy (Takarada et al., 2000b; Yasuda et al., 2004). Secondly, a combination of anaerobic factors such as local ischemia and/or local accumulation of lactate in the legs induced by the



Figure 6. Effects of KAATSU on heart rate variability. The parameters (HF_{RR} and LF_{RR}/HF_{RR}) are shown in control (pre) and during a set pressure of 45 mmHg, and a cuff pressure of 200 mmHg, and after the release of KAATSU (post). Values are means \pm S.E.M. obtained from 10 subjects. *P<0.05, **P<0.01 vs. control (pre)

restriction of muscle blood supply may stimulate peripheral afferent nerves, resulting in enhancing GH secretion (Takarada et al., 2000a; Takano et al., 2005a, 2005b). GH stimulates the liver to secrete insulin-like growth factor-1 (IGF-1) (Abe et al., 2005) and both GH and IGF-1 can contribute to muscle hypertrophy. Thus, the effects of KAATSU on muscle strength and hypertrophy may be related to the severity of the restriction of muscle blood flow and/or accumulation of anaerobic factors. The present study provided the quantitative data about the relationships between the cuff pressure and femoral arterial blood flow. Even 100 mmHg KAATSU decreased femoral arterial blood flow by approximately 22 %. Arterial blood flow decreased with increasing levels of KAATSU, and disappeared at greater than 250 mmHg in most subjects. But, the degree of restriction of arterial blood flow by KAATSU was different among individuals. Besides of the pressure-dependent inhibition of arterial blood flow, the marked venous dilation and pooling of blood in the legs were observed under the conditions with KAATSU. Application of KAATSU on both legs induced venous pooling, and reduced venous return with the reduction of IVC diameter and cardiac size and CO. Even in cases of a set pressure of 45 mmHg, CO, SV, and LVDd were decreased. Application of 200 mmHg induced much larger decrease in CO, SV, LVDd and IVC. The decrease in central venous pressure and changes in cardiac wall stress increased the sympathetic activities measured by HR variability, and HR also tended to increase during the KAATSU. Mean arterial blood pressure did not change significantly under the KAATSU (45~200 mmHg), suggesting that the primary mechanism responsible for sympathoexcitation during the pressure levels used in the present study involved the influence of the cardiac receptor afferents. Thus, KAATSU appears to be a unique method to promote a state of blood pooling in the capillaries within the limb musculature.

Lower body negative pressure (LNBP) has been known to induce the retention of blood flow in lower extremities, and induce subsequent hemodynamic changes (Stevens and Lamb, 1965; Bonde-Petersen et al., 1984). The cardiovascular response to LBNP is thought to involve a complex sequence of steps that occur at different rates. The initial rapid onset and readily reversible steps include increased transmural pressure, reactive arterial tonus, and venous pooling that occur in the lower body with the initiation of LBNP. The slower mechanisms of interstitial and lymphatic sequestration follow with the continued orthostatic stress and result in an increased calf circumference and decreased central vascular filling (Tomaselli et al., 1987; Lathers and Charles., 1993). Melchior et al. (1994) reported that LBNP ramp test from 0 to -40 mmHg reduces central venous pressure (CVP) by about -5~-6 mmHg and venous return, resulting in a decrease in SV and CO by about 37 %and 32 %, respectively. In the present study, we demonstrated that KAATSU of 200 mmHg on both legs produces 26.9 % reduction of SV and 25.7 %reduction of CO, which has an equal effect to LNBP of approximately 30 mmHg. It has been also reported that LBNP (0 to -40 mmHg) produced clear changes in HR variability; LBNP induces sympathetic activation and vagal withdrawal, mediated by unloading of both cardiopulmonary and arterial baroreceptors (Franke et al., 2000; Lucini et al., 2004). Lucini et al. (2004) reported that LFRR progressively rose from 49 ± 7 nu to 83 ± 3 nu by LBNP of -40 mmHg. The HFRR component was reduced from 43 ± 6 to 13 ± 3 nu and the LFRR/HFRR was increased from 1.6 ± 0.6 to 17.5 ± 12.1 . In the present study, the application of 200 mmHg KAATSU on both legs increased the LFRR component (58.7 \pm 4.9 to 76.6 \pm 4.2 nu) and the LFrr/HFrr (1.8 \pm 0.3 to

 4.9 ± 1.0), while the HF_{RR} component was decreased from 41.3 ± 4.9 to 23.4 ± 4.2 . Compared with the effect on autonomic nervous activities with LNBP, pressurization of 200 mmHg on both legs also has an effect equal to a LNBP of about -20~-30 mmHg.

LBNP is known to be a useful method to prevent orthostatic intolerance after space flight and bed rest, probably through its effect as an orthostatic stimulus (Güell et al., 1992; Murthy et al., 1994; Buckey et al., 1996; Lee et al., 1997; Watenpaugh et al., 2000; Watenpaugh et al., 2001). Lee et al. (1997) reported that supine treadmill exercise against LBNP during 5 days of 6° head-down bed rest maintained submaximal exercise responses such as submaximal heart rate, respiratory exchange ratio, and ventilation after bed rest. Watenpaugh et al. (2000) also reported that daily supine exercise in a LBNP chamber at 1.0-1.2 body weight (58 \pm 2 mmHg LBNP) maintains aerobic fitness and sprint speed during 15 days of 6° head-down bed rest. Such preservation of submaximal responses after bed rest suggests that exercise combined with LBNP may be effective in maintaining upright exercise capacity during longer bed rest periods (Perhonen et al., 2001; Schneider et al., 2002). LBNP has been also used for preventing orthostatic intolerance and micogravity-induced cardiac remodeling/atrophy after space flight and for training astronauts (Watenpauch, 2001). The present study clearly indicated that KAATSU is a noninvasive technique that induces venous dilation and pooling of blood in the legs like LBNP. In addition, the KAATSU training may also be used by astronauts to prevent the loss of muscle mass and strength as well as the loss of bone density (Yamazaki Y, 2004). Thus, it is very likely that KAATSU training may be a useful method to counter symptoms of orthostatic intolerance and muscle atrophy in patients, bed rest subjects, and astronauts, but further studies are needed to clarify these interesting possibilities.

In conclusion, the restriction of femoral blood flow induced by the application of KAATSU on both legs caused the marked pooling of blood with the pressure-dependent reduction of femoral arterial blood. KAATSU training appears to be a useful potential countermeasure against orthostatic intolerance for long-term bed rest or space flight as well as strength training to induce muscle strength and hypertrophy.

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ORIGINAL ARTICLE

Use and safety of KAATSU training: Results of a national survey

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KAATSU training is a novel training, which is performed under conditions of restricted blood flow. It can induce a variety of beneficial effects such as increased muscle strength, and it has been adopted by a number of facilities in recent times. The purpose of the present study is to know the present state of KAATSU training in Japan and examine the incidence of adverse events in the field. The data were obtained from KAATSU leaders or instructors in a total of 105 out of 195 facilities where KAATSU training has been adopted. Based on survey results, 12,642 persons have received KAATSU training (male 45.4%, female 54.6%). KAATSU training has been applied to all generations of people including the young (<20 years old) and the elderly (>80 years old). The most popular purpose of KAATSU training is to strengthen muscle in athletes and to promote the health of subjects, including the elderly. It has been also applied to various kinds of physical conditions, cerebrovascular diseases, orthopedic diseases, obesity, cardiac diseases, neuromuscular diseases, diabetes, hypertension and respiratory diseases. In KAATSU training, various types of exercise modalities (physical exercise, walking, cycling, and weight training) are used. Most facilities have used 5-30 min KAATSU training each time, and performed it 1-3 times a week. Approximately 80% of the facilities are satisfied with the results of KAATSU training with only small numbers of complications reported. The incidence of side effects was as follows; venous thrombus (0.055%), pulmonary embolism (0.008%) and rhabdomyolysis (0.008%). These results indicate that the KAATSU training is a safe and promising method for training athletes and healthy persons, and can also be applied to persons with various physical conditions. Key words: KAATSU training; side effects; rehabilitation

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INTRODUCTION KAATSU training is a novel method for muscle training, originally developed by Sato (2005). Under the conditions of restricted muscle blood flow, even short-term, low-intensity exercise can induce muscle strength, and hypertrophy (Takarada et al, 2000b; Takarada et al., 2002; Takarada & Ishii, 2002; Yasuda et al., 2004; Abe et al., 2005a, b). In addition, KAATSU training increases the amount of circulating growth hormone (GH) (Takarada et al., 2000a; Takano et al., 2005a,b; Sato et al., 2005), which may enhance lipolysis and bone formation, resulting in a reduction of obesity and bone diseases as well as improving strength and inducing hypertrophy of muscle (Beekley et al., 2005). Also, GH stimulates the liver to secrete insulin-like growth factor-1 (IGF-1) (Abe et al., 2005a), which may improve the function of endothelium and insulin sensitivity. Thus, until now, KAATSU training has been widely used in healthy subjects and athletes, and has also been applied to various kinds of conditions such as orthopedic diseases, obesity and diabetes. However, the present state of KAATSU training remains

unclear.

Apart from the beneficial effects of KAATSU training, the pressurization of blood vessels may cause the formation of thrombus, and induce microvascular occlusion (no-flow phenomenon) even after releasing blood flow restriction, resulting in muscle cell damage and necrosis (Harmon et al., 1948; Strock & Majno, 1969; Kawada, 2005). Therefore, we are concerned about the formation of thrombus, muscle damage, and the occurrence of pulmonary embolism in the subjects, when KAATSU training is applied. In addition, although KAATSU training restricts venous flow, induces the pooling of venous blood, and subsequently reduces cardiac preload during exercise, which may be useful in rehabilitation in patients with cardiac diseases (Takano et al., 2005a, b; Iida et al., 2005), the excessive inhibition of cardiac preload may decrease cardiac output, and thereafter blood flow to the brain and coronary circulation. Consequently, side effects such as dizziness, fainting, cerebral anemia and the deterioration of ischemic heart disease may occur. But, detailed reports about the side effects of KAATSU training have not yet been reported.

Therefore, to recognize the present state of KAATSU training and its side effects, we mailed questionnaires and obtained results from KAATSU leaders or instructors in a total of 105 facilities where KAATSU training has been adopted, and described them in this paper.

METHODS

Subject

Facilities where KAATSU training has been adopted participated in this study (see the appendix). We mailed questionnaire about KAATSU training to a total of 195 facilities in Japan, which had qualification of KAATSU leaders or instructors. The questionnaire asked about the distribution of age, training methods, training periods, side effects etc as described in Appendix 1.

RESULTS

We obtained an answer from KAATSU leaders or instructors in 105 out of 195 facilities (approximately 53%). As shown in Fig.1A, the number of bonesetter's and osteopath's offices and training gyms is larger than that of hospitals/clinics and rehabilitation centers.

Fig. 1B shows the period since the facilities have started KAATSU training. Most of the facilities (84%) have started KAATSU training within 5 years. Until now, total 12,642 persons have been received it (male



45.4%, female 54.6%).

Fig. 2 shows the age distribution of persons who have received KAATSU training in 105 facilities. The training was distributed over a wide range of ages. Persons aged under 20 years old accounted for 17.8%, and persons aged over 70 years old accounted for 14.6% of the total. Thus, the KAATSU training appears to be widely applied to all ages.

Fig. 3 shows the number of the facilities classified by the object of the training (Fig. 3A) and the purpose of the use of KAATSU training with regard to



Figure 2. The age distribution of persons who have received KAATSU training in a total of 105 facilities.



Figure 1. A: The kinds of the facilities (n=105), who participated in this study. B: The distribution of the period since the facilities introduced KAATSU training.

Figure 3. The object of the KAATSU training in each facility (A) and the symptom aimed to be improved with the use of KAATSU training (B). Numbers of facilities are indicated in each figure.

symptoms (Fig. 3B). As illustrated in Fig. 3A, KAATSU training has been applied to various kinds of conditions; sports, healthy persons, cerebrovascular diseases, orthopedic diseases, obesity and countering aging. In addition, it has been also used for training patients with cardiac diseases, muscle diseases, diabetes, hypertension and respiratory diseases. Thus, KAATSU training is used for training persons with various kinds of physical conditions. The purpose of the use of KAATSU training by the various facilities was as follows (Fig. 3B); improvement of muscle strength or countering muscle atrophy, improvement of paralysis, build-up of bone-structure, reduction of obesity and prevention of old persons from becoming bedridden. Other purposes include improvement of the symptoms of Berger's disease, ateriosclerotic obliterans (ASO), bone atrophy, lumbago and shoulder stiffness, relief of pain including menstrual pain, countering aging, and maintenance of health.

The total number of persons per year who have received KAATSU training is shown in Fig. 4A. The annual number of visits to the facilities for KAATSU training was about 31,754, and the mean annual



number of visits in one facility was 345. KAATSU training has been applied to athletes (n=5,311), orthopedic patients (2,776), the elderly (5,382) and healthy people (15,284) as shown in Fig. 4B. Thus, KAATSU training has been mainly used for



Figure 5. A: The distribution of type of exercises combined with KAATSU training in each type of facility. B: The distribution of part of body pressurized with KAATSU in each type of facility.



Figure 4. A: The annual number of visits in each type of facility. Mean numbers per facility and total numbers are indicated. B: The improvement with KAATSU training expected by subjects. Numbers of persons classified by expected improvement with KAATSU training are indicated.

Figure 6. Training hours distribution of each KAATSU training.






Figure 7. A: Frequency of KAATSU training in each type of facility. B: Period distribution of KAATSU training in each type of facility.

Figure 9. The most frequent side effects of KAATSU training.

Table 1.	Side effects	of KAATSU	training and	their occur	rence in each	n type of facility.
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	Total	Hospitals and Clinics	Bonesetter's and osteopath's offices	Acuouncturist's and moxa-cauterizer's office	Rehabilitation centers	Training gyms	Others
Subcutaneoushemorrhage	1651	156	300	86	2	1105	2
Numbness	164	6	67	42	1	48	
Cerebral anemia	35	10	3	21			1
Coldfeeling	16	2	10	1		3	
Venousthrombus	7	1	3	3			
Pain	5	1	4				
Itch	3					3	
Deteriorationofischemicheart disease	2		2				
Feeling sick	2		1			1	
Increasingofbloodpressure	2		1				1
Physical weariness	2		1	1			
Dizziness	2	2					
Pulmonaryembolism	1		1				
Rhabdomyolysis	1		1				
Palpitation	1		1				
Nosebleed			1				
Deteriorationofdiabetic retinopathy	1		1				
Fainting	1		1				
Cerebral infarction	1		1				
Hypoglycemia	1	1					
Edema	1	1					
Chafe	1	1					



strengthening of muscle in the field of sports, and health promotion in elderly and healthy subjects.

KAATSU training can be combined with various types of exercises. Fig. 5A shows types of exercises used with KAATSU training. As shown in Fig. 5A, it has been applied to stretch exercise, walking, cycling, and weight training. Many facilities have used both legs and arms as the sites of KAATSU (Fig. 5B). Figs. 6 and 7 show the percentage of the facilities classified by the training hours, frequency and duration of KAATSU training. Most of the facilities have been applying 5-30 min KAATSU training each time (Fig. 6), and have been doing it 1-3 times a week (Fig. 7A). In many cases, KAATSU training has been continued for at least 3-12 months as shown in Fig. 7B.

The reasons why subjects ended KAATSU training were as follows: improvement of their diseases or symptoms, achievement of goals, end of previously planned period, and shift to training at home. In 30 facilities, subjects have been continuing KAATSU training without stopping it. Fig. 8 shows the proportion of the facilities that are satisfied with the effects of KAATSU training (approximately 80%).

Fig. 9 and table 1 summarize the side effects of KAATSU training. The occurrence ratio of side effects was as follows: subcutaneous hemorrhage (13.1%), numbness (1.297%), cerebral anemia (0.277%), cold feeling (0.127%), venous thrombus (0.055%), pulmonary embolism (0.008%), rhabdomyolysis (0.008%), deterioration of ischemic heart disease (0.016%) (Fig. 9 and table 1). In addition, fainting has occurred in rare cases, and hypoglycemia has been reported.

DISCUSSION

The major findings of the present study obtained from 105 out of 195 facilities (approximately 53%) are as follows: 1) KAATSU training has been used for a wide generation of people including the young (under 20 years old) and the elderly (over 80 years old), and has mainly been used for strengthening of muscle in the field of sports and health promotion in healthy subjects; 2) It has been also applied to various kinds of conditions; cerebrovascular diseases, orthopedic diseases, obesity, cardiac diseases, neuromuscular diseases, diabetes, hypertension, and respiratory diseases; 3) It can be combined with various types of exercises (e.g., walking, cycling, and weight training). In most of the facilities, subjects have received 5-30 min KAATSU training each time, 1-3 times a week; and 4) approximately 80% of the facilities are satisfied with the results of KAATSU training and there have been only a small number of side effects. Thus, KAATSU training is a safe and promising method of training not only for athletes, but also for ordinary people, and it can also be applied to persons with various physical conditions.

KAATSU training is a novel method for muscle training (Sato, 2005). Under conditions of restricted muscle blood flow, even short-term and low-intensity exercise can induce muscle strength and hypertrophy (Takarada et al, 2000b; Takarada et al., 2002; Takarada & Ishii, 2002; Yasuda et al., 2004; Abe et al., 2005a, b). Therefore, as shown in the present study, many facilities have introduced KAATSU training for strengthening muscle in the fields of sports. The field of sports is indeed widespread, such as hockey, kendo, mountain climbin, jet skiing, triathlon, rugby, gymnastics, softball, handball, rock climbing, table tennis, badminton, marathon, skating, bicycle [cycle] race, skiing, snowboarding, bodybuilding, aerobics, judo, swimming, dance, basketball, tennis, volleyball, boxing, soccer, karate, golf, baseball, and combative sports. KAATSU training is also applied for health promotion in healthy subjects, even in the elderly. In addition, it has also been used to improve muscle strength in various conditions, including cerebrovascular diseases, respiratory diseases, orthopedic diseases, neuromuscular diseases, cases of being bedridden, and effects of old age.

Moreover, it is likely that KAATSU training is a useful method for training patients with a variety of diseases such as diabetes, hypertension and obesity. KAATSU training has additional effects, such as increasing GH secretion (Takarada et al., 2000a; Takano et al., 2005a, b; Sato et al., 2005). GH enhances lipolysis and bone formation, which may contribute to the reduction of obesity and bone diseases such as osteoporosis (Beekley et al., 2005). It also stimulates the liver to secrete insulin-like growth factor-1 (IGF-1) (Abe et al., 2005a), resulting in improvement of function of the endothelial cells and insulin sensitivity. Considering such effects of KAATSU training, some facilities have used KAATSU training for these patients.

In KAATSU training, various types of exercises can be used. In the present study, 30% of the facilities have used only KAATSU training. Even only KAATSU can prevent muscle atrophy observed in patients at bed rest (Takarada et al., 2000c). However, in most of facilities, various types of exercises (walking, cycling, and weight training) have been combined with KAATSU training. It is especially interesting to note that 70% of the facilities have used walking combined with KAATSU (commonly called "KAATSU walking"). Abe et al (2005b) reported that KAATSU walking can enhance GH responses, and then induce muscle strength and hypertrophy. In addition, no special machine is required in case of KAATSU walking. Therefore, KAATSU walking can be more widely adopted. Concerning duration and frequency, subjects have completed 5-30 min KAATSU training each time, 1-3 times a week in most of the facilities. However, since KAATSU training produces minimal muscle damage (Takarada et al., 2000a; Abe et al., 2005a), less recovery time is necessary, which means that the frequency of KAATSU training can therefore be increased according to the degree of its effect.

The most noteworthy finding is that the number of severe side effects due to KAATSU training is very low based on this survey results. The most frequent side effect was subcutaneous hemorrhage. It has been observed in 13.1% of cases, and more frequently occurred in arms than legs. However, subcutaneous hemorrhage is usually transient and diminished as the time goes on, even if training is continued. Therefore, subjects can continue the training with little cause for concern. Numbness has been observed in 1.297% persons who received KAATSU training, probably due to the compression of peripheral nerves of extremities. The numbress is also only temporary and abolished immediately after the release of KAATSU pressure. No cases of leg paralysis have occurred in any facility. Therefore, this symptom also does not interfere with KAATSU training. Several papers showed that KAATSU training combined with low-intensity exercise does not cause severe muscle injury, and there is no elevation of creatine phosphokinase (CPK) (Takarada et al., 2000a; Abe et al., 2005a) or rhabdomyolysis, as compared with heavy exercises (Chiu et al., 1976; Sorichter et al., 1999; Clarson & Hubal, 2002). In fact, rhabdomyolysis was noted in only 1 case.

Occlusion of blood vessels sometimes causes the formation of thrombus, and induces microvascular occlusion (no-flow phenomenon) even after releasing blood flow restriction, resulting in muscle damage and cell necrosis (Harmon et al., 1948; Strock & Majno, 1969; Kawada, 2005). However, fortunately, the incidence of serious side effects was low. Venous thrombus was observed in 7 cases (0.055%). Pulmonary embolism was noted in only 1 case (0.008%), but this subject had not been diagnosed definitely and had not serious problems. Recently, Tanimoto et al (2005) showed that the muscle oxygenation level during exercise with KAATSU training, measured with the near-infrared continuous-wave spectroscopy, was larger than that observed in completely-occluded conditions. Thus, it is likely that the blood flow is not completely restricted under the usual conditions with KAATSU exercise, which may help to avoid the severe side effects such as venous thrombus, pulmonary embolism and rhabdomyolysis.

Dizziness, fainting and cerebral anemia were observed in some cases as shown in table 2. Also, the deterioration of ischemic heart disease has been described in rare cases (2 cases). The conceivable reason is as follows; Application of KAATSU training on both legs caused the pooling of venous blood with the pressure-dependent reduction of femoral arterial blood flow (Iida et al., 2005). The inhibition of venous return during the KAATSU exercise reduces cardiac preload during the exercise, which may be beneficial in rehabilitation of certain patients with cardiac diseases (Takano et al., 2005a, b). On the other hand, the excessive inhibition of cardiac preload decreases cardiac output, and subsequently blood flow to the brain and coronary circulation. Therefore, KAATSU training should be performed carefully in patients with cardiac diseases such as ischemic heart diseases, severe aortic stenosis and hypertrophic obstructive cardiomyopathy. Also, the excessive application of KAATSU pressure may cause larger increases in both blood pressure and catecholamine levels as compared with low-intensity exercise without KAATSU training (Takano et al., 2005a, b). Therefore, we also need to be cautious when treating patients with hypertension and after cerebral hemorrhage as well as cardiac diseases. To minimize the side effects and complications, the KAATSU leaders and instructors should determine the duration and degree of KAATSU training as well as KAATSU pressure carefully. Of course, the informed consent about the side effects including subcutaneous hemorrhage should be given to all persons receiving KAATSU training. However, comparing with heavy physical exercises, where mortality rates of 0 to 2.5% per 10,000 have been reported, especially in older persons (ACSM, 2000; Kallinen M, 2005), any fatal complications have not been occurred in KAATSU training. Thus KATSU training is a safe and promising method for training persons including older persons.

In conclusion, KAATSU training is a safe and promising method for training in the field of sports and healthy persons, and can also be applied to persons with various kinds of physical condition including cerebrovascular diseases, orthopedic diseases, obesity, cardiac diseases, neuromuscular diseases, diabetes, hypertension, and respiratory diseases.

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KATTSU Training Group, See the Appendix for a list of respondents.

Appendix 1. Questionnaire

1 About your facility

- 1-1 What kind of facility is yours?
 - (1) Hospitals and Clinics
 - 2 Bonesetters' and osteopath's offices
 - ③ Acupuncturist's and moxa-cauterizer's offices
 - 4 Rehabilitation
 - **⑤** Training gyms
 - 6 Others

1-2 When did your facility introduce KAATSU training?

- (1) < 1 year ago
- (2) $1 \sim 3$ years ago
- $3 \sim 5$ years ago
- (4) > 5 years ago

1-3

(1) How many KAATSU leaders does your facility have?

____ persons

(2) How many KAATSU instructors does your facility have? ____ persons

2 About your cases or patients

2-1 How is the distribution of age of your subjects who have ever taken KAATSU training in your facility?

<19 years	persons
$20 \sim 30$ years	persons
$30\sim40$ years	persons
$40 \sim 50$ years	persons
$50\sim 60$ years	persons
$60 \sim 70$ years	persons
$70 \sim 80$ years	persons
>80 years	persons

2-2 How is the proportion of sex (%) in your facility?

Male

Female

2-3 What is the object of KAATSU training in your facility?

_____ %

____ %

- Training or health promotion
 - 1 Athletes
 - (2) Astronauts
 - ③ Ordinary healthy persons
- Rehabilitation or prevention
 - 1 Cardiovascular disease
 - 2 Cerebrovascular disease
 - ③ Neuromuscular disease
 - ④ Orthopedic disease
 - (5) Diabetes mellitus
 - 6 Obesity
 - 7 Renal disease (including hemodialysis)
 - **8** Hypertension
 - 9 Depression
 - 10 The elderly
 - 1 Respiratory disease
 - 12 Others (
-) 2-4 What is the symptom aimed to be improved with the use of KAATSU training?
 - ① Muscle atrophy
 - ⁽²⁾ Hypertension
 - ③ Diabetes mellitus
 - **④** Paralysis
 - (5) Bone disease (build-up of bone-structure)
 - 6 Obesity
 - 7 Bedridden

- (8) Increase of muscle strength
- 10 Others (
- 2-5 How many visits are there in your facility a year?
- 3 About KAATSU training
- 3-1 What types of exercises are combined with KAATSU training?

)

- ① Only KAATSU
- ⁽²⁾ Physical exercise
- ③ Walking (including treadmill)
- (4) Cycling
- ⁽⁵⁾ Weight training
- ⁽⁶⁾ Others (
-) 3-2 What parts of subject's body do you apply KAATSU training?
 - Legs: persons
 - Arms: persons
 - Both legs and arms: persons
- 3-3 How often do your subjects visit your facility?
 - 1 Everyday
 - 2 2-3 times a week
 - ③ Once a week
 - (4) 2-3 times a month
 - (5) Once a month
 - (6) Others (
-) 3-4 How is the distribution of the duration to treat each subject in your facility?
 - (1) < 3 months ____% 2 3-6 months _____% ③ 6 months - 1 year _____ % ④ 1-3 years _____ %
 - (5) > 3 years %
- 3-5 How long is each training period in your facility?
 - (1) < 5 minutes
 - (2) 5-10 minutes
 - ③ 10-20 minutes
 - (4) 20-30 minutes
 - **(5)** > 30 minutes
- 4 About effect of KAATSU training
- 4-1 How large is the proportion of your subjects who is satisfied with the effect of KAATSU training?
- 4-2 How many subjects have suffered from the side effect of KAATSU training?
 - _ persons

_ %

- 4-3 What is the side effect of KAATSU training that your subjects suffered from?
 - ① Cold feeling
 - (2) Numbness
 - ③ Subcutaneous hemorrhage
 - ④ Venous thrombus
 - ⁽⁵⁾ Pulmonary embolism
 - ⁽⁶⁾ Rhabdomyolysis
 - ⑦ Myocardial infarction
 - ⑧ Others (
- 4-4 To what point do you pay attention while you treat your subjects? _____ persons

)

(9) No particular symptom

Appendix 2

List of Respondents; Denenchofu-iin (clinic), Kohno Internal medicine clinic (clinic), Kamo hospital (hospital), Tokuyama-seikeigeka (orthopedic clinic), Kurihara seikeigeka (orthopedic clinic), Endo Kikyo Children's clinic (clinic), Konfogarden clinic (clinic), Ikujinkai Medical Corporation of Odagiri hospital (hospital), Social Insurance Shiga Hospital (hospital), Inoue hospital/clinic (hospital and clinic), Keiyu-seikeigeka (orthopedic hospital), Fukuda-seikeigeka (orthopedic clinic), Sata-seikeigeka hospital (hospital), Medical corporation Koueikai Inoue Orthopedic Surgery Clinic (clinic), Department of Ischemic Circulatory Physiology, KAATSU Training, University of Tokyo (hospital), Takeuchi-seikotsuin (osteopath's office), Synthetic Therapy & Conditioning Plaza (osteopath's and acupuncturist's office), Yoshimura-sekkotsuin/Kenshin-club (bonesetter's office), Yomeido Acupuncture & judo therapist clinic (osteopath's office), Kobayashi-seikotsuin (osteopath's office), WAKABA SEKKOTUIN (bonesetter's office), Nishiodo seikotuin (osteopath's office), Itosekkotsuin (bonesetter's office), Kusaka bonesetting office (bonesetter's office), Kuga bonesetter's office (bonesetter's office), Nemoto-sekkotsuin (bonesetter's office), Tanaka-seikotsuin (osteopath's office), Ekaku-Mizuno-seikotsuin (osteopath's office), Koga JUDO Therapy Office (osteopath's office), ARISAWA BONESETTER (bonesetter's office), Hayashi-sekkotsuin (bonesetter's office), GOKISO SEKKOTSUIN (bonesetter's office), Kanai-sekkotsuin (bonesetter's office), KOMATA BONESETTER'S OFFICE (bonesetter's office), Mizuno-sekkotsuin (bonesetter's office), Shonan-sekkotsuin (bonesetter's office), Magaribuchi Judo Therapy Centre, Yuri-seikotsuin (osteopath's office), KINOSITA SEIKOTUIN (osteopath's office), Mori-Shuichi-seikotsuin (osteopath's office), A spin treatment House (acupuncturist's and moxacauterizer's office), Athlete support/KAATSU training Toshima-branch (acupuncturist's and moxa-cauterizer's office), YUWA-acupuncture (acupuncturist's and moxa-cauterizer's office), Taiyo-shinkyuin/KAATSU training Fukui school (acupuncturist's and moxa-cauterizer's office), FURUYA ACUPUNCTURE (acupuncturist's and moxa-cauterizer's office), First step (acupuncturist's and moxa-cauterizer's office), TOKOROZAWA SPORTS CARE CENTER (acupuncturist's and moxa-cauterizer's office), RITTAI CHIROPRACTIC (acupuncturist's and moxa-cauterizer's office), MAEDA CHINESE MEDICAL/KAATSU training Osaka-Daito-shi-branch (acupuncturist's and moxa-cauterizer's office), Roppongi conditioning (acupuncturist's and moxa-cauterizer's office), Osaka-sakai-chiropractic (acupuncturist's and moxa-cauterizer's office), Inoue-conditioning (rehabilitation center), Armring Physical Plaza (rehabilitation center), Training center SUNPLAY (training gym), Maruyu-training-center (training gym), KAATSU-sports-seitai-seikotsusho (training gym), ATLAS (training gym), DO SPORTS. PLAZA (training gym), Training-college-Kubira (training gym), BODYCRAFT Sports Conditioning HIRATSUKA (training gym), KAATSU training Yotsukaichi-branch (training gym), Fitness-care-Hokuetsu (training gym), Asahi KAATSU Training Studio (training gym), Hoasen Sports Plaza (training gym), Able-fitness-club-Okayama (training gym), Asurie-sekkotsuin/Asurie-sports-club (training gym), GOLF & FITNESS CLUB S.I.H (training gym), Maruya-gym (training gym), TOTAL conditioning (training gym), KAATSU training Kiryu-branch (training gym), K Medical Training Center (training gym), KENKO PLAZA SKIP (training gym), Shinkyuin-Gen/TRAINING GYM Genkijuku (training gym), TAIL TOP (training gym), Conditioning & Cure support (training gym), B-sports (training gym), SPORTS CLUB NAS, ROX (training gym), Conditioning Club P.C.P (training gym), TIPNESS, TOKYU SPORTS OASIS (training gym), SPORTS GYM COSMOS Åitraining gym), Arugo Sports Service CO. Ltd. (training gym), Sato sports plaza, Taro Ikezaki, Niigata university, KAATSU training Kobe-Nishi-ku-branch, KAATSU training Tomakomai-branch, KAATSU training Okinawa-branch (Ryukyu university), KAATSU training Muroran-branch /Kyokushin-kaikan Hokkaido-Kimohuri-branch, Genkisports, Satoshi Tanabe, KAATSU training Oita-branch, KAATSU training Kashiwa-branch, Orange garden, SAGINUMA SWIMMING CLUB, AVISPA FUKUOKA, Noriko Konuma, SPORTS SHOP XEBIO DOME SAPPORO, KAATSU training Takasaki-branch (Miho Harada), and others.

Can KAATSU be used for an orthostatic stress in astronauts?: A case study

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The application of an orthostatic stress such as lower body negative pressure (LBNP) during exercise has been proposed to minimize the effects of weightlessness on the cardiovascular system and subsequently to reduce the cardiovascular deconditioning. The KAATSU training is a novel method for strength training to induce muscle strength and hypertrophy. KAATSU induces venous pooling of blood in capacitance vessels by restricting venous blood flow. Therefore, to investigate whether KAATSU can be used as an orthostatic stress, we examined the effects of KAATSU on the hemodynamic, autonomic nervous and hormonal parameters in one subject. The several parameters were measured by impedance cardiography; heart rate (HR), mean blood pressure (mBP), stroke volume (SV), cardiac output (CO), total peripheral resistance (TPR), and heart rate variability (HRV). These data were obtained before (pre), during and after (post) pressurization (50 and 200 mmHg) on both thighs with KAATSU mini belts, and compared with those in standing. The serum concentration of noradrenaline (NA) and vasopressin (ADH), and plasma rennin activity (PRA) were also measured. The application of 200 mmHg KAATSU decreased SV, which was almost equal to the value in standing. HR and TPR increased in a similar manner as standing with slight change of mBP. High frequency (HFr,R), a marker of parasympathetic nervous activity, decreased during both 200 mmHg KAATSU and standing, while LFRR/HFRR, a quantitative marker of sympathetic nervous activity, increased significantly. During KAATSU and standing, NA, PRA and ADH increased. These results indicate that the application of KAATSU on both thighs simulates systemic cardiovascular effects of orthostasis in one gravity (1G), and that KAATSU training appears to be a useful method for potential countermeasure like lower body negative pressure (LBNP) against orthostatic intolerance in space flight as well as strength training to induce muscle strength and hypertrophy.

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Key words: KAATSU training; lower body negative pressure; hemodynamics; cardiac output; autonomic function; power spectral analysis; space flight; deconditioning

INTRODUCTION

During space flights, several serious adaptive changes in cardiovascular function occur and consequently crew's health and safety are endangered. When gravitational hydrostatic gradients are abolished, there is a shift of intravascular fluid from the capacitance vessels of the legs and lower body centrally toward the head. Then, elevation of capillary blood pressure and increased capillary perfusion pressure in tissues of the head cause facial, intracranial edema and headache, which probably distresses astronauts or space travelers. After shortand long- duration spaceflights, some crew members experience orthostatic hypotension and reduced upright exercise capacity, which may be attributed in part to microgravity-induced hypovolemia, decreased baroreflex responsiveness, decreased skeletal muscle tone and increased venous compliance. On return to Earth, orthostatic intolerance is the most serious symptom of cardiovascular deconditioning, in addition, significantly reduced exercise capacities and increased resting heart rate are also observed (Blomqvist et al., 1994; Buckley et al. 1996). Accordingly, effective countermeasures during spaceflight are critical in order to maintain the cardiovascular system, as well as the musculoskeletal structure and function and ensure the well-being and safety of crew members during and after return to Earth. A passive countermeasure called "bracelets", which are specially designed elastic thigh cuffs developed in Moscow, has been reported to reduce cephalic edema and make the adaptation to zero gravity (0 G) more comfortable (Arbeille et al., 1995; Lindgren et al., 1998). This equipment contributes to reducing the edema and the venous stasis in the cephalic region by pooling blood into the vascular and extravascular compartment of the legs. Consequently, it can compensate partially for the cardiovascular changes induced by exposure to 0 G, but does affect the cardiovascular deconditioning induced by microgravity (Herault et al., 2000). It would seem that the most effective countermeasure regimen to prevent cardiovascular deconditioning would be a gravitation-like stress combined with exercise.

Lower body negative pressure (LBNP) can induce the retention of blood flow in lower extremities, and causes subsequent hemodynamic changes including autonomic nervous activities (Stevens and Lamb, 1965; Bonde-Petersen et al., 1984; Tomaselli et al., 1987; Lathers and Charles, 1993, Lucini et al., 2004). Until now, when combined with intensive exercise, it has been known to be a useful method to prevent such orthostatic intolerance after space flight, probably through its effect as orthostatic stimulus (Güell et al., 1992; Lee et al., 1997; Watenpaugh et al., 2000).

KAATSU training is a novel method for muscle training, originally developed by Sato (2005). Under the conditions of restricted muscle blood flow, even short-term, low-intensity exercises such as resistance training and walking can induce increased muscle strength, hypertrophy and increased muscle mass (Takarada et al, 2000a; b, c; Abe et al., 2004). Recently, KAATSU training has been used to improve muscle mass and strength in patients with cardiovascular and orthopedic diseases as well as healthy subjects and athletes in Japan (Nakajima et al., 2006). Since KAATSU can decreases cardiac output (CO) and stroke volume (SV), due to the pooling of blood into legs and inhibition of venous return (Iida et al., 2005), it may be an effective and unique method for applying an orthostatic stress during spaceflight.

In the present study, to investigate whether

KAATSU can be used as an orthostatic stress, we examined the effects of KAATSU on the hemodynamic, autonomic nervous and hormonal parameters in one subject.

METHODS

Subject

The subject was a normal healthy adult male, aged 34 years. His height was 172.5 cm and his weight was 59.4 kg. He had no disease. This study was approved by the ethics committee of the University of Tokyo.

Experimental studies

All studies were performed in the afternoon at least 4 h after lunch. An indwelling heparin-lock catheter was inserted into the superficial antecubital vein of left arm. After a 30 minutes rest in a supine position, control blood samples were collected. Then, after taking rest measurements of hemodynamic parameters in this position for 3 minutes by using an impedance method (see below), both legs were pressure-applied with a specially-designed belt, named 'KAATSU mini' in Japanese (Fig. 1, see below). During KAATSU, the hemodynamic parameters were continuously monitored (Fig. 2). Then, after 15 minutes of KAATSU (50 mmHg), the banding pressure was released and the hemodynamic parameters were taken again during a 5-10 minutes recovery period. Blood samples were obtained at 0 to 1 minute and 10 minutes after the KAATSU. All blood samples were processed to serum or plasma before storage at -20 °C until analysis. After more than an hour recovery time, a similar study was done at a KAATSU pressure of 100-200 mmHg. Finally, after a 30 minutes rest time, the hemodynamic



Figure 1. KAATSU Mini apparatus and mini belt (60 mm in width and 605 mm in length) developed in September 2005.



Figure 2. A healthy person wearing the apparatus of KAATSU mini and impedance cardiography. Both thighs were pressure-applied by a special-designed belt, named KAATSU mini.

parameters were taken at rest and during standing (5 minutes). The blood samples were also taken at rest and 5 minutes after standing.

Reduction of femoral muscle blood flow by KAATSU

A method for inducing the reduction of muscle blood flow as shown in Fig. 2 is somewhat similar as previously reported (Takarada et al., 2000a, b, c; Takano et al., 2005a, b). Both sides of subject's thighs were pressure-applied at the proximal ends by means of specially designed belts named KAATSU belts (Fig.1) to restrict venous blood flow and cause pooling of blood in capacitance vessels distal to the cuff, and restrict arterial blood flow. In this study, a KAATASU mini belt, recently developed, was used. The subject did not complain of any side effects.

Measurement of hemodynamic parameters

To evaluate hemodynamic parameters, the Task Force Monitor (CNSystmes Medizintechnik, Graz, Austria) (Gratze et al., 1998; Fortin et al., 1998), which includes surface electrocardigrams (ECG), impedance cardiography (ICG), beat-to-beat blood pressure by vascular unloading technique (Penaz, 1973) and oscillometric blood pressure were sampled at 1,000 Hz. These data were then used to calculate online all hemodynamic parameters, which induced heart rate (HR), mean blood pressure (mBP), SV, CO and total peripheral resistance (TPR). The calculation of CO and TPR was as follows;

CO=SV*HR

TPR=mBP*80/CO

Histograms of RR intervals were computed and pseudo-digitized at 10 samples per second. Autoregressive modeling (Burg method) was used to construct frequency domain spectrograms of the heart rate variability (HRV) (Bailey et al., 1994; Burklow et al., 1999). Parameters extracted from the variability spectra were low-frequency power (LF_{RR}, 0.03 to 0.15 Hz) and high-frequency power (HF_{RR}, 0.16 to 0.50 Hz), normalized to total power over the range from 0.01 to 0.50 Hz. LF_{RR}/HF_{RR} have previously been demonstrated to measure changes in sympathetic activity (Malliani et al., 1991). These data were obtained every one beat, and data are expressed as mean \pm S.E.M.

Measurement of noradrenaline, plasma renin activity and vsasopressin

Blood samples for hormone determination (7ml) were collected in pre-heparinised syringes. Blood was drawn into test tubes containing 10.5 mg of EDTA-2Na. Samples were kept in ice-cold water and centrifuged (3000 rpm) for 10 minutes and the plasma stored at -20°C until the assays were

performed. Plasma concentrations of noradrenaline (NA) were measured using high performance liquid chromatography (HPLC) method. The lower limit of detection of the assay was 6 pg/ml. Plasma renin activity (PRA) was measured by the Radioimmunoassay (RIA) method with a lower detection limit of 0.1 ng/ml/hr. Vasopressin (ADH) was also determined by RIA method. The lower limit of detection of the assay was 0.2 pg/ml.

RESULTS

Hemodynamic responses to the restriction of femoral blood flow by KAATSU

Figures 3 and 4 show the time course of SV and HR from standing as well as from the applicaton of pressure on both legs as determined by impedance cardiography. Table 1 shows the hemodynamic changes following standing and the 2 KAATSU conditions (50 and 200 mmHg). As shown in Fig. 3A, immediately after beginning of standing SV rapidly decreased and reached a steady-level within several minutes (from 71.71 ± 0.14 ml to 56.87 ± 0.17). Also pressurization of 50 and 200 mmHg gradually decreased SV (62.30 ± 0.07 ml at 50 mmHg and 57.50 ± 0.10 at 200 mmHg) within several minutes, and the decreased SV reached a quasi-steady state level. SV at 200 mmHg KAATSU (Fig. 3C, Table 1) was much lower than that at 50 mmHg and at standing position. The decrease in SV continued during the application of KAATSU. After the release of pressure, SV rapidly returned to the pre test level within several minutes.

After an orthostatic stress (standing), HR promptly increased with decreasing SV (Fig. 4A). Pressurization of 50 mmHg (Fig. 4B) did not affect HR. On the other hand, 200 mmHg KAATSU increased HR gradually from 64.89 ± 0.25 bpm at supine rest to 75.34 ± 0.24 . After the release of pressure, HR returned to the pre test level (69.16 ± 0.27 bpm) within several minutes. MBP decreased slightly during standing (from 94.18 ± 0.29 mmHg to 88.60 ± 0.82). But the pressurization of both 50 and 200 mmHg increased mBP (from 81.69 ± 0.34 mmHg to 88.10 ± 0.55 at 50 mmHg, and from 90.04 ± 0.20 to 94.98 ± 0.40 at 200 mmHg).

CO decreased during standing (from 4.83 ± 0.02 l/min at supine rest to 4.19 ± 0.02). The pressurization of KAATSU (50 and 200 mmHg) also decreased CO as shown in Table 1, but the degree of change in CO did not differ much between 50 and 200 mmHg because of the compensatory increase in HR for the decrease in venous return followed by the decrease SV. TPR increased during standing and pressurization. Standing increased TPR from 1515.74 \pm 8.53 dyne*sec/cm-5 to 1638.30 \pm 18.37. The pressurization of 50 and 200 mmHg also increased TPR from 1378.78 \pm 5.12 to 1811.69 \pm 9.36 at 50



Figure 3. Effects of orthostatic stress (standing) and KAATSU on stroke volume (SV). The time courses of SV before and during standing (A) or the pressurization of both legs by KAATSU (50 (B) and 200 mmHg (C)) and after the release of KAATSU. Note that pressurization of both legs by KAATSU gradually decreased and reached a quasi-steady state level within several minutes, and the decrease in SV continued during application of KAATSU. After the release of KAATSU, SV returned to a control level immediately.

Figure 4. Effects of orthostatic stress (standing) and KAATSU on heart rate (HR). The time courses of HR before and during standing (A) or the pressurization on both legs by KAATSU (50 (B) and 200 mmHg (C)) and after the release of KAATSU. Note that HR increased during orthostatic stress (standing). HR also increased during the pressurization of both thighs at 200 mmHg, but not 50 mmHg. Immediately after the release of KAATSU, the increased HR returned to a control level.

Table 1.	Hemodynamic	parameters during	standing, and	KAATSU (50 and 200 mmHg)	
----------	-------------	-------------------	---------------	----------	------------------	--

	HR	mBP	SV	CO	TPR
	(bpm)	(mmHg)	(ml)	(l/min)	(dyne*sec*cm ⁻⁵)
pre	67.48±0.45	94.18±0.29	71.71±0.14	4.83±0.02	1515.74±8.530
standing	73.80±0.28	88.60±0.82	56.87±0.17	4.19±0.02	1638.30±18.37
pre	66.53±0.23	81.69±0.34	76.37±0.17	5.07±0.02	1378.78±5.120
50 mmHg	65.22±0.12	88.10±0.55	62.30±0.07	4.06±0.01	1811.69±9.360
post	67.48±0.25	83.75±0.43	71.71±0.14	4.83±0.02	1515.74±8.530
pre	64.89±0.25	90.04±0.20	75.69±0.16	4.90±0.02	1293.78±10.04
200 mmHg	75.34±0.24	94.98±0.40	57.50±0.10	4.33±0.01	1585.17±11.67
post	69.16±0.27	94.18±0.29	69.63±0.17	4.80±0.02	1349.11±10.07

mmHg and from 1293.78 \pm 10.04 to 1585.17 \pm 11.67 at 200 mmHg.

Autonomic nervous responses to the restriction of femoral blood flow by KAATSU

Figure 5 depicts the changes in the power spectra of heart rate variability (HRV) in control (pre), during 200 mmHg KAATSU, after the release of KAATSU and subsequently during standing. The HF_{RR}

component, which is a marker of parasympathetic activity, was reduced by both 50 and 200 mmHg KAATSU (Fig. 6A). On the other hand, LF_{RR}/HF_{RR} component was increased by both 50 and 200 mmHg as shown in Fig. 6B. HF_{RR} markedly decreased from 55.30 \pm 0.38 % to 34.64 \pm 0.25 and LF_{RR}/HF_{RR} increased from 1.18 \pm 0.02 before KAATSU to 4.55 \pm 0.12 at 200 mmHg KAATSU.



Figure 5. The time courses of heart rate variability (HRV) during KAATSU (200 mmHg and standing). Note that during KAATSU (200 mmHg), LF_{RR} increased, compared with the control (shown by blue arrow), while HF_{RR} decreased (shown by red arrow) as standing.



Figure 6. Effects of orthostatic stress (standing) and KAATSU on the heart rate variability (HRV). A: Effects of orthostatic stress (standing) and KAATSU on HF_{RR}/LF_{RR}. B: Effects of orthostatic stress (standing) and KAATSU on HF_{RR}.

Hormonal responses to the restriction of femoral blood flow by KAATSU

Figure 7 depicts hormonal changes induced by pressurization and standing. NA, a well-known neurotransmitter released from sympathetic nerve, increased under the pressurization. 50 mmHg of KAATSU increased from 183 ng/ml to 229 ng/ml, and 200 mmHg increased from 239 ng/ml to 350 ng/ml. The standing also raised NA from 175 ng/ml to 321 ng/ml. PRA also was raised by both KAATSU



Figure 7. Effects of orthoststic stress (standing) and KAATSU on the concentration of noradrenaline (NA, A), plasma rennin activity (PRA, B) and the concentration of vasopressin (ADH, C). The blood was obtained before (supine rest), after 5 minutes standing and 15 minutes after the pressurization (50 and 200 mmHg), and 10 minutes after the release of KAATSU.

and standing. 50 mmHg of pressurization increased PRA from 1.4 ng/ml/hr to 1.9 ng/ml/hr, and 200 mmHg of KAATSU had more effect on PRA (from 1.3 ng/ml/hr to 2.2 ng/ml/hr). In addition, ADH was also increased during 5 minutes standing and during the application of KAATSU at 200 mmHg.

DISCUSSION

We studied the effects of KAATSU on the hemodynamic, autonomic nervous and hormonal system in one subject. Application of KAATSU on both legs induced the hemodynamic, hormonal and autonomic alterations that were similar to standing.

During an orthostatic stress (i.e. standing), part of the blood and plasma volume can pool in the capillary bed of the legs. Subsequently, SV decreased to about 79.3 % of the control in this case. Application of KAATSU on both thighs by a specially designed KAATSU mini belt also decreased SV by pooling blood into the vascular and extracellular compartment of the legs, which depends on the KAATSU pressure. The increasing cutaneous blood volume in the legs could be observed by the dark-red coloration of the skin in the lower body. At 200 mmHg, the decrease in SV (76.0 %) was almost equal to that observed in standing. The addition of KAATSU on both thighs gradually decreased SV, and reached to a quasi-steady level within several minutes. The decrease in SV remains stable during the application of KAATSU. This suggests that venous return is almost consistent during KAATSU. In other words, it is likely that the amount of arterial blood flow into the legs and the amount of venous flow out of the legs are almost equal under the pressurization. As previously reported, KAATSU suppresses the arterial blood flow to the legs in pressure-dependent manner (Iida et al., 2005), so in this case, at 200 mmHg of KAATSU, venous blood flow and therefore also arterial blood flow may not be completely blocked. Immediately after the release of KAATSU, the depressed SV promptly returned to the control level.

In the hemodynamic response to orthostatic stress, initially SV is decreased, but the decrease in SV is successfully compensated for by an increased standing HR and TPR via baroreceptor control of circulation. Since BP varies with both TPR and CO, no remarkable change of mean BP was found in standing. Even in cases of a set pressure of 50 mmHg, SV was decreased, and TPR was elevated. Mean BP and HR did not change significantly. It suggests that the primary mechanism responsible for sympathoexcitation during the low pressure is primarily due to the cardiopulmonary baroreceptors, which are located in the atria and ventricles of the heart and in the pulmonary artery and veins and are responsive to changes in central venous pressure (Furlan et al., 2001; Brown et al., 2003). On the other hand, application of 200 mmHg induced a much larger decrease in SV, with the increase in HR and TRP. Thus, during high pressure such as 200 mmHg, both arterial baroreceptors and cardiopulmonary baroreceptors were also unloaded. Regarding autonomic nervous response, HFRR/HFRR, a marker of sympathetic activity, and the serum concentration of NA, a well-known neurotransmitter released from sympathetic nerve, increased, which depends on the pressure of KAATSU. On the other hand, HFRR, a marker of parasympathetic activity, decreased. Overall, it seems likely that at low level of pressure the reduced venous return induces a cardiac unloading, resulting in an inhibition of cardiogenic sympathetic excitatory mechanisms and in an increased arterial baroreflex gain, while at high level the arterial baroreceptor unloading is the dominant phenomenon leading to sympathetic excitation. During an orthostatic stress (standing), part of the blood and plasma volume can pool in the legs owing to the shift to the capacitance vessels. The subsequent decrease in the plasma volume and renal blood flow stimulates the secretion of PRA and ADH. The secretion of PRA and ADH were also increased during KAATSU, which also depends on the degree of the pressure. Thus, it is likely that the application of 200 mmHg KAATSU on both thighs simulates systemic cardiovascular, autonomic nervous and hormonal effects of orthostatic in 1G (standing).

In spaceflight, without regular exposure to gravity (G) forces, the cardiovascular functions are

compromised. After short- and long- duration space flights, some crew members experience orthostatic hypotension and reduced upright exercise capacity named cardiovascular deconditioning and severe muscle atrophy (Blomqvist et al., 1994; Buckley et al., 1996; Fritsch-Yelle et al., 1996; Meck et al., 2001). The cardiovascular deconditioning may be attributed in part to microgravity-induced hypovolemia, decreased baroreflex responsiveness, decreased skeletal muscle tone and increased venous compliance. In addition, muscle atrophy produced greater compliance of lower limbs and a predisposition to orthostatic intolerance. To maintain the structure and function of musculoskeletal and cardiovascular systems during space flight, and to eusure well-being and safety of crew members during space flight and after return to Earth, effective countermeasures during space flight are critical (Convertino and Sandler., 1995; Nicogossian et al., 1995). A passive countermeasure called a "bracelet" developed in Moscow has been used to reduce this cephalic edema and make the adaptation to 0 G more comfortable (Arbeille et al., 1995; Herault et al., 2000). The bracelets are fixed at the upper part of each thigh and applying pressure of approximately 20-30 mmHg (where 1 mmHg =133.3 N/m²) on the skin. The bracelets were considered to reduce the venous return by trapping a significant amount of fluid into the lower-limb vascular and interstitial space, subsequently followed by the fluid shift (Lindgren et al., 1998). The KAATSU apparatus can apply the previously planned pressure to the cuff accurately. Thus, it is interesting to know whether the KAATSU with low pressure (20-50 mmHg) can prevent facial edema and make the adaptation to 0 G more comfortable than the Russian bracelets (Arbeille et al., 1995; Lindgren et al., 1998; Herault et al., 2000). Currently, astronauts practice 2-3 h of intensive exercise using treadmill, ergometer and resistance machines. These time-consuming countermeasures cannot completely prevent them from cardiovascular deconditioning. Therefore, alternate countermeasure strategies that are more effective and efficient are necessary. Now, it is likely that the most effective countermeasure regimen would be a gravitation-like stress combined with exercise. The present study indicates that KAATSU can provide this gravitation-like stress in 1G.

A human centrifuge is another possible candidate, but the centrifuge apparatus is relatively expensive and it is technically laborious to accommodate the apparatus on a space craft. In addition, it may be difficult to minimize cetrifugation-induced Coriolis effects on the vestibular system and the consequent motion sickness associated with onboard centrifuge. Therefore, at present, it is likely that exercise against the suction force produced by LBNP may provide a low mass and low-cost alternative procedure to stress the cardiovascular systems (Hargens, 1994). However, LBNP is difficult to apply when combined with several exercise, such as resistance exercises which needs large machines. Furthermore, LBNP without exercise by itself can not protect crew members from cardiovascular deconditioning.

LBNP has been known to induce the retention of blood flow in lower extremities (Wolthuis et al., 1974), and induce subsequent hemodynamic changes such as decreased SV and CO and increased TPR (Stevens and Lamb, 1965; Bonde-Peterson et al., 1984; Güell et al., 1990; Güell et al., 1992; Melchior et al., 1994; Murthy et al., 1994; Lee et al., 1997; Watenpaugh et al., 2000), and produce sympathetic activation and vagal withdrawal (Franke et al., 2000). As previously reported, 200 mmHg of KAATSU has almost equal effect on cardiovascular and autonomic nervous system to -40 - -50 mmHg LBNP (Iida et al., 2005). In addition, the present study clearly indicated that KAATSU simulates orthostatic effects (1G) on cardiovascular, autonomic nervous and hormonal system like LBNP at bed rest. KAATSU training was originally developed as a novel method for muscle training to strength muscle and induce muscle hypertrophy and increase muscle mass. Under the condition with restriction of muscle blood flow by KAATSU, even a short-term and low-intensity exercise can induce muscle strength, hypertrophy and increasing muscle mass (Takarada et al., 2000a; b; c; Takarada et al., 2002a, b; Abe et al., 2005; Yasuda et al., 2005; Abe et al., 2006). Up to now, several mechanisms underlying the effects of KAATSU training are proposed. First, under the ischemic condition with restriction of muscle blood flow by KAATSU, a larger number of fast-twitch muscle fibers are recruited, resulting in muscle hypertrophy. Second, a combination of anaerobic factors such as local ischemia and/or local accumulation of lactate in the legs induced by the restriction of muscle blood supply may stimulate peripheral afferent nerves, resulting in enhanced GH secretion (Takarada et al., 2000a; Takano et al., 2005 a, b). GH stimulates liver to secrete insulin-like growth factor-1 (IGF-1) (Abe et al., 2005). Both GH and IGF-1 can contribute to muscle hypertrophy. Thus, KAATSU training may be able to be used by astronauts in order to protect against both musle atropny and strength and cardiovascular deconditioning. Furthermore, KAATSU training can be easily applied to almost all types of exercises such as treadmill, ergometer, and resistance machines without any major complications (Nakajima et al., 2006). Thus, it is very likely that KAATSU training, if used under the suitable exercise protocol, may be a very promising method to counter symptoms of orthostatic intolerance and muscle atrophy in astronauts. But further studies are needed to clarify these interesting possibilities.

In conclusion, the application of KAATSU on both thighs simulates cardiovascular effects of orthostasis in 1G. The KAATSU training appears to be a useful method for potential countermeasure like LBNP against orthostatic intolerance in space flight as well as strength training to induce muscle strength and hypertrophy when combined with exercise.

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ORIGINAL ARTICLE

Resistance exercise combined with KAATSU during simulated weightlessness

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The application of a gravity-specific stress (e.g. LBNP), in combination with exercise, prevents cardiovascular deconditioning in space flight. KAATSU training is a method to induce blood pooling in capacitance vessels by restricting venous return (as with LBNP) and which when combined with lowintensity resistance (RE) exercise produces remarkable muscle mass and muscle strength gains. The purpose of this study was to investigate the hemodynamic and neurohumonal responses induced by KAATSU in combination with leg RE (30 % 1 RM), during simulated weightlessness (6° head-down tilt for 24 h, n=7). Following 24 h bed rest 6° head-down tilt, body mass was decreased from 75.3 ± 3.9 to 73.3 ± 3.8 Kg (P<0.01). Blood volume (BV) and plasma volume (PV) were reduced by -4.4 ± 1.4% and -7.9 ± 2.5%, respectively. During RE, BV and PV were significantly decreased; the changes with KAATSU induced a lower-body venous pooling, resulting in a sustained decrease in stroke volume (SV; from 77.0 ± 4.4 ml to 55.9 ± 5.1 ml; P<0.01) that was comparable to resting SV while standing. Consequently, RE heart rate (HR) was greater with KAATSU. The serum concentrations of plasma renin activity (PRA), vasopressin (ADH), noradrenaline (NOR), and lactate were also significantly elevated during RE with KAATSU as compared to control RE. These hemodynamic and neurohumoral responses following head-down tilt and during RE closely approximate the gravity-specific stress observed with LBNP. Thus, when used in combination with RE, KAATSU may be a useful countermeasure in microgravity.

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Key words: KAATSU training, leg press, bed rest, plasma renin activity, noradrenaline

INTRODUCTION

Astronauts practice 2-3 h intensive exercise in space. This time-consuming exercise training does not prevent astronauts from cardiovascular deconditioning, and alternative countermeasure strategies are recommended. The most effective regimen is a gravity-simulating stress such as lower body negative pressure (LBNP) or artificial gravity (Güell et al., 1992; Murthy et al., 1994; Watenpaugh et al., 2000; Iwase., 2005) combined with exercise modalities, e.g. treadmill or ergometer. However, these methods are difficult to use in conjunction with resistance exercise.

KAATSU training is a unique technique of lowintensity resistance exercise (RE) training performed in combination with restricted muscle blood flow that results in muscle hypertrophy and muscle strength gains comparable to those observed with highintensity resistance exercise training (Takarada et al. 2000a; b; c; Takarada et al., 2002a,b; Abe et al. 2006; Fujita et al., 2007). The utility of KAATSU training is that it can be applied to most types of exercise. Additionally, KAATSU femoral blood flow restriction induces lower-body venous pooling and reduces venous return (Iida et al., 2007; Nakajima et al., 2008). Thus, the addition of KAATSU to exercise appears to induce an orthostatic stress on the cardiovascular system as observed with LBNP plus exercises, which may be useful in preventing orthostatic intolerance. However, the hemodynamic and neurohumoral responses to exercises combined with KAATSU have not been investigated in a zero gravity environment. The aim of the present study was to investigate hemodynamic and neurohumoral responses to RE with and without KAATSU during the simulated weightlessness using head-down tilt bed rest (Sandler, 1986; Nicogossian, 1994).

MATERIALS AND METHODS Subjects

Seven healthy males (age, 31.6 ± 1.1 ; height, 1.76 ± 1.6 m; weight, 75.3 ± 3.9 kg) participated in this study. This study was approved by the institutional review board (IRB) of human research of Japan Aerospace Exploration Agency (JAXA) and the ethics committee of the University of Tokyo. All volunteers had no prior experience with RE and informed consent was obtained prior to the study. The subjects were paid by the grant from JAXA for participating in this study. None of the subjects had any diseases nor took any medications.

Protocol

Subjects maintained a 24-hour period of bed rest in -6° head-down tilt position (-6° bed rest). Transportation and toilet procedures were restricted to the head-down recumbent position. Subjects were allowed to rest on their elbows during meals and could move volitionally but remained horizontal to the bed. Subject's diet, fluid intake, and urine volume were monitored.

After 24 h -6° bed rest, control blood samples were taken. Following bed rest subjects were randomly assigned to one of two groups: one group performed leg press RE without KAATSU, followed by a 2-h rest interval and then repeated the leg press RE with KAATSU (\sim 150-160 mmHg cuff pressure; Fig.1). The other group performed leg press RE with KAATSU followed by a 2-h rest interval and then repeated the leg press RE without KAATSU. Hemodynamic parameters and venous blood samples were collected prior to, during, and following RE. All exercise was performed in the head-down tilt position. Immediately following RE, the KAATSU was released and hemodynamic parameters and blood samples were again collected during a 5-10 min recovery period. After 60 min recovery, the subjects stood up and hemodynamic response was recorded continuously for 5 min in standing position.

Resistance Exercise Protocol

Subjects performed leg press RE on a speciallydesigned leg press machine with -6° head down tilt. Knee and hip range of joint motion during exercise was 0 to 90° (0° being full extension). RE consisted of 4 sets of leg press. The sets consisted of the following repetition pattern; 30 repetitions (1/3 s), 15 repetitions, 15 repetitions, 15 repetitions. There was a 1 min rest interval between sets. Contraction intensity was 30% of predetermined one-repetition was 3.0 sec with a 1.5:1.5 sec shortening-lengthening contraction duty cycle as controlled by a metronome (40 beats per min).



Figure 1. Experimental design. Subjects maintained 6° head-down tilt position during the bed rest period. After 24 h at -6° bed rest, subjects performed leg press exercises with and without KAATSU (see methods).

KAATSU Blood Flow Restriction

Femoral blood flow was impaired using the KAATSU technique which also restricts venous blood flow causing pooling of blood in capacitance vessels distal to the cuff and partial occlusion of arterial blood flow (Takano et al., 2005; Iida et al., 2007; Nakajima et al., 2008). KAATSU belts, specifically designed for space flight (65 mm in width and 650 mm in length), were applied to the proximal ends of both thighs as near to the hip joint as possible. The cuff pressure was $150 \sim 160$ mmHg, which was controlled by the KAATSU apparatus.

Cardiovascular Hemodynamics

Hemodynamic parameters were determined using the Task Force Monitor (CNSystmes Medizintechnik, Graz, Austria; Fortin et al., 1998; Gratze et al., 1998). Analysis included electrocardiogram (ECG), impedance cardiography, beat-to-beat blood pressure by vascular unloading technique (Penaz, 1973) and oscillometric blood pressure. Data were obtained every beat with a 1,000 Hz sampling rate and used to calculate all hemodynamic parameters in real time. Data included heart rate (HR; bpm), mean arterial blood pressure (MAP; mmHg), systolic blood pressure (sBP), diastolic blood pressure (dBP), stroke volume (SV; ml), cardiac output (CO; l·min⁻¹) and total peripheral resistance (TPR; dyn·s·cm⁻⁵). TPR was calculated in relative units as MAP·CO⁻¹, and the calculation of CO and TPR was as follows:

CO=SV·HR

TPR=MAP·80·CO⁻¹

Hormone Metabolite Levels

An indwelling catheter was inserted into the superficial antebrachial vein of left arm and was maintained patent with a heparin-loc solution. Blood samples were obtained before, 0-1 min and 10 min recovery period. Venous blood samples were collected and analyzed for hematocrit, hemoglobin, lactate, noradrenaline, plasma renin activity and vasopressin. For measurement of hemoglobin and hematocrit, 2 ml of blood was placed into test tubes containing EDTA-2Na. Blood hemoglobin (Hb; g·dl⁻¹) was determined by the cyanomethemoglobin method (Coulter hemoglobinometer) and hematocrit (Hct; %) by the micro-hematocrit ultra centrifugation technique. Plasma level of lactate was measured at S.R.L. Inc (Tokyo, Japan) by the use of an enzyme system employing lactate oxidase combined with Nethyl-N-(3-methylphenyl)-N'-acetyl ethylenediamine and an auto-analyzer, HITACHI Type 7170. For hormone determination, blood (7 ml) was placed in test tubes containing 10.5 mg of EDTA-2Na. All samples were kept in ice-cold water and centrifuged (3000 rpm) for 10 min and the plasma stored at -20° C until the assays were performed.

Plasma concentrations of noradrenaline (NOR; lower limit of detection 6 pg·ml⁻¹) were measured using high performance liquid chromatography. Plasma renin activity (PRA; lower limit of detection 0.1 ng·ml⁻¹·h⁻¹) and vasopressin (ADH; lower limit of detection 0.2 pg·ml⁻¹) were determined by radioimmunoassay. These assays were completed at commercially available laboratories (SRL Inc., Tokyo, Japan).

Changes in blood and plasma volume (%) were derived from the following equation (Iida et al., 2007):

$$\begin{split} BV_{B} \cdot BV_{A}^{-1} &= Hb_{A} \cdot Hb_{B}^{-1} \\ \% \Delta PV &= 100 \cdot ((Hb_{B} \cdot Hb_{A}^{-1}) \cdot ((1 - Hct_{A} \cdot 10^{-2})/(1 - Hct_{B} \cdot 10^{-2})) - 100 \end{split}$$

where A is the initial value and B is the value at the corresponding time.

Data Analysis

All values are expressed as means \pm S.E.M. Student's paired *t*-test and one-way ANOVA for repeated measures were used, and differences were considered significant if P < 0.05.

RESULTS

Table 1 shows the changes of various parameters following bed rest. Body mass significantly decreased from 75.3 \pm 3.9 Kg (Pre, 0 h bed rest) to 73.3 \pm 3.8 Kg (n=7, P<0.01) 24 h after -6° bed rest. Hct significantly increased from 46.4 \pm 1.2% to 48.5 \pm 0.8% (n=7, P<0.01). Blood volume and plasma volume decreased by -4.4 \pm 1.4% and -7.9 \pm 2.5% (n=7), respectively. The urine volume (2052 \pm 249 ml·d⁻¹) markedly exceeded the volume of water intake (1320 \pm 67 ml·d⁻¹), suggesting that the central fluid shift of blood induced by -6° bed rest enhanced

Table 1. Changes of various parameters during 24 h bed rest

Parameters	0 h bed rest	24 h bed rest
Body weight (Kg)	75.3 ± 3.9	73.3 ± 3.8**
Water balance intake (ml)		1320 ± 67
Urine volume (ml)		2052 ± 249
Hct (%)	46.4 ± 1.2	$48.5 \pm 0.8*$
Hb (mg/dl)	15.0 ± 0.3	$15.7 \pm 0.3*$
Blood volume (%)		-4.4 ± 1.4
Plasma volume (%)		-7.9 ± 2.5
PRA (ng/ml/h)	1.51 ± 0.48	0.86 ± 0.18
ADH (pg/ml)	1.81 ± 0.26	1.50 ± 0.12
NOR (pg/ml)	201 ± 45	157 ± 24
Dopamin (p g/ml)	7.1 ± 1.5	5.4 ± 0.4
HR (/min)	59.2 ± 4.0	58.9 ± 3.7
sBP (mmHg)	121.9 ± 5.1	126.0 ± 5.6

*P<0.05 vs. 0 h bed rest **P<0.01 vs. 0 h bed rest

urine volume, resulting in the loss of plasma volume and an increase in Hct. The serum concentration of PRA and ADH tended to decrease during -6° bed rest for 24 h. NOR and dopamine tended to decrease following -6° bed rest, but was not statistically significant. HR and sBP did not significantly change.

Fig 2 summarizes the effects of leg press RE with and without KAATSU on hemodynamic parameters. The increase in HR with RE was significantly greater with KAATSU than without KAATSU (Fig. 2A). The peak HR during exercise reached to 107.1 ± 9.4 bpm (Fig. 2B). sBP (Fig. 2C), dBP, and mBP increased (P<0.01) during KAATSU exercise, and reached peak values of 154.2 ± 8.2 mmHg, 99.2 ± 6.0 mmHg, and 116.1 \pm 6.5 mmHg, respectively (Table 2). In leg press RE with KAATSU, SV significantly decreased from 77.0 ± 4.4 ml to 55.9 ± 5.1 ml (P<0.01) compared with the exercises without KAATSU (Fig. 2D). The level of SV during KAATSU was approximately equal to that in standing. During leg press RE with KAATSU, CO increased from $4.8 \pm 0.6 \text{ l} \cdot \text{min}^{-1}$ to $5.8 \pm$ 0.6 l·min⁻¹ (P<0.01; Fig. 2E). During RE without KAATSU, CO increased from $4.6 \pm 0.4 \text{ l} \cdot \text{min}^{-1}$ to $6.0 \pm$



Figure 2. Effects of leg press exercises with and without KAATSU on the hemodynamic parameters (HR (B), sBP (C), SV (D), CO (E), TPR (F)). The changes of HR during exercises are indicated in A. Hemodynamic parameter in control (Pre, 24 h bed rest), at peak exercise (EX) and 10 min after the exercises (Post) with and without KAATSU. Effects of standing on these parameters are also indicated before and after standing.*p<0.05, **p<0.01 vs. control (pre). Significant differences between exercises with and without KAATSU are also shown.

		Pre	Peak exercise	Post
HR (bpm)	(-)	61.3 ± 4.9	85.6±5.7** - **	$69.4 \pm 6.1 **$
	(+)	61.9 ± 4.9	107.1 ± 9.4**	$81.7 \pm 8.1 **$
SV (ml)	(-)	76.4 ± 5.7	70.9 ± 6.6	71.7 ± 4.9
	(+)	77.0 ± 4.4	55.9 ± 5.1** —	67.7 ± 5.8
CO (l/min)	(-)	4.6 ± 0.4	$6.0 \pm 0.5^{**}$	4.9 ± 0.4
	(+)	4.8 ± 0.6	$5.8\pm0.6^{**}$	5.3 ± 0.4
sBP (mmHg)	(-)	124.8 ± 6.0	$139.6 \pm 8.6 **$	126.6 ± 9.2
	(+)	127.5 ± 4.9	$154.2 \pm 8.2^{**}$	130.3 ± 5.9
dBP (mmHg)	(-)	79.8 ± 4.8	$90.8 \pm 5.4^{**}$	79.3 ± 5.8
	(+)	96.4 ± 4.3	$99.2 \pm 6.0 **$	93.5 ± 4.6
mBP (mmHg)	(-)	92.9 ± 5.1	$105.7 \pm 6.4 ^{**}$	92.4 ± 7.1
	(+)	77.0 ± 4.4	116.1±6.5**	78.5 ± 4.3
TPR	(-)	1672 ± 238	$1488 \pm 221 **$	1586 ± 263
(dyne*s/cm ⁻⁵)	(+)	1666 ± 179	1655 ± 192	1431 ± 131

Table 2. Hemodynamic responses to acute leg-press exercise in healthy volunteers after 24 h-bed rest

*P<0.05 vs.Pre **P<0.01 vs. Pre *P<0.05, **P<0.01 KAATSU (-) vs.

KAATSU (+)

0.5 l·min⁻¹ (P<0.01). The increase in CO was not statistically different between both exercises. TPR did not change significantly at the peak exercises with and without KAATSU (Fig. 2F).

Fig 3 summarizes the effects of leg press RE with and without KAATSU on neurohumoral parameters. Following RE, there was a significant increase in Hct (Fig.3A) and Hb which was greater during KAATSU. These changes reflected a significant decrease in BV and PV that was greater during KAATSU (Fig.3B). The increase in lactate concentration after exercise with KAATSU was much higher than that without KAATSU (Fig. 3C). In leg press exercises with KAATSU, NOR increased from $140 \pm 20 \text{ pg} \cdot \text{ml}^{-1}$ at rest to $514 \pm 110 \text{ pg} \cdot \text{ml}^{-1}$ (P<0.01) immediately after the exercise, and gradually decreased after the exercise (Fig. 3D). On the other hand, it increased from $131 \pm 16 \text{ pg} \cdot \text{ml}^{-1}$ to $239 \pm 47 \text{ pg} \cdot \text{ml}^{-1}$ (P<0.01) in the control exercise. Thus, the increase in NOR concentration attained in the leg press RE with KAATSU was significantly higher than that without KAATSU.

Figs. 3E and 3F show the effects of KAATSU on serum concentration of PRA and ADH. PRA significantly increased from $0.7 \pm 0.1 \text{ ng} \cdot \text{ml}^{-1} \cdot \text{h}^{-1}$ to $1.3 \pm 0.3 \text{ ng} \cdot \text{ml}^{-1} \cdot \text{h}^{-1}$ at 10 min after the RE without KAATSU (P<0.05), and ADH also tended to increase from $1.5 \pm 0.1 \text{ pg} \cdot \text{ml}^{-1}$ to $1.8 \pm 0.2 \text{ pg} \cdot \text{ml}^{-1}$ immediately after the exercises. PRA and ADH were more markedly raised by the application of KAATSU. The leg press exercises combined with KAATSU increased PRA from $1.0 \pm 0.2 \text{ ng} \cdot \text{ml}^{-1} \cdot \text{h}^{-1}$ to $2.2 \pm 0.8 \text{ ng} \cdot \text{ml}^{-1} \cdot \text{h}^{-1}$ (n=7, P<0.01). In addition, ADH was also increased during the application of KAATSU (from $1.7 \pm 0.2 \text{ pg} \cdot \text{ml}^{-1}$ to $9.2 \pm 3.0 \text{ pg} \cdot \text{ml}^{-1}$, n=7, p<0.01). In

these exercises, CPK did not change significantly (Table 3). In addition, under the simulated weightlessness, all subjects complained no symptoms, and completed the experiments.



Figure 3. Effects of leg press exercise with and without KAATSU on Hct (A), plasma volume (B), lactate (C), NOR (D), PRA (E), and ADH (F). The changes of plasma volume are shown as compared with the pre level.

		Pre	0-1 min	10 min	30 min
Hct (%)	(-)	48.8 ± 0.3	50.2±0.6**	48.8.±0.5	48.1 ± 0.6
	(+)	48.5 ± 0.6	51.6±0.5**	49.6. ± 0.5**	48.1 ± 0.5
Hb (mg/dl)	(-)	15.9 ± 0.1	16.3 ± 0.2**	15.9±0.1	15.7 ± 0.1
	(+)	15.7 ± 0.2	16.7±0.2**	$16.1 \pm 0.1^{**}$ \square *	15.7 ± 0.1
BV (%△)	(-)	-	-2.9 ± 0.3**	-0.4 ± 0.4	$1.3 \pm 0.3*$
	(+)		-5.9 ± 0.4**	$-2.3 \pm 0.7 **$	0.2 ± 0.9
PV (%△)	(-)	-	-5.6 ± 1.0****	-0.5 ± 0.8	$2.7\pm0.8*$
	(+)		-11.6±0.8**	-4.4 ± 1.1**	0.8 ± 1.7
NOR	(-)	131 ± 16	239 ± 47**	161 ± 23 *	126 ± 17
(pg/ml)	(+)	140 ± 20	514 ± 110** —	224 ± 42	144 ± 23
Lactate	(-)	8.9 ± 0.7	20.9 ± 2.6**	15.0±1.9***	9.2±0.6
(mg/dl)	(+)	9.7 ± 0.7	27.4 ± 3.8**	27.1 ± 4.1**	15.6±2.4
PRA	(-)	0.7 ± 0.1	0.9±0.2	$1.3 \pm 0.3*$	1.0 ± 0.2
(ng/ml/h)	(+)	1.0 ± 0.2	1.6±0.3	$2.2\pm0.8*$	1.0 ± 0.2
ADH	(-)	1.5 ± 0.1	1.8±0.2	1.5±0.2	1.6 ± 0.2
(pg/ml)	(+)	1.7 ± 0.2	9.2 ± 3.0** - *	2.4 ± 0.3 *	1.7 ± 0.1
СРК	(-)	103 ± 13	108 ± 14	103 ±13	102 ±13
(IU/l)	(+)	105 ± 14	115 ± 16	107 ±14*	103 ± 14
1					

Table 3. Hemostatic responses to acute leg-press exercise in healthy volunteers after 24 h-bed rest

*P<0.05 vs. Pre **P<0.01 vs. Pre *P<0.05, **P<0.01 KAATSU (-) vs.

KAATSU (+)

DISCUSSION

The present study shows that following 24 h of bed rest with -6° head-down tilt, a model simulating microgravity effects on the cardiovascular system, resistance exercise (RE) combined with KAATSU mimic the exercise hemodynamic response to exercise during standing at 1 G. Thus, KAATSU combined with resistance exercise appears to stimulate the cardiovascular system during simulated weightlessness and may provide an appropriate countermeasure stimulus for the cardiovascular system associated with weightlessness.

6° head-down tilt bed rest (a model used to simulate zero G) eliminates the normal downward hydrostatic pressure gradients and causes an immediate central fluid shift from lower extremities toward the thoracic-cephalic region (Norsk et al., 1993). The central hypervolemia affects hormonal regulation of fluid excretion and stimulates central cardiac volume receptors, resulting in a loss of plasma volume (Norsk et al., 1993; Duranteau et al., 1995). Some papers have reported that plasma volume (PV) decreases during bed rest within 1-2 d and this lower level was maintained during subsequent bed rest (Volicer et al., 1976; Nixon et al., 1979; Fortney et al., 1991; Norsk et al., 1993; Johansen et al., 1997). In the present study, 24 h 6° head-down tilt bed rest resulted in a total urine volume $(2052 \pm 249 \text{ ml}\cdot\text{d}^{-1})$

that was in excess of fluid intake $(1320 \pm 67 \text{ ml} \cdot \text{d}^{-1})$. Blood and plasma volume was decreased by a mean value of 4.4% and 7.9%, respectively. This fluid volume loss was reflected in the significant decrease in body mass, which was comparable with previous results (Nixon et al., 1979; Gaffney et al., 1985). They reported a significant reduction in PV of up to 500 ml in 24 h. Furthermore, head-down bed rest induces an initial cephalad fluid shift with an inhibition of the renin-angiotensin system and ADH. Hughson et al. (1995) showed a 40% decrease in PRA after 10 h head-down tilt. In the present study, concentration of PRA (1.51 \pm 0.48 to 0.86 \pm 0.18 ng·ml⁻¹·h⁻¹, p=0.08) and ADH (1.81 \pm 0.26 to 1.50 \pm 0.12 pg·ml⁻¹, p=0.08) tended to decrease following 24 h 6° head-down tilt bed rest, but were not statistically different. NOR (201 \pm 45 to 157 \pm 24 pg·ml⁻¹, p=0.09) also tended to decrease following 24 h 6° bed rest, which has been reported throughout spaceflight missions and bed rest (Leach et al., 1983; 1985). Thus, it is likely that physiological alterations caused by 6° head-down tilt bed rest condition observed here effectively mimicked the weightless condition of space flight.

During the simulated weightlessness, we investigated the effects of KAATSU on hemodynamic and neurohumoral responses to leg press RE. The addition of KAATSU to RE induced lower-body venous pooling and reduced venous return, which resulted in a sustained decrease of SV comparable to the value observed in standing at rest. Additionally, a greater increase in HR, blood lactate, NOR, PRA, and ADH concentration was observed when RE was conducted with KAATSU. Thus, the present study shows that the combination of RE and KAATSU during simulated microgravity, elicits hemodynamic and neurohumoral responses that approximate a gravity-specific stress on the cardiovascular system. Thus it appears that RE and KAATSU may provide a unique countermeasure regimen to prevent cardiovascular deconditioning.

Currently, astronauts practice 2-3 h of intensive exercise using treadmill, ergometer and resistance machines. These time-consuming countermeasures cannot completely prevent astronauts from cardiovascular deconditioning. Therefore, alternate countermeasure strategies are necessary. A human centrifuge is a possible candidate, but the centrifuge apparatus is relatively expensive and it is technically laborious to accommodate the apparatus on a space craft. Now, the most effective countermeasure regimen appears to be a gravity-like stress combined with exercise. LBNP has been shown to be a useful method to prevent orthostatic intolerance after space flight, probably through its effect as orthostatic stimulus (Buckey et al., 1996; Güell et al., 1992; Lee et al., 1997; Murthy et al., 1994; Watenpaugh et al., 2000). Supine treadmill exercise combined with LBNP has been reported to maintain submaximal exercise responses such as maximal heart rate, respiratory exchange ratio, and ventilation after bed rest (Lee et al., 1997). In addition, supine exercise in a LBNP chamber (58 \pm 2 mmHg LBNP) has been reported to maintain aerobic fitness and sprint speed during 15 d of 6° head-down bed rest (Watenpaugh et al., 2000). Thus, exercise combined with LBNP appears to provide an effective procedure to stress the cardiovascular system (Hargens, 1994), and may be useful in maintaining upright exercise capacity and prevent orthostatic intolerance during longer bed rest periods or space-flight (Watenpaugh et al., 2000; Perhonen et al., 2001; Schneider et al., 2002). LBNP has been combined with many exercise modalities, e.g. treadmill (Hargens et al., 1991; Murthy et al., 1994), but not resistance-type exercise. This may be important as resistance training specifically promotes muscle enlargement and muscular strength, which are negatively impacted by weightlessness (Akima et al., 2003).

KAATSU training is widely used in Japan (Nakajima et al., 2006) and was originally developed as a novel method for muscle training to increase muscle mass and strength. Under the condition of restricted muscle blood flow with KAATSU, even a short-term and low-intensity exercise bout (treadmill walking, RE, etc) can induce increased muscle mass and muscular strength (Takarada et al. 2000a; b; c; Takarada et al., 2002a,b; Abe et al. 2006). KAATSU training can be applied to most types of exercises such as treadmills, ergometer, and resistance machines. This low-load KAATSU training technique could be easily applied to space flight and utilized by astronauts. Given the previous findings (increased muscle hypertrophy and muscular strength and the present findings), RE with KAATSU appears to provide an interesting combination of effects which may serve as a countermeasure to both cardiovascular and musculoskeletal deconditioning associated with weightlessness. However, the latter needs to be specifically addressed.

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Effects of low-intensity KAATSU resistance training on skeletal muscle size/strength and endurance capacity in patients with ischemic heart disease

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KAATSU training induces muscle hypertrophy and strengthens muscle in athletes and healthy subjects through short-term and low-intensity exercise. However, it remains uninvestigated whether lowintensity KAATSU resistance training (LIKRT) induces muscle strength and hypertrophy in patients with cardiovascular diseases. We examined the effects of LIKRT on skeletal muscle size/strength and endurance capacity in patients with ischemic heart disease (IHD). Seven male patients with stable IHD performed three kinds of resistance exercises (leg press, leg curl and leg extension) with their femoral muscle blood flow restricted by KAATSU belt two times/week for three months. We measured one RM (1-RM) in each resistance exercises, and evaluated muscle cross-sectional areas (CSA) by MRI before training and after the training. We used cardiopulmonary examinations to measure endurance capacity (Peak VO₂ (VO₂peak), VO₂ at anaerobic threshold (VO₂AT)). We performed blood sampling to measure resting plasma level of insulin growth factor-1 (IGF-1) and serum high-sensitive C-reactive protein (hsCRP). LIKRT significantly increased leg press (15%), leg curl (18%) and leg extension (17%) 1-RM strength. Increases of muscle CSA in quadriceps femoris at the proximal lower leg (30%), the mid-thigh (50%), and the proximal lower leg (70%) were 5.1%, 4.6% and 10.4%, respectively. Similarly, hamstring and adductor CSA were also increased by LIKRT. LIKRT significantly increased VO2peak and VO2AT by 10.7% and 10.9%, respectively. IGF-1 and hsCRP were not altered before or after the training. These results suggest that LIKRT increases muscle strength/mass and endurance capacity in patients with IHD. LIKRT appears to be a promising and effective resistance method in cardiac rehabilitation.

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Key words: KAATSU training, ischemic heart disease, muscle hypertrophy, aerobic capacity, blood restriction

INTRODUCTION

Cardiac rehabilitation is a well-established method for improving quality of life and cardiopulmonary function in patients with cardiovascular diseases. Traditionally, aerobic exercise has been generally used for cardiac rehabilitation in patients with cardiovascular diseases. This kinds of exercise improve exercise endurance capacity assessed by using parameters measured during a cardiopulmonary exercise test like peak oxygen consumption (VO₂) (VO₂peak), but has a rather minor effect on skeletal muscle weakness or mass. However, especially in patients with muscle atrophy and elderly patients, it is difficult to improve muscle endurance capacity by using only aerobic exercises. Therefore, resistance exercises combined with aerobic exercises have been recommended (Pollock et al., 2000; Leon et al., 2005). According to the American College of Sports Medicine (ACSM) guidelines, the conditions for inducing muscle hypertrophy and increasing muscle mass are as follows:

- (1) More than 65~70% work load of one repetition maximum (1-RM) is needed.
- (2) Three to four sets until exhaustion.
- (3) Frequency is two \sim three times per week.

However, the adequate high-intensity loads can not be applied to patients with diseases, especially in patients with cardiovascular diseases and elderly patients.

The KAATSU training is a novel exercise that restricts muscle blood flow, by binding the proximal portion of lower or upper extremities with a speciallydesigned belt. It has been reported to induce muscle hypertrophy and strengthen muscle in athletes and healthy subjects through short-term low-intensity exercises (Takarada et al. 2000a; b; c; Takarada et al., 2002a,b; Abe et al. 2006; Fujita et al., 2007; Karabulut et al., 2007, Wernbom et al., 2008; Karabulut et al., 2010). Thus, KAATSU training appears to be a useful muscle training method for promotion of healthy subjects and muscle strength in fields of sport. To date, 200,000 people have performed KAATSU training, and serious side effects have not been reported (Nakajima et al., 2006), suggesting that it is safe under the supervision of the instructors. However, it remains uninvestigated whether low-intensity KAATSU resistance training strengthens muscle and induces muscle hypertrophy in patients with cardiovascular diseases.

Therefore, we investigated the effects of lowintensity KAATSU resistance exercise on skeletal muscle size/strength and endurance capacity and its safety in patients with stable ischemic heart disease.

MATERIALS AND METHODS

Subjects

Seven stable male patients with ischemic heart disease (post-coronary artery bypass grafting (p-CABG) 2, post-percutaneous coronary intervention (p-PCI) 5, 52 ± 4 years old) participated in this study. These patients had no organic stenosis after CABG or PCI, and had no symptoms at the start of this study. This study was approved by the Ethics Committee of the University of Tokyo. All subjects were informed of the methods, procedures and risks, and signed an informed consent document before participation. None of the subjects had participated in strength/resistance-type training before the start of the study. During the study, they did not receive any strength/resistance-type training other than KAATSU training.

Training Protocol

Patients performed three kinds of leg resistance exercises (leg press, leg extension, and leg curl) for three months. The sets consisted of the following repetition pattern: 30 repetitions, 15 repetitions, 15 repetitions, 15 repetitions. There was a one-minute rest interval between sets. Contraction intensity was 20-30% of predetermined 1-RM (leg press 30% 1-RM, leg extension 20% 1-RM, and leg curl 20% 1-RM). Individual contraction duration was 3.0 seconds with a 1.5:1.5 sec shortening-lengthening contraction duty cycle as controlled by a metronome (40 beats per minute). Training was conducted two times per week by the KAATSU method, which restricts muscle blood flow. We monitored the symptom score (Borg scale), blood pressure, and heart rate during the training.

Reduction of Femoral Muscle Blood Flow by KAATSU

This method for inducing the reduction of muscle blood flow is similar to those described in previous papers (Takarada et al. 2000a; b; c; Takarada et al., 2002a,b; Takano et al., 2005; Abe et al. 2006; Fujita et al., 2007; Iida et al., 2007; Nakajima et al., 2008). A specially-designed KAATSU belt applies pressure at the proximal ends of both sides of the thighs, to restrict venous blood flow. The cuff pressure was first set at a low-pressure of 100 mmHg, and gradually increased to 160-250 mmHg within two to three weeks, depending on the subjects and Borg scale during the training. The training was performed within Borg scale of 16. The cuff pressure can be controlled by a KAATSU apparatus.

Estimation of Muscle Cross-Sectional Area

Muscle cross-sectional area (CSA) was estimated using magnetic resonance imaging, (MRI) technology before KAATSU training and three months after KAATSU training ("Pre" and "Pst" in the Figures, respectively). They were evaluated at the proximal lower leg (30%), the mid-thigh (50%), and the proximal lower leg (70%). The % change in CSA was calculated at the quadriceps femoris, hamstring and adductor, separately. The total CSA (cm²) was also measured at the proximal lower leg (30%), the mid-thigh (50%), and the proximal lower leg (70%).

Measurement of 1-RM and VO₂

We measured 1-RM voluntary force, before the training and three months after the training. We measured per-breath gas exchange and determined VO₂, using a standard increment cycle ergometer protocol, with a ramp pulse (20 watt increase/min) with AE 300S (Minato Medical Science CO., LTD., Tokyo, Japan). We measured VO₂ at the anaerobic threshold (AT) and VO₂peak as well as the work load at the AT level and the peak exercise. The VO₂peak value in this study was 1463 \pm 93 ml/min (n=7). One RM evaluated by 8-10 RM was measured at three different stations of a resistance exercise circuit (leg press, leg curl, and leg extension).

Blood Sampling and Hormonal Analyses

We collected venous blood samples before the training and three months after the training. We obtained blood samples by venipuncture from the antecubital vein. High-sensitive C-reactive protein (hsCRP) was measured at SRL, Inc. (Tokyo, Japan) in 500 μ l serum samples, using a method of latex turbidimetric immuno assay (LTIA). The limit of detection of hsCRP was 50 ng/ml. Plasma level of insulin-like growth factor-1 (IGF-1) was determined using immunoradiometric assays (IRMA) specific for the human peptides at SRL, Inc..

Data Analysis

All values are expressed as means \pm S.E.M. Student's paired t-test and one-way ANOVA for repeated measures were used, and differences were considered significant if P<0.05.

RESULTS

All participating patients had no side effects and completed the study. None of them had any complaints, chest pain. There were no hospitalizations, or no exacerbations of ischemic heart disease during the study period.

Fig. 1 shows the effects of KAATSU training on resting plasma IGF-1 (Fig. 1A), and serum hsCRP, an inflammatory marker (Fig. 1B). There were no significant changes (P>0.05) in resting IGF-1 and hsCRP between control (Pre) and three months after the training (Post).

During the KAATSU training, muscle CSA increased significantly for quadriceps femoris (QF, Fig. 2). Increases of muscle CSA in quadriceps femoris at the proximal lower leg (30%), the mid-thigh (50%), and the proximal lower leg (70%) were 5.1%, 4.6%



Figure 1. Changes in serum IGF-1 (A) and high-sensitive CRP (hsCRP) (B) concentration following 3 months KAATSU resistance training. Mean \pm S.E.M. value is shown. Pre: Pre-training Post: Post-training



Figure 2. Changes in skeletal muscle size following three months KAATSU training. Muscle cross sectional area (CSA) was evaluated at the proximal lower leg (30%), the mid-thigh (50%), and the proximal lower leg (70%). The % change in cross sectional areas (CSA) was calculated at the quadriceps femoris (QF), hamstring (HAM), and adductor (ADD), separately. Mean ± S.E.M. value is shown. *P<0.05, **P<0.01 vs. pre (before)-KAATSU training

and 10.4%, respectively. Similarly, CSA of hamstring (HAM) and adductor (ADD) CSA were increased by the KAATSU training. A representative MRI finding is shown in Fig. 3.

Fig. 4 shows the effects of KAATSU training on total muscle CSA for the proximal lower leg (30%), the mid-thigh (50%), and the proximal lower leg (70%). After KAATSU training, muscle CSA at 30%, 50% and 70% position of femur length increased significantly to 4.2%, 3.0% and 7.5%, respectively.

The KAATSU training significantly increased 1-RM strength for leg press (15%), leg curl (18%) and leg extension (17%) (Fig. 5).

Fig. 6 shows the effects of KAATSU training on aerobic capacity in cardiopulmonary examination. KAATSU resistance training significantly increased the work load of the peak and AT exercise (Fig. 6A), and significantly increased VO₂ peak and VO₂ AT by 10.7% and 10.9% significantly (Fig. 6A). However, resting HR and systolic blood pressure (sBP) were not significantly changed (Fig. 6B).



Figure 3. A typical MRI finding. The MRI finding is illustrated at the proximal lower leg (30%), the mid-thigh (50%), and the proximal lower leg (70%) before (left part) and three months after the training (right part).



Figure 4. Effects of KAATSU resistance training on CSA of 30%, 50% and 70% position of femur length. Mean \pm S.E.M. value is shown. *p<0.05 vs. pre (before)-KAATSU training



Figure 5. Effects of KAATSU resistance training on 1-RM of leg press, leg curl, and leg extension. The % change in 1-RM is shown in each exercise. Mean \pm S.E.M. value is illustrated. *p<0.05 vs. pre (before)-KAATSU training

DISCUSSION

These results suggest that low-intensity KAATSU resistance exercises safely increase muscle strength/mass and endurance capacity in patients with ischemic heart diseases. Low-intensity KAATSU resistance training using low-intensity resistance exercise appears to be a promising and useful resistance method in cardiac rehabilitation.

Several studies investigate the effects of heavy resistance exercise on muscle strength in healthy subjects or patients. Hakkinen et al. (2000) showed that the increase in maximal isometric resistance strength of the knee extensor was 28% in middle healthy aged men (42 years), and 21% in elderly men (72 years) 6 months after 50-80% 1-RM resistance exercise. Brochu et al (2002) also reported that the resistance training of 50-80% (three times per week for six months) in older women with coronary heart disease improved upper body strength (18%) and lower body strength (23%). In the present study, we investigated the effects of low-



Figure 6. Effects of KAATSU training on the parameters determined by cardiopulmonary examinations. A: Change in work load (watt) at anaerobic threshold (AT) and peak exercise (peak). B: Change in resting HR, systolic blood pressure (sBP), and VO₂AT and VO₂ Peak. The resting HR (bpm), systolic blood pressure (sBP, mmHg), and VO₂AT (ml/min) and VO₂ peak (ml/min) are shown before and three months after the training. Mean \pm S.E.M. value is illustrated. *P<0.05 vs. pre (before)-KAATSU training

intensity KAATSU resistance exercise on muscle strength in male patients with ischemic heart diseases, by using only 20-30% 1-RM. They had no organic stenosis after the therapy (CABG and PCI). The KAATSU resistance training twice a week for three months, even by using 20-30% load of 1-RM, significantly increased leg press (15%), leg curl (18%) and leg extension (17%) 1-RM strength. The magnitude of the increase in the strength was 0.17% per day (leg press), 0.2% per day (leg curl), and 0.19% per day (leg extension). These values were larger than those reported by Hakinnen et al. (2000) (0.15% per day) and Brochu et al. (2002) (0.13% per day), and similar to the previous papers using healthy subjects and athletes under the restricting muscle blood flow (see review, Karabulut et al., 2007; Wernbom et al., 2008). Thus, low-intensity KAATSU training appears to effectively increase muscle strength comparable to the high-intensity exercise, even in patients with ischemic heart disease.

In the present study, significant increase of muscle CSA in quadriceps femoris was observed. Similarly, KAATSU training also increased hamstring and adductor CSA. The estimated thigh CSA in the present study increased by approximately 4-10% (P<0.05) following three months of the KAATSU training (2 days per week, 32 total training sessions). Increases of muscle CSA in quadriceps femoris at 30%, 50% and 70% position of femur length were 5.1%, 4.6% and 10.4%, respectively. Therefore, the magnitude of the hypertrophic potential (percent increase in muscle CSA divided by total training sessions) in the thigh was about 0.17-0.31% per training session. Recently, Wernborn et al. (2008) reviewed the effects of high-intensity resistance exercise and low-intensity KAATSU resistance exercise (leg extension) on muscle hypertrophy of quadriceps femoris in healthy subjects. High-intensity extension exercise two to three times per week, using 80% 1-RM (three to four sets, 6-10 repetitions, interval 60-120 s), increased muscle mass by 0.03-0.26% per day and 1-7% per month (3-21% per three months). On the other hand, low-intensity KAATSU training using 20-50% 1RM (3-4 sets, 15-30 repetition, interval 30-60 s) increased it by 0.04-0.22% per day and 1.2-6.6% per month (3.6-18% per three months). Thus, low-intensity KAATSU resistance training appears to increase muscle mass in a similar way to heavy resistance exercise in healthy subjects. In the present study, increases of muscle CSA in quadriceps femoris at mid-thigh were 4.6% per three months, suggesting that even low-intensity KAATSU resistance training may be a useful method for inducing muscle hypertrophy as well as improvement of muscle strength in patients with ischemic heart diseases.

The mechanisms of the hypertrophic response and strength gain from KAATSU training are still poorly understood. It has been well known that acute bout of KAATSU training sessions significantly increase growth hormone secretion (Takarada et al., 2000a; Takano et al., 2005; Abe et al., 2006), leading to the increased secretion of IGF-1. The enhanced IGF-1/GH secretion may be involved in the hypertrophic effects of KAATSU training. However, in the presents study, IGF-1 did not increase significantly during the threemonth low-intensity KAATSU resistance training. Therefore, it is unlikely that IGF-1 was mainly involved in the muscle hypertrophy induced by KAATSU training. In fact, the involvement of GH/IGF-1 on the hypertrophy induced by resistance exercise has recently been denied (West et al., 2009). The further studies are needed to clarify the basic mechanisms of the hypertrophic response and strength gain from KAATSU training.

It is generally known that aerobic exercises improve exercise endurance capacity assessed by using parameters measured during a cardiopulmonary exercise test like VO₂peak. However, especially in patients with muscle atrophy and elderly patients, it is difficult to improve muscle endurance capacity by using only aerobic exercises. Therefore, resistance exercises combined with aerobic exercises have been recommended (Pollock et al., 2000; Leon et al., 2005). In the present study, KAATSU resistance training also significantly increased VO₂peak and VO₂AT by 10.7% and 10.9% significantly. The present study showed that the KAATSU resistance exercises significantly increased skeletal muscle mass. An increase of 1 kg skeletal muscle mass in the lowerbody skeletal muscle mass would predict an increase in maximal oxygen consumption (VO₂max) of about 200 ml per minute (Frontera et al., 1990). In the present study, KAATSU resistance training also significantly increased the work load of the peak and AT exercise. Thus, the increase of muscle mass and then improvement of muscle strength might play a role in the improvement of aerobic endurance capacity induced by KAATSU resistance training in patients with ischemic heart disease.

KAATSU training consists of exercises performed with restricted venous blood flow. Therefore, occlusion of blood vessels may affect the haemostasis, and cause the formation of thrombus, though serious side effects of KAATSU training such as pulmonary embolism have not been reported (Nakajima et al. 2006). During our study using patients with ischemic heart disease, no venous thrombosis had developed. This might be compatible with the previous papers showing that vascular occlusion alone stimulates the fibrinolytic activity without the coagulation activity and clot formation (Nakajima et al., 2007; Madarame et al., 2010; Clark et al., 2010). The most common side effects in KAATSU training are petechia and temporary numbness as described previously (Nakajima et al., 2006). The petechia is frequently developed during the KAATSU training of upper extremities (Nakajima et al., 2006). In the present study, all of the patients had received acetylsalicylic acid or ticlopidine hydrochloride, an antiplatelet drug. But, no petechia had occurred during the training of lower extremities. Umbel et al. (2009) reported that delayed-onset muscle soreness was induced by lowload blood flow restricted exercise in healthy subjects. But, in the present study, no serious delayed onset muscle soreness was occurred. In addition, highsensitive CRP, an inflammatory marker (Nakajima et al., 2010), did not change during the KAATSU training. Thus, low-intensity KAATSU resistance training using low-intensity exercise appears to be a safe method for increase in muscle strength and muscle mass in patients with ischemic heart disease. However, the addition of KAATSU to resistance exercise induces a greater increase in blood pressure and noradrenaline, compared to the exercise without KAATSU (Takano et al., 2005; Abe et al., 2006; Kubota et al., 2009). Therefore, we must take this

into account when the KAATSU method is applied to training in patients with cardiovascular disease.

In summary, three months of KAATSU resistance training (leg press. leg curl and leg extension, 20%-30% 1-RM) safely induces muscle hypertrophy/ strength and increases aerobic capacity in patients with stable ischemic heart disease. Low-intensity KAATSU resistance training appears to be a promising and useful resistance method in cardiac rehabilitation.

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Key considerations when conducting KAATSU training

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KAATSU training is a novel training method conducted under special conditions of restricted muscle blood flow of the limbs with a specially-designed KAATSU belt. In 2006, we conducted a questionnaire survey across the whole of Japan to investigate the status of KAATSU training. After that, we have performed KAATSU training mainly for patients with cardiovascular diseases at a total of approximately 700 people per year, and no serious side effects of note have been found. However, it has also been applied for various purposes such as to increase the muscle strength and prevent the muscular atrophy of all kinds of patients including older people. And, in near future, it may be used to train more seriously affected patients with cachexia or sarcopenia. Therefore, in this review, we introduce the main matters reported over the five years since the questionnaire survey was conducted. In addition, we outline key considerations concerning the implementation of KAATSU training centered mainly on our previous experience and reports reached to us.

Key words: KAATSU training, side effects, petechial hemorrhage, contraindication, restricted muscle blood flow

INTRODUCTION

KAATSU training refers to training conducted when pressure is applied in moderation to the base of the limbs with a specially-made belt and the muscle blood flow is restricted (Sato et al., 2007). Differing to hemostasis with a tourniquet, which completely stops the flow of blood in arteries and veins, KAATSU training is undertaken while accumulating blood (pooling) in the upper or lower limbs. A wide variety of loading methods include stretching, self-weight, walking, cycling, strength-training machine, and thera-band. It is said that over 200,000 people are currently conducting KAATSU training to improve the muscle strength of able-bodied people, sportsmen and older people, and for health maintenance purposes. There are also now hopes that it can be applied to the rehabilitation of patients with a variety of diseases (Sato et al., 2007; Nakajima et al. 2010). However, because it is a training method conducted under special circumstances, such as restricting blood flow, abundant experience and caution is believed necessary for implementation and indication. Newlydeveloped KAATSU devices may be launched in the near future, and to conduct safely, spread and develop KAATSU training, we will outline key considerations concerning the implementation of KAATSU training centered mainly on our previous experience and reports reached to us. However, there is a possibility that key considerations will be added or changed in the future due to the increased number of cases.

I) Key considerations concerning KAATSU training

The characteristics of KAATSU training are as follows. :

Point 1) Short-term and low-intensity loads

KAATSU training with high-intensity loads has little effect, but it may be rather dangerous. Needless to say, restricting blood flow for a long time should be avoided.

Point 2) Hemostasis with a tourniquet should be avoided

Point 3) KAATSU training should, in principle, be conducted by KAATSU trainers and instructors

This is believed to be one of the reasons why serious complications have been seldom occurred until now.

We conducted a questionnaire survey in 2006 across the whole of Japan to investigate the status of KAATSU training (Nakajima et al., 2006). According to responses gained from 106 facilities across Japan, KAATSU training was widely provided for people in their teens to their eighties. Main side effects include petechial hemorrhage beneath the skin, chills, numbness, and dizziness. Petechial hemorrhage beneath the skin was discovered when applying pressure, particularly to the upper limbs, at the beginning of KAATSU training. However, this disappeared as the training progressed and was not a problematic side effect. Paralysis caused by nerve compression was also not discovered.

We have conducted KAATSU training mainly for patients with cardiovascular disease at a total of approximately 700 people per year since 2007, and no serious side effects of note have been found. However, it has been applied for various purposes such as to increase the muscle strength and prevent the muscular atrophy of all kinds of patients including older people, and it is speculated that it will be used to train more seriously affected patients with cachexia or sarcopenia in near future.

We will therefore introduce the main matters reported over the five years since the questionnaire survey was conducted.

1) Brain hemorrhage: there was one reported case of brain hemorrhage during KAATSU training. It is known that sudden deaths very rarely occur while playing sports. Sudden deaths have even been reported in golf, running, and gateball. While there have been no reported sudden deaths during KAATSU training to date, sufficient caution is required. As underlying diseases of sports-related sudden deaths, it is said that hypertrophic cardiomyopathy, cardiomegaly (cause unknown), coronary artery malformation, coronary artery sclerosis, aortic rupture, and brain hemorrhage are common among people aged 35 years and below, while 80% of people aged over 35 years are affected by coronary artery sclerosis and brain hemorrhage. Accordingly, the discovery of cardiocirculatory diseases is vital when giving medical checkups to sportsmen and, needless to say, blood pressure management is particularly important. Consideration must also be given to breathing methods during muscle training (exhale when applying effort).

KAATSU training uses low-intensity loads which raise blood pressure less than high-intensity loads (rise to 250-300mmHg or more with high-intensity loads). In addition, hormones (catecholamine) which cause increased heartbeat and blood pressure during exercise increase slightly during KAATSU training, compared to exercises when muscle blood flow is not restricted (Takano et al., 2005; Iida et al., 2007). Therefore, caution is required when dealing with patients with high blood pressure, heart disease, and other diseases.

2) Petechial hemorrhage beneath the skin: This refers to red or purple bodily spots caused by microbleeding (collapsed capillary vessels). Petechial hemorrhage beneath the skin is normally harmless and disappears within a few days. But, there were a few cases of petechial hemorrhage beneath the skin not disappearing and continuing for a while, however, they disappeared in the course. Although it may be unconnected, there were some cases of purpura, such as purpura pigmentosa chronica, when conducting KAATSU. This continued for a few months and then disappeared. They sometimes emerge as symptoms of thrombocytopenia (caused by side effects from treatment for certain types of infectious diseases),

caused by interference with platelet function and as a clotting factor defect, so careful examination by a dermatologist is recommended.

Point 4: Explain prior to KAATSU training that petechial hemorrhage beneath the skin may occur after KAATSU training, particularly of the upper limbs.

This normally disappears after a few days and does not really obstruct continuation of KAATSU training, but it is vital to explain that there are very rare cases of it becoming serious. This can be particularly problematic if not explained to young women. Specifically, cases of people visiting for beauty are a problem. Even patients taking anti-coagulant therapy are not really a problem, but if their condition is serious then training should be stopped. Patients that are administered vibration machines concurrently with medicine have not been a problem so far, but there has been one case of worsening petechial hemorrhage beneath the skin after KAATSU training.

3) Rhabdomyolysis: One case with a serum CPK level > 10,000 IU/L has been reported both in Japan and abroad. When feeling unwell in a hot and humid environment, repeated hard training may lead to rhabdomyolysis. In addition to the hardness of the training, patients are more susceptible when dehydrated. KAATSU training uses low-intensity loads so rhabdomyolysis is considered less likely to occur than when using high-intensity loads. Nevertheless, it is necessary to be cautious such as cancel training when the patient is sick and provide frequent fluid replacement during training. In addition, heat stroke can occur more easily as it becomes hotter, so ensure that patients are replenished with sufficient fluids and electrolytes.

4) Cases of numbness lasting for days: While there have been no reports of paralysis, be sure to adhere to the KAATSU training time and not take too long, and attach the KAATSU belt to the correct area.

5) Venous thrombosis: One case has been reported. However, appropriate application of pressure in KAATSU training does not lead to a worsening coagulation system. Rather, it induces a fibrinolytic state, which helps to restrict thrombus formation (Nakajima et al., 2007; Madarame et al., 2010). In addition, because KAATSU training is 15 to 20 minutes long, it is believed to contribute to the safety of this treatment by not restricting blood flow for a long time. But, out of 10,000 people, 1 to 3 ordinary people, 3 to 11 women in a normal pregnancy, 30 to 40 women after normal childbirth, and 100 women that give birth by Caesarean section have venous thrombosis. Therefore, while KAATSU training is not thrombogenic unless reckless things are done, some of the subjects receiving KAATSU training have venous thrombosis from the beginning. Echocardiography is recommended for suspected cases before starting KAATSU training.

6) Venous injury and induration: This has continued for one to two months in women in their thirties and forties, and is sometimes accompanied by pain. After cancelling the KAATSU training and taking a wait-and-see approach, it disappeared after one to two months. When KAATSU pressure becomes strong or the appropriate pressure is not applied especially to the upper limbs, this may eventually lead to venous damage.

Pay attention to the following.

O Repeat pressurization and depressurization, get blood vessels accustomed to this training, and apply the appropriate pressure ○ The blood vessels of people who take no exercise will not be accustomed to this training, so take care when applying high pressure

○ Adhere to the KAATSU training time.

II) Key considerations when conducting KAATSU training

We will now provide information on basic treatment information required for general exercise therapy as it will serve as a useful reference for safety purposes when conducting KAATSU training. Basic treatment information required for general exercise therapy is shown in Table 1. Basic treatment information includes: 1) subjective symptoms; 2) previous medical history; 3) existence of lifestylerelated diseases; 4) family medical history; 5) lifestyle habits. It also includes resting electrocardiograms if possible and careful examination based on exercise tolerance tests will also be required as appropriate.

Table 1. Basic treatment information required for exercise therapy

	Treeessity of an excisise tolerance test Other action
Subjective symptoms	
Chest pain / chest discomfort / palpitation / shortness of breath	Yes
Dizziness / fainting / intermittent claudication	Yes
Spondylosis symptoms / joint symptoms	Orthopedic examination and guidance
History of disorder	
Cardiovascular disease	Yes
Orthopedic disorder	Orthopedic examination and guidance
Existence of lifestyle-related diseases	
High blood pressure	Assessed severity
Diabetes	Assessed severity
Hyperlipidemia	Assessed severity
Obesity	Assessed severity
Family medical history*	
Myocardial infarction and sudden deaths in first degree relatives	Yes
Lifestyle habits	
Exercise / diet / smoking / alcohol	Lifestyle guidance
Resting electrocardiogram	
Myocardial infarction	Yes
ST-T segment abnormality	Yes
Ventricular arrhythmia	Yes
	Yes

* A family medical history of relatively young sufferer such as famile of hist degree male relative aged under 55 of mother of hist degree female relative aged under 65 who have undergone myocardial infarction and coronary revascularization or died suddenly. Table 1 and 2 are cited from the following papers, and changed: (1) Edited by Japanese Circulation Society et al. Guidelines for diagnosing and treating cardiovascular disease. 2000-2001 Joint Research Group Report. Guidelines for exercise therapy for cardiovascular disease (JCS 2002) (Group head: Saito) (2) Manual for prescribing exercise therapy. Journal of the Japan Medical Association, 116 (3), 1996.

Disease	Indication	Conditional indication	Contraindication
High blood pressure	140-159/90-94 mmHg	160-179/95-99 mmHg	180/100 mmHg or more
		Men aged over 40 or women aged over	CTR of 55% or more visible on a chest
		50 that are in treatment and don't have a	roentgenogram
		contraindication value should undergo	Life-threatening arrhythmia or ischemic
		an exercise tolerance test if possible.	change shown by an electrocardiogram
			(excluding times when safety was
			confirmed by an exercise tolerance test)
			Uric protein of 100 mg/dl or hypertensive
			change in the fundus oculi (more than IIb)
Diabetes	Fasting blood glucose -	Fasting blood glucose - 140 - 249 mg/dl	Fasting blood glucose –
	110 - 139 mg/dl	Men aged over 40 or women aged over	250 mg/dl or more
		50 that are in treatment and don't have a	Urinary ketone body (+)
		contraindication value should undergo	Diabetic retinopathy (+)
		an exercise tolerance test if possible.	
Hyperlipidemia	TC:220 - 249 mg/dl	TC: 250 mg/dl minimum or	
	or	TG: 300mg/dl or more	
	TG:150 - 299 mg/dl	Men aged over 40 or women aged over	
		50 that are in treatment should undergo	
		an exercise tolerance test if possible.	
Obesity	BMI:24.0 - 29.9	BMI: 24.0 - 29.9 and lower limb joint	BMI: 30 or more
		damage	
		Orthopedic examination and exercise	
		restriction	

Table 2. Indications and contraindications for exercise therapy for lifestyle-related diseases

TC: Total cholesterol; TG: Triglycerides; BMI: Body Mass Index (body weight (kg) / height (m)²)

Recently, KAATSU training is often used to train metabolic syndrome and obese patients. Table 2 shows indications and contraindications of normal exercise therapy for lifestyle-related diseases. At a blood pressure of 180/100 mmHg or more, exercise therapy is generally contraindication, so it would be better to give treatment at 160-179/95-99 mmHg. For cases of poorly-controlled diabetic retinopathy and extreme obesity with a BMI of 30 or greater, careful examination for the coexistence of ischemic heart disease is recommended. It is believed that such indications will serve as a useful reference when conducting KAATSU training.

Key considerations when administering KAATSU training to older people and a variety of patients are listed as follows.

1) Pay attention to restricted blood flow

- Adhere to the basics of KAATSU to prevent hemostasis with a tourniquet
- Take care not to allow overload

- Neurally-mediated syncope; refer to the paper (Sato et al., 2007) for details
- Search for complications (heart diseases, etc.)
- 2) During training, pay attention to the following: Sufficient stretching and fluid replacement Patients falling
- 3) Get an expert opinion for high-risk matters and don't provide training for unknown cases.

III) Clinical conditions and diseases to watch out for when conducting KAATSU training

Vascular endothelial damage, stagnation of blood, and hypercoagulability are origins of pulmonary infarction and deep-vein thrombosis. KAATSU training is not a tourniquet, which completely stops the flow of blood in arteries and veins, and according to our past examinations of able-bodied people, it does not lead to an impairment of the coagulation system. Rather, it induces a fibrinolytic state, which has an antithrombotic effect (Nakajima et al., 2007; Madarame et al., 2010). In addition, there have been no reports of pulmonary infarction. However, due to the stagnation of blood occurring during KAATSU training, pulmonary infarction and deep-vein thrombosis risk scores used by surgeons to conduct safer KAATSU training will be introduced.

- Point 5: Using risk factors in determining KAATSU training indication
- 5 points History of deep-vein thrombosis (DVT); hereditary thrombotic tendency; antiphospholipid antibody syndrome
- 4 points Pregnant women
- 3 points 1) Varicose veins of legs; 2) prolonged immobility (incapable of 8 hours thromboprophylaxis rehabilitation);
 3) atrial fibrillation or heart failure
- 2 points 1) People aged over 60 years old; 2) BMI > 30; 3) hyperlipidemia; 4) malignancy; 5) using lower limb tourniquet; 6) using oral contraceptives or adrenocortical steroids; 7) quadriplegia; 8) high hemoglobin level
- 1 point 1) People aged 40 to 58 years old; 2) women; 3) 25 < BMI < 30

The higher the number of points the greater the risk, and the higher the combined number of points for several risks the greater the risk. KAATSU training for people corresponding to 5 points (history of deepvein thrombosis; hereditary thrombotic tendency; and antiphospholipid antibody syndrome) should be avoided. Caution is required when dealing with pregnant women, who have impairment of the coagulation system in the latter stages of pregnancy. We do not conduct training for these pregnant women in principle. While there have been no reports of KAATSU affecting varicose veins of legs, a review of this is needed in the future. Older people and bedridden patients are considered to be suitable for KAATSU training, but it is necessary to exercise care when providing KAATSU training for people that originally had thrombosis. In addition, some patients get thrombosis in the early postoperative period and extreme caution needs to be exercised for such patients.

Since KAATSU training predominately involves the restriction of blood flow, reduced stroke volume and cardiac output may be discovered depending on the extent of applied pressure (Takano et al., 2005; Iida et al., 2007). Even though KAATSU training reduces the preload and places little stress on the heart, it can encourage a lower cardiac output for patients with a significantly reduced heart pumping ability, so extreme caution is required. It is therefore necessary to carefully consider reducing KAATSU pressure or only applying it to upper limbs or one-side, as well as

liaising with doctors for such cases. In addition, it is necessary to stop loading if overload is suspected, depending on the patient's symptoms. In any case, it can only be carried out under close supervision, and even more careful examination is required in the future.

Point 6: In principle, KAATSU training should not be provided or expert advice should be obtained when dealing with hemodynamically unstable patients, especially patients suffering from cardiovascular diseases etc.

CONCLUSION

As we move increasingly towards an aged society, it is estimated that rehabilitation for patients with low exercise capacity and various complications such as cardiovascular diseases will increase more and more. KAATSU training is expected to attract increasing attention. Accordingly, we hope that you fully understand and pay heed to the characteristics of KAATSU training, and conduct sensible and effective training.

Point 7: To conduct safe as well as effective KAATSU training

- 1. Confirm there are no contraindications that are identical to ordinary exercise therapy
- 2. KAATSU training should either not be provided or expert advice should be obtained when dealing with hemodynamically unstable patients
- 3. KAATSU training is a contraindication or caution should be exercised when dealing with thrombotic disease patients
- 4. Explain about petechial hemorrhage beneath the skin and numbness etc. when starting training
- Training tailored to individuals' physical capacity and condition Apply appropriate as well as safe and effective pressure Never be too eager to gain results

Avoid hemostasis with a tourniquet.

- 6. Build a relationship of trust with patients
- 7. There is a possibility that presyncope and fainting will occur, but pay attention to prodromal symptoms and take preventive measures such as fluid intake.
- 8. Older people, bedridden patients and postoperative patients often have venous thrombosis prior to the training so exercise caution.
- 9. Ensure blood pressure is (< 160 /> 95 mm Hg)
- 10. Implement safety measures such as AED
- Avoid conducting KAATSU training over a long time. Upper limbs: 10-15 minutes; Lower limbs: 15-20 minutes
- 12. In principle, never conduct training when the patient is sick. Never continue performing

KAATSU training, when a guest or patient feels bad during the training.

13. When unsure about a patient's medical condition, consult a specialist or go straight to a medical institution if you need to seek other medical help

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A case of dementia presenting remarkable improvement in activities of daily living through KAATSU training

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The number of patients with dementia is increasing markedly, and effective treatment and methods for prevention are needed. Moderate exercise decreases the progression of dementia. We report a case of dementia presenting remarkable improvement in activities of daily living (ADL) through KAATSU training. The patient was a 78-year-old woman with brain atrophy detected by magnetic resonance imaging (MRI) at the age of 73. She had a remarkable decrease in ADL and vitality at the age of 76 and was diagnosed with frontotemporal dementia (FTD) at the age of 77. In initial presentation, she had a humpback and Parkinsonian gait. Her muscle strength in the abdomen, lower back, and thighs was remarkably decreased, and her trunk rotation and sense of equilibrium were also reduced. At first, the training protocol was low-intensity resistance exercises using self-weight workouts and KAATSU-walk training. For upper extremities exercises, dynamic movement using the truncal muscle group was performed, and for lower extremities exercises, the sense of equilibrium was stimulated using standing exercises. The load and degree of difficulty were increased gradually. The average 10meter walk time, the number of steps, and the average timed up and go test time were lower after 6 months than after 2 months. The average bilateral femoral circumference increased after 6 months compared with after 2 months. In conclusion, a patient with FTD performed KAATSU training for 6 months and had a remarkable improvement in motor function. Thus, KAATSU training may be effective in improving ADL in patients with dementia.

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Key words: elderly people, frontotemporal dementia, KAATSU training, activities of daily living

INTRODUCTION

Approximately 24.3 million people suffer from dementia around the world, and this number is expected to double every 20 years to 81.1 million by 2040 owing to the rapid increase in the number of elderly people (Ferri et al., 2005). Senile dementia is predominantly caused by neurodegenerative diseases and cerebrovascular diseases. Alzheimer's disease (AD) is the most common neurodegenerative disease, and various symptoms occur, including memory and orientation disturbance, depression, aggressive behavior, hallucination, and delusion. Recently, early diagnosis of dementia has been encouraged, owing to the increase of older men living alone, home-nursing insurance, and the emergence of anti-dementia drugs such as Donepezil hydrochloride, a cholinesterase inhibitor. Frontotemporal dementia (FTD), characterized by progressive aphasia and curious behavior with personality change, is also a neurodegenerative dementia, and it has been gaining some attention as a differential diagnosis (Grossman et al., 2002).

It has been reported that aerobic training for elderly people without dementia may delay the onset of dementia (Larson et al., 2006). KAATSU training is performed under conditions using low-intensity and short time loading (Takarada et al., 2000a, b). It can now be applied to muscle training in healthy subjects, elderly people, and patients with various diseases (Karabulut et al., 2010; Nakajima et al., 2010; Ozaki et al., 2011).

In this report, we present a case of dementia presenting remarkable improvement in activities of daily living through KAATSU training.

CASE REPORT

The patient was a 78-year-old Japanese woman that had been diagnosed with depression at the age of 53. In her MRI findings, brain atrophy was detected at the age of 73. She had a remarkable decrease in ADL and vitality at the age of 76 and then was diagnosed with FTD when she fell down at home at the age of 77. Although the idea of a home nurse had been introduced by a local doctor, she refused to take part. However, she was later introduced to KAATSU training, and she received a check-up at this hospital. She displayed neither an even temper nor cooperativeness at that time. At initial presentation, she had a humpback and Parkinsonian gait. Her muscle strength in the abdomen, lower back, and thighs was remarkably decreased, and her trunk rotation and sense of equilibrium were also reduced. Since she could hardly walk without assistance, several evaluations were difficult to perform. This study was approved by the ethics committee of the University of Tokyo.

METHODS

The patient performed KAATSU training one day per week. The training protocol began with lowintensity resistance exercises using self-weight workouts and KAATSU-walk training. For upper extremities exercises, dynamic movement using the truncal muscle group was performed, and for lower extremities exercises, the sense of equilibrium was stimulated using standing exercises. The load and degree of difficulty were increased gradually. Specifically, she initially performed three sets of upper extremities, chest presses, rowing, three sets of lower extremities, leg extensions, knee ups, and KAATSUwalk training (Table 1). Initially, however, she could hardly perform the training menu. In the middle of the training period (after 3 months), she performed three sets of upper extremities, chest presses, rowing, side raises, three sets of lower extremities, leg extensions, sitting leg curls, knee ups, and KAATSUwalk training. Furthermore, later on (after 5 months), she performed three sets of upper extremities, chest presses, rowing, side raises, three sets of lower extremities, squats, standing leg curls, leg extensions, and KAATSU-walk training.

RESULTS

There were no side effects during this study.

Bilateral mid-upper arm circumference increased after 6 months compared with after 4 months (Fig. 1A; right: +1.0 cm, left: +1.5 cm). Bilateral lower leg circumference also increased after 6 months compared with after 2 months (right: +1.0 cm, left: +1.0 cm). In particular, bilateral thigh circumference also increased after 6 months compared with after 2 months (right: +2.0 cm, left: +3.0 cm), suggesting enlargement of the femoral muscle group.

Shoulder range of motion increased after exercise compared with before exercise, not only as an immediate effect (Fig. 1B; right flexion: $+5.7^{\circ}$, left flexion: $+2.1^{\circ}$, right horizontal abduction: $+4.6^{\circ}$, left horizontal abduction: $+2.5^{\circ}$), but also as a long-term effect after 6 months compared with after 2 months (right flexion: $+17.5^{\circ}$, left flexion: $+15.0^{\circ}$, right horizontal abduction: $+22.5^{\circ}$, left horizontal abduction: $+2.5^{\circ}$). Posture in KATSU-walk training also improved as shown in Fig. 2.

The 10-meter walk time was lower after 6 months than after 2 months (Fig. 3A; before exercise: -18.5 sec, after exercise: -9.4 sec). The number of steps for the 10-meter walk was also lower after 6 months than after 2 months (before exercise: -24, after exercise: -14). These findings suggest improvement in walking speed. The timed up and go test time also improved after 6 months compared with after 2 months (Fig. 3B; before exercise: -16.3 sec, after exercise: -2.4 sec).

Initial period (Before 3 Months)	Middle period (From 3 Months to 5 Months)	Late period (After 5 Months)
3 sets of upper extremities chest presses	3 sets of upper extremities chest presses	3 sets of upper extremities chest presses
rowing	rowing	rowing
3 sets of lower extremities	side raises	side raises
leg extensions	3 sets of lower extremities	3 sets of lower extremities
knee ups	leg extensions	squats
KAATSU walking	sitting leg curls	standing leg curls
	knee ups	leg extensions
	KAATSU walking	KAATSU walking

Table 1. Training menu during KAATSU training





Figure 1. A Changes in brachial, femoral, and lower leg circumference through KAATSU training. B Change in range of shoulder motion through KAATSU training. Pre, before exercise; post, after exercise; RF, right flexion; LF, left flexion; RHAB, right horizontal abduction; LHAB, left horizontal abduction.

Figure 1B



4Mo

6Mo

2Mo





Figure 2. Change of posture in KAATSU-walk through KAATSU training.





Figure 3. A Changes in 10-meter walk time and number of steps through KAATSU training. B Change in timed up and go test time through KAATSU training. Pre, before exercise; post, after exercise.

DISCUSSION

KAATSU training is a novel form of strength training which not only affects the endocrine system, including growth hormone secretion, but also stimulates many muscle fibers and brings about muscle hypertrophy and muscle power enhancement (Takarada et al., 2000a, b). It also has pleiotropic effects including the improvement of obesity and an effect on bones. It can also be applied to muscle training in healthy subjects, elderly people, and patients with various diseases (Karabulut et al., 2010; Nakajima et al., 2010; Ozaki et al., 2011). Furthermore, we have performed KAATSU training on a total of 2,000 patients mainly with cardiovascular diseases, and serious adverse side effects have not been observed. Thus, KAATSU training is safe under a director familiar with appropriate methods (Nakajima et al., 2006). In this paper, we reported for the first time that an elderly patient with dementia, who had muscle atrophy and decreased quality of life, had a remarkable improvement in quality of life through KAATSU training.

Regular exercise has been reported to delay the onset of dementia in a prospective cohort study (Larson et al., 2006). In this study, moderate exercise induced an approximately 40% decrease in the risk of dementia. They conducted a follow-up survey on 1,750 subjects (aged 65 years and older) without cognitive disturbance over a period of 6 years, and

the subjects declared their exercise patterns, including walking, hiking, aerobics, setting-up exercises, swimming, water aerobics, weight training, and stretching every 2 years. A total of 158 of the 1740 subjects developed dementia during 6.2 ± 2.0 years. Of this number, 107 subjects were diagnosed with AD. The incidence rate of dementia was 13.0/1,000 person-year [hazard ratio 0.62 (p=0.004, 95% confidential interval 0.44-0.86)] in subjects who performed exercise three times or more every week, whereas it was 19.7/1,000 person-year in those that exercised less than three times every week. Furthermore, the decrease in risk associated with exercise was greater in subjects with a poor performance level than those with a maintained performance level.

Regular exercise also improves cognitive function in patients with dementia. The basic mechanisms remain unclear, but brain-derived neurotrophic factor (BDNF) may be involved. BDNF, which is produced mostly in the cerebral cortex and hippocampus, is known to play an essential part in the life, growth, and maintenance of neurons and is strongly related to study, memory, and cognitive function (Zigova et al., 1998; Lee and Son, 2009). In the hippocampus of patients with AD, a decrease in BDNF has been reported (Pedersen et al., 2009). Recent studies showed that BDNF plays an important role in the beneficial effects of exercise training on dementia (Ding et al., 2006; Griesbach et al., 2004; Kim et al., 2010; Vaynman et al., 2003, 2004). It remains unexamined whether KAATSU training can enhance BDNF secretion. However, there is a possibility that KAATSU training may improve cognitive function via BDNF. In addition, we measured cerebral blood flow in healthy subjects using near-infrared spectroscopy and compared KAATSU training to low-intensity training without KAATSU. In this study, cerebral blood flow increased significantly in the KAATSU training group, suggesting favorable influence on the cerebral center (Morita et al., 2010). From these observations, it is likely that KAATSU training may improve cognitive function and delay the onset of dementia by promoting BDNF secretion and improving cerebral blood flow. However, further studies are needed to clarify this possibility.

The pleiotropic effect and safety of KAATSU training has been established. However, how KAATSU training is provided for elderly people and patients with dementia is important, since they often find it difficult to continue performing training in terms of understanding instructions and their ability to concentrate. In an NIH Consensus Statement (NIH Consensus Development Panel on Physical Activity and Cardiovascular Health, 1996), tips on continuing exercise training included being conscious of the effects of exercise training, performing pleasant exercise activities, satisfying exercises, and recognizing the safety of exercise. In these points, KAATSU training, a man-to-man training program involving communication with a partner, appears to be a very suitable and effective method.

In conclusion, a patient with FTD who performed KAATSU training one day per week over 6 months had increased extremity circumference, increased range of shoulder motion, improved static and dynamic position, and improved 10-meter walk and timed up and go tests, resulting in the improvement of ADL. In addition, KAATSU training appears to be a useful method for improving ADL and cognitive function in patients with dementia.

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The effects of low-intensity KAATSU resistance exercise on intracellular neutrophil PTX3 and MPO

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Myeloperoxidase (MPO) has been suggested to have a role in atherosclerosis through its strong oxidative capacity. MPO-mediated formation of reactive oxidants is enhanced after exhaustive exercise. Low-intensity resistance exercise (LIRE) under the conditions of restricting muscle blood flow (KAATSU) has been reported to strengthen muscles, in a way similar to high-intensity resistance exercise. We investigated the effects of LIRE-KAATSU on neutrophil intracellular MPO and pentraxin 3 (PTX3), an inflammatory marker. Five control subjects and six patients with ischemic heart diseases (IHD) (pPCI 5, pCABG 1) have participated in this study. Each subject performed LIRE (20% 1RM leg extension, 4 sets, 30-15-15-15) under the conditions with or without KAATSU. Neutrophils were immunostained with a specific antibody to PTX3 and MPO and the density of these markers was measured by flow cytometry analysis (FACS). FACS and immunostaining studies showed the existence of PTX3 and MPO in neutrophils. LIRE did not particularly release PTX3 and MPO from neutrophils in healthy subjects under the conditions with or without KAATSU. The similar results were obtained in patients with IHD. These results suggest that the addition of KAATSU to LIRE did not enhance PTX3 and MPO release from neutrophils even in patients with IHD, which may be related to the safety of KAATSU, compared to high-intensity resistance exercises

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Key words: KAATSU training, Myeloperoxidase; Pentraxin 3; neutrophils; resistance exercise

INTRODUCTION Moderate-intensity exercise induces transient oxidative stress, stimulates redox-sensitive signals, and then increases the antioxidant defense system, leading to cytoprotection. However, heavy exercise induces excessive oxidant stress, resulting in tissue injury. The increase of myeloperoxidase (MPO), an oxidant stress marker belonging to the heme peroxidase superfamily, has been also reported as a result of acute heavy exercise in healthy subjects (Suzuki et al., 1996; Nakajima et al., 2010a; Reihmane et al., 2012). MPO is found in myeloid cells, particularly in neutrophils (Ikitimur and Karadag, 2000; Tavora et al., 2009; Nakajima et al., 2010a), and plays an important role in the host defense against bacteria and viruses. On the other hand, it has been suggested to have a role in atherosclerosis through its strong oxidative capacity. MPO-mediated formation of highly reactive oxidants, hypochlorous acid (HClO), causes consumption of nitric oxide (NO) on endothelial cells and the oxidation of LDL on vascular walls in atherosclerotic regions. Therefore, the excessive generation of MPO leads to the formation of atheroma, then plaque rupture and acute coronary syndrome as well (Nicholls and Hazen, 2005; Tavora et al, 2009). Thus, MPO is a key component in the development and progression of atherosclerosis and other forms of

cardiovascular diseases (Ikitimur and Karadag, 2010).

In addition, daily regular exercise is known to induce anti-inflammatory effects and reduce the risk of cardiovascular diseases associated with chronic low-grade systemic inflammations such as atherosclerosis (Petersen and Pedersen, 2005; Plaisance and Grandjean, 2006), resulting in a decrease of inflammatory markers such as C-reactive protein (CRP) and pentraxin 3 (PTX3) (Kasapis and Thompson, 2005; Fukuda et al., 2012). However, in contrast to the anti-inflammatory effects of exercise, acute high-intensity and prolonged exercise causes production of stress hormones and alterations in the circulating quantity and function of various immune cells, including leukocyte mobilization and leukocytosis (Hoffman-Goetz and Pedersen, 1994; Pedersen and Hoffman-Goetz, 2000; Peake et al., 2004; 2005a; Buttner et al., 2007), and increase of inflammatory markers, CRP and PTX3 (Nakajima et al., 2010a). The low-intensity resistance exercise under the conditions with the restriction of muscle blood flow named as KAATSU has been reported to strengthen muscles in athletes and healthy subjects, results similar to high-intensity resistance exercise (Takarada et al., 2000b; 2002; Karabult et al., 2007; Wernbom et al., 2008; Karabult et al., 2010), which is a promising method for rehabilitation in patients including cardiovascular diseases (Nakajima et al.,

2006; Nakajima et al; 2010). We have previously reported that KAATSU training using 20-30% 1RM resistance exercises increase muscle strength and muscle mass in patients with cardiovascular diseases (Nakajima et al., 2010) and applying blood flow restriction does not affect exercise-induced hemostatic and inflammatory responses of CRP (Madarame et al., 2012). However, responses to KAATSU including neutrophils have not been investigated.

Therefore, the purpose of this study is to investigate the effects of low-intensity resistance exercise with KAATSU on neutrophil intracellular MPO and PTX3 conducted on patients with ischemic heart diseases (IHD) in comparison to control subjects. Results have been derived by assessing the PTX3 and MPO levels in neutrophils isolated from the peripheral blood by using flow cytometry analysis as a result of the exercises.

MATERIALS AND METHODS

Subjects

Five male control subjects $(37 \pm 5 \text{ years})$ and six male patients with IHD (post-coronary artery bypass grafting (p-CABG) 1, post-percutaneous coronary intervention (p-PCI) 5, 60 ± 3 years old) participated in this study. These patients had no organic stenosis after CABG or PCI and had no symptoms at the start of this study. This study was approved by the Ethics Committee of the University of Tokyo. All subjects were informed of the methods, procedures and risks, and signed an informed consent document before participation.

Exercise Protocol

Each subject performed low-intensity leg extension resistance exercise (20% 1RM, 4 sets) under the conditions either with or without blood flow restriction – the order of which was counterbalanced across subjects. In each exercise session, one set with 30 repetitions was followed by 3 sets with 15 repetitions. A rest period of 1 min was taken between sets. We measured 1RM voluntary force before the exercise.

Femoral Muscle Blood Flow Reduction Method by KAATSU

The method for inducing the reduction of muscle blood flow is similar to that described in previous papers (Takarada et al. 2000a; b; Takarada et al., 2002; Takano et al., 2005; Abe et al. 2006; Fujita et al., 2007; Iida et al., 2007; Nakajima et al., 2008). A specially-designed KAATSU belt applies pressure at the proximal ends of both sides of the thighs to restrict venous blood flow. The cuff pressure is set to 200 mmHg.

Blood Sampling

Blood samples were obtained using an indwelling heparin-lock catheter inserted into a superficial antebrachial vein on the left arm. The subjects were instructed to sit quietly before taking blood sample for at least 30 min. The baseline (resting/preexercise) blood sample was taken before the application of KAATSU or the start of the exercises. Post-exercise blood samples were further obtained within 1-2 min after the exercise and the release of the belt.

Neutrophils, Monocytes, and Lymphocytes Isolation

Heparinized whole blood (5 ml) from peripheral blood was layered over 5 ml of Polymorphprep (Axis-Shield, Oslo, Norway) and spun for 35 min at 500 g at 20 °C. Mononuclear cells (MNCs) were harvested from the top band of the sample-medium interface, and polymorphonuclear cells (PMNs) were harvested from the second interface. One ml of 0.45% NaCl solution was added into each fraction to restore normal osmolarity, washed with phosphate-buffered saline (PBS, Nacalai tesque Co., Kyoto, Japan) and spun for 10 min at 270 g at room temperature.

PMNs, mainly composed of neutrophils, were resuspended in a 0.5 ml buffer (3% FBS and 0.05% NaN₃ in PBS), and mixed with a 5 ml ice-cold 70% ethanol for 30 min and spun for 10 min at 270 g at room temperature. Subsequently, PMNs were resuspended in a 1 ml 70% ethanol, dropped on a slide glass, and was dried. MNCs, composed of monocytes and lymphocytes, were resuspended in a 1 ml buffer. Then, the cells were performed on a cell sorter (EPICS ALTRA, Beckman Coulter, Fullerton, CA) to sort out monocytes and lymphocytes from the fraction of MNCs. Each sorted portion was spun for 10 min at 270 g at room temperature. Subsequently, monocytes and lymphocytes were resuspended in a 1 ml 70% ethanol, dropped on a slide glass, and dried in a similar manner to PMNs.

Immunofluorescent Staining

The fixed cells were washed in PBS, and then blocked for 15 min with 2% horse serum in PBS, and were incubated with the primary antibody in a humid chamber overnight at 4 °C. The primary antibodies used were a rabbit polyclonal anti-human myeloperoxidase (Novus Biological, Littleton, CO) and a rat monoclonal antibody against human PTX3 (clone MNB4, Alexis Biochemicals, San Diego, CA). After the preparation of the antibodies, the cells were washed in PBS and the secondary antibody was incubated for 60 min at room temperature. An Alexa Fluor 488-conjugated donkey anti-rabbit IgG (H+L) antibody (Molecular Probes, Eugene, OR), and a Cy3-conjugated goat anti-rat IgG (Jackson ImmunoResearch, West Grove, PA) were used to visualize the MPO and PTX3 expression. For negative controls, normal rabbit IgG and normal rat IgG (Vector laboratories, Burlingame, CA) were used instead of primary antibodies. The nuclei were stained with Hoechist 33258 (Sigma Aldrich). A confocal laser scanning microscope (Olympus FluoView FV300, Olympus Co., Tokyo, Japan) was used to examine the specimens.

Flow Cytometry Analysis

For the flow cytometry analysis (FACS) using neutrophils as previously reported (Nakajima et al., 2010a), whole blood (300 μ l) was lysed with a Versalyse reagent (Beckman Coulter, Fullerton, CA) and spun for 5 min at 270 g. Then, cells were resuspended in a 100 μ l of 4% paraformaldehyde for 15 min. Subsequently, 1 ml of the buffer (see above) was added and spun for 5 min at 270 g. The cells were resuspended in a 100 μ l buffer containing 0.1% saponin for 5 min and incubated for 30 min at room temperature with a mixture of primary antibody (the rabbit polyclonal anti-human myeloperoxidase (Novus Biological, Littleton, CO) and rat monoclonal antibody against human PTX3 (clone MNB1, Alexis Biochemicals, San Diego, CA). Then, the cells were added with a 1 ml buffer containing 0.1% saponin, spun at 270 g and incubated with a secondary antibody (Alexa Fluor 488-conjugated donkey anti-rabbit IgG (H+L) antibody (Molecular Probes Inc., Eugene, OR) and a PE-conjugated Goat anti-rat IgG (H+L) antibody (Beckman Coulter, Fullerton, CA). Finally, the cells were again, resuspended in the buffer. Flow cytometry (FACS) was performed on a flow cytometer (EPICS XL-MCL, Beckman Coulter,

Fullerton, CA).

Figure 1 shows a typical example of PTX3 in neutrophils by FACS. The expression of PTX3 was identified in neutrophils. The right side shows neutrophils counterstained with Hoechst33258 which helps visualize the nuclei. The mean fluorescence intensity of PTX3 and MPO from blood taken immediately after the exercise and taken before the exercise was compared. Assuming the mean florescence density of MPO and PTX3 in neutrophils isolated from pre-exercise blood is 100%, the relative value of the mean florescence density of neutrophils isolated from blood immediately after the exercises is calculated.

Data Analysis

All values are expressed as means \pm S.E.M. Student's pared *t* test was used to compare two sets of data from the same subjects. Differences were considered significant if *p* values were less than 0.05.

RESULTS

First, we investigated the existence of MPO and PTX3 in human neutrophils. Immunostaining studies demonstrated the presence of MPO (Fig. 2A) and PTX3 (Fig. 2B) in human neutrophils taken from control subjects. Flow cytometry analysis also showed the existence of both PTX3 (Fig. 1) and MPO (Fig. 5) using a specific antibody. The data were obtained from a control subject (Fig. 1) and a patient with IHD (Fig. 5), respectively. The expression of MPO and PTX was also separately investigated in neutrophils, monocytes, and lymphocytes. As shown in Fig. 3, the expression of MPO was particularly observed in neutrophils (A), and no expression was detected in negative controls without the antibody (B). It was also observed to a lesser extent in



Figure 1. Flow cytometry analysis of PTX3 expression in human neutrophils isolated from peripheral blood (see methods). The neutrophils were counterstained with Hoechst33258 to visualize nuclei shown on right.



Figure 2. Immunostaining of MPO (A) and PTX3 (B) in neutrophils.



Figure 3. Immunostaining of MPO in neutrophil, monocytes, and lympocytes. A: neutrophil B: negative control (without antibody) C: monocytes D: lympocytes. Note that the expression of MPO in healthy subjects is particularly observed in neutrophils, and to a lesser extent in monocytes.



Figure 4 Immunostaining of PTX3 in neutrophils, monocytes, and lympocytes.A: neutrophil B: negative control (without antibody) C: monocytes D: lympocytes.Note that the definite expression of PTX3 in healthy subjects is observed only in neutrophils.



fluorescence intensity

Figure 5. Effects of low-intensity resistance exercise with KAATSU on neutrophil MPO by using flow cytometry. The typical data obtained from a patient with ischemic heart disease were shown before and after exercising. Note that neutrophils isolated from blood immediately after the exercise (Post) exhibit similar mean fluorescence for MPO compared to those from pre-exercise blood (without KAATSU).

monocytes (C). The very weak staining was observed in human lymphocytes (D). The marked expression of PTX3 was observed in neutrophils (A) only as shown in Fig. 4. No expression was detected in negative controls without the antibody (B). The definite expression of PTX3 was not observed in human monocytes (C) and lymphocytes (D), compared with neutrophils (A).

Secondly, we investigated the effects of 20% 1RM low-intensity resistance exercise with or without the restriction of muscle blood flow (KAATSU) on neutrophil MPO and PTX3, by using FACS. The typical data under the KAATSU condition obtained from a 67-year old patient are shown in Fig. 5. The mean fluorescence density of MPO in neutrophils isolated from post-exercise blood was similar to that obtained at rest (pre-exercise blood).

Five control male subjects and 6 patients with IHD were also examined as shown in Figs. 6 and 7. Neutrophils isolated from blood immediately after exercise without KAATSU exhibited no significant differences in the mean fluorescence for MPO (Fig. 6)



Figure 6. Effects of 20% 1RM resistance exercise with or without KAATSU on neutrophil MPO by using flow cytometry in healthy subjects (left part) and patients with ischemic heart diseases (IHD, right part). The mean fluorescence density of MPO in neutrophils isolated from pre-exercise blood without KAATSU is considered 100%, and the relative value of the mean fluorescence density of neutrophils isolated from blood immediately after exercises is shown. The data were obtained from 5 control subjects (left) or six patients with IHD (right part).



Figure 7. Effects of 20% 1RM resistance exercise with or without KAATSU on neutrophil PTX3 using flow cytometry in healthy subjects (left) and patients with ischemic heart diseases (IHD, right). The mean fluorescence density of PTX3 in neutrophils isolated from pre-exercise blood without KAATSU is considered 100%, and the relative value of the mean fluorescence density of neutrophils isolated from blood immediately after exercises is shown. The data were obtained from 5 healthy subjects (left) or six patients with IHD (right).

and PTX3 (Fig. 7) compared to that isolated from preexercise blood in control subjects (Figs. 6 & 7, left side) and patients with IHD (Figs. 6 & 7, right side). Similar results were obtained from patients with IHD under the conditions with KAATSU (Figs. 6 & 7). Neutrophils isolated from blood immediately after exercise with KAATSU also exhibited no significant changes in the mean fluorescence for MPO (Fig. 6) and PTX3 (Fig. 7) from control subjects and patients with IHD.

DISCUSSION

The major findings of the present study are as follows; 1) FACS and immunostaining studies have shown the existence of PTX3 and MPO in neutrophils. 2) 20% 1RM low-intensity resistance exercise did not significantly release PTX3 and MPO from neutrophils in control subjects under the conditions with or without KAATSU. Similar results have been observed in patients with IHD. Thus, the addition of muscle blood flow (KAATSU) restrictions to low-intensity resistance exercise did not enhance PTX3 and MPO release from neutrophils in patients with IHD, which may be related to the safety of KAATSU, compared to high-intensity resistance exercises.

First, the present study using FACS and immunostaining studies has shown the existence of MPO and PTX3 in neutrophils, which is consistent with the previous papers (Ikitimur and Karadag, 2000; Jaillon et al., 2007; Tavora et al., 2009; Nakajima et al., 2010a). As shown in Fig. 3, the expression of MPO was particularly observed in neutrophils and to a lesser extent in monocytes. Also, the expression of PTX3 has only been observed in neutrophils as shown in Fig. 4. Therefore, we have investigated the effects of low-intensity resistance exercise on intracellular MPO and PTX3 in neutrophils using flow cytometry analysis. Similarly, changes in neutrophil surface receptor expressions including CD16 and degranulation have been investigated immediately after a high-intensity exercise (a treadmill for 1 h at 80% VO₂max) (Peake et al., 2004).

Acute exercise is an important stressor to the body, leading to an activation of immune responses (Hoffman-Goetz and Pedersen, 1994; Pedersen and Hoffman-Goetz, 2000; Peake et al., 2004; 2005a; Buttner et al., 2007) depending on the intensity. The exercise-induced acute phase response consists of typical changes including leukocytosis and release of cytokines and acute phase proteins, similar to acute phase responses evoked by other stressors such as trauma and sepsis (McCarthy et al., 1992; Ostrowski et al., 2000; Shephard, 2001; Yamada et al., 2002; Natale et al., 2003; Peake et al., 2005b; Mooren et al., 2006; Zaldivar et al., 2006). PTX3 and CRP have been shown to be independent high-sensitive markers for inflammation under the various pathophysiological conditions (Presta et al., 2007; Mantovani et al., 2008), and the increase of inflammatory markers such as CRP and PTX3 have been reported in acute heavy exercises (Castell et al., 1997; Nakajima et al., 2010a). High-intensity exercises also increase serum MPO concentration which depends on the exercise intensity (Peake et al., 2004; Reihmane et al., 2012).

Recently, we have shown that neutrophils isolated from blood immediately after high-intensity resistance exercise (70% 1RM) releases MPO and PTX3 in control subjects using flow cytometry analysis (Nakajima et al., 2010a). However, the present study showed that 20% 1RM low-intensity resistance exercise did not significantly release neutrophil MPO and PTX3 in control subjects. Thus, the exercise-induced neutrophil MPO and PTX3 release appears to depend on exercise-intensity. The present study also investigated the responses to KAATSU in healthy subjects. The application of KAATSU did not enhance neutrophil MPO and PTX3 significantly in healthy subjects. These results were in contrast with the results from high-intensity exercise, but somewhat consistent with the previous papers showing that applying blood flow restriction does not affect exercise-induced inflammatory responses of CRP, another inflammatory marker (Madarame et al., 2012)..

MPO has a role in atherosclerosis through its strong oxidative capacity. MPO-mediated formation of highly reactive oxidants, hypochlorous acid (HClO), causes consumption of nitric oxide (NO) on endothelial cells and the oxidation of LDL on vascular wall in atherosclerotic regions. The excessive formation of MPO leads to the formation of atheroma, then plaque rupture, and acute coronary syndrome as well (Tavora et al, 2009). Thus, MPO is a key component in the development and progression of atherosclerosis and other forms of cardiovascular diseases (Ikitimur and Karadag, 2010). Therefore, we also investigated the effects of lowintensity resistance exercise with or without KAATSU on neutrophil MPO and PTX3 in patents with cardiovascular diseases as well as healthy subjects. The present study has shown that KAATSU training using low-intensity exercise did not significantly release neutrophil MPO and PTX3 release in these patients. Similarly, we have recently reported that applying blood flow restriction did not enhance exercise-induced inflammatory responses of CRP in patients with IHD (Madarame et al., 2012). Thus, the addition of KAATSU to low-intensity resistance exercise did not enhance inflammatory responses (CRP and PTX3) and neutrophil MPO release from neutrophils even in patients with IHD, which may be related to the safety of KAATSU compared to high-intensity resistance exercise. In fact, we have reported that KAATSU resistance training using 20-30% 1RM resistance exercises can increase muscle strength and muscle mass in patients with cardiovascular diseases. Furthermore, no obvious side effects including coronary events have occurred (Nakajima et al., 2010b). However, since the patients used in this study are very stable, and have normal cardiac function and no significant

In conclusion, the addition of KAATSU to lowintensity resistance exercise did not enhance PTX3 and MPO release from neutrophils in patients with cardiovascular diseases, which suggests the safety of KAATSU in cardiac rehabilitation compared to highintensity resistance exercise.

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Effect of KAATSU training on a patient with benign fasciculation syndrome

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[Objective]

Benign fasciculation syndrome (BFS) is a neurological disorder characterized by involuntary and repeated contractions of synergetic muscles and commonly occurs in the eyelids, arms and legs. BFS is also associated with pain, which may interfere with everyday activities. This report describes the case of a BFS patient who performed KAATSU exercise, a type of exercises performed under the conditions of restricting muscle blood flow.

[Methods]

The patient performed KAATSU exercise of the bilateral upper and lower extremities at a frequency of twice a week for 3 months. Knee extensor strength as measured with a hand-held dynamometer (HHD), QOL scores as assessed by the SF-36v2 questionnaire form, and bilateral femoral muscle mass as measured by MRI were compared before and after exercise. [Results]

After a 3-month KAATSU exercise program, the right and left knee extensor strength as measured with a HHD increased by about 26% from 30.9 to 38.8 kgf and by about 44% from 39.9 to 57.4 kgf, respectively, and the bilateral femoral muscle mass as measured by MRI increased by about 23% from 8,730 to 10,709 cc. Overall improvement in QOL was also observed, as assessed by the SF-36v2 questionnaire form.

[Conclusion]

For patients with neurological disorders with pain, such as the present patient, active introduction of KAATSU-based exercise is likely to result in improved health-related QOL, as well as increased muscle mass and strength.

Key words: Benign Fasciculation Syndrome, KAATSU training, exercise therapy

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Fasciculation can be seen in healthy people, especially after high-intensity exercise, but is also observed in patients with severe neuromuscular diseases, such as amyotrophic lateral sclerosis (ALS), and thus should be considered in differential diagnosis. Benign fasciculation syndrome (BFS) is a benign disease characterized by fasciculation as a prominent symptom, such as pain, anxiety, fatigability, and paresthesia, and often lasts for several months to years (Fettel et al., 1982). Major causes of BFS include severe stress and anxiety. For treatment, exercise therapy may be performed, but no effective rehabilitation program has been established. KAATSU exercise, a novel, attention-drawing method of low-intensity resistance exercise performed under the condition of restricting muscle blood flow (Takarada et al., 2000b; Abe et al., 2006; Karabulut et al., 2007; Wernbom et al., 2008), causes minimal strain on the musculoskeletal and cardiovascular systems compared to high-intensity resistance exercise and thus is increasingly applied to rehabilitation programs for patients with various disorders. Meanwhile, its effect on BFS patients has yet to be examined.

This report describes the case of a BFS patient in which KAATSU exercise exerted beneficial effects on the muscles, pain, and quality of life (QOL) of the patient.

Patient

Patient: a 44-year-old man

Diagnosis: benign fasciculation syndrome

Comorbidity: none

Previous medical history: none

Chief complaint: systemic fasciculation, pain

History of present illness: The patient noted fasciculation of the right upper and lower extremities in May 2010. The symptom worsened thereafter and he started using a wheelchair in mid-August. The symptom continued to worsen gradually until a reduction in pain was noted around March 2011 and the patient became able to walk 500-600 m on a flat road without resting. With the expectation for further improvement of symptoms, the patient experienced an introductory session of KAATSU exercise at another hospital in

Exercise menu	Month	6		7			8			9	
Triple sets		•	•	•	•	•	•	•	•	•	•
Rowing			•				•	٠	•	•	•
Side raises			•								
Depressurization						•					

 Table 1.
 Upper extremity exercise program

 Table 2.
 Lower extremity exercise program

Exercise menu	Month	6		7			8			9	
Triple set		٠	•	•	•	•	•	•	•	•	•
Leg extensions			•	•	•	•	•	•	•	•	•
Leg curls			٠	٠	٠	٠	٠	٠	•	•	•
Squats		٠									
Step-up exercises			٠	٠	٠						
Ladder						•					

early May 2011, followed by the initiation of a KAATSU exercise program at our hospital in June 2011.

MATERIALS AND METHODS

This study was approved by the ethics committee of the University of Tokyo. The patient was informed of the methods, procedures and risks, and signed an informed consent document before participation.

- Frequency of exercise: 2 sessions of 30 minutes of KAATSU exercise of the upper and lower extremities each week (for 3 months)
- Upper extremity exercise program: The KAATSU belt was set at 40-50/140-210 mmHg, exercise menu consisting of triple sets, rowing, side raises, and depressurization (Table 1)
- Lower extremity exercise program: The KAATSU belt was set at 40-50/270-400 mmHg, Exercise menu consisting of leg extensions, leg curls, and step-up exercises (Table 2)

RESULTS

Through the 3-month KAATSU exercise program, bilateral thigh muscle volume enhanced by about 23% (0.26%/day) from 8,730 to 10,709 cc (Fig. 1) and right and left knee extensor strength enhanced by about 26% (0.29%/day) from 30.9 to 39.9 kgf and by about 44% (0.49%/day) from 38.8 to 57.4 kgf, respectively (Fig. 2).



Figure 1. Change in femoral muscle volume before and after KAATSU exercise



Figure 2. Change in bilateral knee extensor strength before and after KAATSU exercise



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Figure 3. Results of SF-36v2 questionnaire before and after KAATSU exercise

The results of the questionnaire survey using SF-36v2, a measure of health-related QOL, showed improvements in all 8 domains, including physical functioning, role functioning-physical, bodily pain, general sense of well-being, vitality, social functioning, role functioning-emotional and mental health (Fig. 3).

DISCUSSION

We experienced the case of a patient with benign fasciculation syndrome (BFS) in which low-intensity resistance exercise (KAATSU) resulted in a significant enhancement in muscle strength and myopachynsis, as well as improved QOL, including reduced pain, as assessed by a questionnaire survey. These findings suggest the usefulness of low-intensity KAATSU exercise for BFS patients.

Several studies have evaluated the effect of highintensity resistance exercise on the muscle strength of healthy subjects and patients with various disorders. Hakkinen et al.(2000) applied a 6-month highintensity knee extension resistance exercise program to middle-aged and elderly subjects and reported 28% and 21% enhancements in maximum muscle strength, respectively. Brochu et al.(2000) also applied a 6-month high-intensity resistance exercise program to an elderly female patient with ischemic heart disease and observed 18% and 23% enhancements in the muscle strength of the upper and lower extremities, respectively. The present patient exhibited 26% and 44% enhancements in right and left knee extension strength, respectively, after a 3-month exercise program. These values were higher than those reported by Hakkinen et al.(2000) or Brochu et al.(2002) and comparable to those reported from studies that applied KAATSU exercise to healthy subjects or athletes (Karabulut et al., 2007; Wernbom et al., 2008). These studies include one reported by Wernborn et al. (2008), which reviewed the effects of high-intensity lower

extremity resistance exercise and low-intensity lower extremity resistance exercise with KAATSU on quadriceps femoris myopachynsis of healthy subjects. A high-intensity exercise program performed 2-3 times/week (3-4 sessions, each consisting of 6-10 repetitions at a 60-120-second interval) resulted in a 3-21% enhancement in muscle mass in 3 months while a low-intensity KAATSU resistance exercise (3-4 sessions, each consisting of 15-30 repetitions at a 30-60-second interval) resulted in a 3.6-19.8% enhancement in muscle mass in the same period. This result demonstrates that low-intensity KAATSU resistance exercise results in a comparable degree of myopachynsis to that achieved by high-intensity resistance exercise. The present patient exhibited a significant enhancement in the cross-sectional area (CSA) of the quadriceps femoris muscle. The CSAs of the biceps femoris and adductor muscles also enhanced after KAATSU exercise. These observations suggest that low-intensity KAATSU exercise is beneficial for BFS patients, as it can induce enhanced muscle strength as well as myopachynsis in these patients.

BFS is a benign disease characterized by fasciculation as a prominent symptom, but is also accompanied by other symptoms, such as pain, anxiety, fatigability, and paresthesia, and often lasts for several months to years (Fettel et al., 1982). Major causes include strong stress and anxiety. Exercise therapy may be performed for treatment, but no effective rehabilitation program has been established. Differential diagnoses include amyotrophic lateral sclerosis (ALS), a serious neuromuscular disorder. Decreased exercise tolerance is also observed as a symptom. In the present case of a BFS patient, low-intensity KAATSU resistance exercise also resulted in improved QOL, as assessed by the SF-36 questionnaire form. Conventional exercise methods have been shown to induce minimal improvement in SF-36-based QOL in elderly subjects (Chou et al., 2012). In contrast, KAATSU exercise is performed under the conditions of low-intensity strain and stress, which might have led to improved QOL. It is also possible that the exercise program reduced stress, a precipitating factor of BFS, and thereby improved fasciculation.

Exercise is the most effective therapy for sarcopenia and also greatly contributes to improve QOL of patients through such effects as preventing lifestyle-related diseases and delaying the onset of dementia. Aging has often been associated with hyposecretion of growth hormone (GH), and GH hyposecretion has been associated with muscle atrophy and depressive symptoms, thereby affecting patients' QOL (Hull and Harvey, 2003). Although it is unclear how KAATSU exercise induces enhanced muscle strength and the mechanism of myopachynsis, it has been shown that significant increase in GH level is observed а immediately after KAATSU exercise (Takarada et al., 2000a; Takano et al., 2005; Abe et al., 2006). This observation indicates the possible involvement of increased GH level in the mechanism of myopachynsis in BFS patients. It is also possible that increased GH level might have led to improved depressive symptoms and subsequent improvement in QOL, although GH level was not measured in the present case. Future studies should examine whether GH has a role in the improvement of QOL.

In conclusion, KAATSU exercise was safely performed on a patient with BFS and improved muscle strength, muscle volume, and health-related QOL, including reduced pain, as assessed by the SF-36v2 questionnaire form. For patients with neurological disorders with pain, such as the present patient, active introduction of KAATSU-based exercise is likely to result in improved health-related QOL, as well as enhanced muscle mass and strength.

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Low-intensity kaatsu resistance exercises using an elastic band enhance muscle activation in patients with cardiovascular diseases

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Int. J. KAATSU Training Res. 2013; 9: 1-5

We examined the effects of blood flow-restricted, low-intensity resistance exercise (termed kaatsu), using an elastic band for resistance, on muscle activation in patients with cardiovascular diseases. Six patients with cardiovascular diseases [male, 69±12 (SD) years old, five old myocardial infarction and one dilated cardiomyopathy] performed biceps flexion exercises (four sets) using an elastic band for resistance with blood flow restriction [BFR (kaatsu training)] or CON (unrestricted blood flow). During a BFR (kaatsu training) session, subjects wore a kaatsu belt inflated to 110-160 mmHg on the proximal region of both arms. Surface electromyography (EMG) was recorded from the biceps brachii muscles, and mean integrated EMG (iEMG) was analyzed. Ratings of perceived exertion (RPE) were measured using the Borg scale immediately after the last set of each exercise (Post). During exercises, muscle activation increased progressively under BFR (kaatsu training) (approximately 40%), but not under CON; muscle activation was higher under BFR (kaatsu training) than that under CON in all exercises (P < 0.001). RPE at Post was also higher (P < 0.05) under BFR (kaatsu training) than that under CON in all exercises. RPE at Post was significantly correlated with increased iEMG in biceps flexion exercises (r = 0.68, P < 0.001). We conclude that kaatsu training using elastic bands for resistance enhances muscle activation in patients with cardiovascular diseases and may be an effective method to promote muscle hypertrophy in patients with cardiovascular diseases.

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Key words: elastic band, electromyography, kaatsu training

INTRODUCTION

Muscle wasting results from sarcopenia due to aging and from cachexia caused by diseases such as chronic heart failure (CHF), chronic obstructive pulmonary disease (COPD), cancer, chronic kidney disease and other diseases (Thomas 2007). Above all, CHF is a disease of remarkable muscle wasting, and preventing it by exercise training is important. Nakajima et al. (2010) recently reported that low-intensity kaatsu resistance exercises safely increase the muscle strength/mass and endurance capacity in patients with ischemic heart disease. Kaatsu training using a low-intensity resistance exercise appears to be a promising and useful resistance method for cardiac rehabilitation.

According to the American College of Sports Medicine guidelines, the conditions for inducing muscle hypertrophy and increasing muscle mass are as follows: (1) More than 65-70% work load of one repetition maximum (1-RM) is needed. (2) Three to four sets until exhaustion. (3) Frequency is two-three times per week. However, such high-intensity loads cannot be applied to patients with diseases, especially to patients with cardiovascular diseases and to elderly patients. In the past decade, several studies have reported that muscle hypertrophy can be produced with low-intensity (20–30% 1-RM) kaatsu training, regardless of age (Takarada et al. 2000; Abe et al. 2006; Fujita et al. 2007). As this type of exercise does not require lifting heavy weights, it might be a feasible method for cardiac rehabilitation requiring resistance exercise (Pollock et al. 2000; Balady et al. 2007).

As an elastic band is inexpensive, compact, and easy to use compared with machines/free weights, elastic band resistance training is feasible for use in homebased training programs for older subjects or for patients with a lower level of activity (Zion et al. 2003; Ribeiro et al. 2009; Colado et al. 2010). We examined the effects of low-intensity kaatsu exercise using an elastic band for resistance on muscle activation in patients with cardiovascular diseases.

MATERIALS AND METHODS Subjects

Six patients with cardiovascular diseases [male, 69±12 years old, five old myocardial infarction and one dilated cardiomyopathy] participated in this study. Left ventricular ejection fraction was 55±10% by

ultrasonic cardiogram. All subjects received a verbal and written description of the study and provided written, informed consent prior to participating in the study. This study was approved by the Ethics Committee of the University of Tokyo.

Protocol

One week prior to experiments, all subjects completed an orientation session, which included familiarization with BFR and the elastic band exercises using the "medium" bands (i.e., Red Thera-Bands; Hygenic Corporation; Akron, Ohio, USA) for 5 subjects and the "thin" band (i.e., Yellow Thera-Band) for 1 subject. During the orientation session, subjects sat in a chair with the testing arm placed on a table at heart level. During biceps flexion exercise, subjects were seated comfortably on a chair. Both exercises were performed using an elastic band. The exercise duration was 2.4 sec and included a 1.2-sec concentric and 1.2sec eccentric exercise cycle controlled by a metronome (50 beats/min). The exercise session (30 repetitions followed by three sets of 15 repetitions, with rest periods of 30 sec between sets and exercises) was determined based on previous studies (Yasuda et al. 2009, 2012).

BFR

In the orientation session, all subjects received instructions on how to wear the kaatsu belt (30-mm width; Kaatsu-Master, Sato Sports Plaza, Tokyo, Japan) at the most proximal region of both arms. Prior to exercise with BFR, subjects were seated on a chair and the kaatsu arm cuff was tightened around the arm to a belt pressure of 40 mmHg. The cuff was then inflated to a pressure of 100 mmHg for 30 sec and then deflated for 10 sec. This procedure was repeated, until the final cuff restriction pressure of 110–160 mmHg was achieved. Once the cuffs were inflated, they remained inflated for the entire experimental session, including rest periods between sets and exercises.

ELECTROMYOGRAPHY (EMG)

The skin was abraded with a skin preparation gel (Skinpure, Nihon Kohden, Tokyo, Japan), and cleaned with alcohol wipes. During the experiment, skin impedance was less than 2 k Ω . The ground electrode was positioned on the lateral epicondyle. Bipolar (1-cm center-to-center) surface EMG (sEMG) electrodes (Ag/AgCl; Vitrode F; Nihon Kohden) were placed along the longitudinal axis of the biceps brachii of the left upper arm. The electrode placement on the biceps brachii was at the mid position of the upper arm limb length. EMG signals were recorded and collected on a personal computer for subsequent analysis. All EMG signals were digitized at a sampling rate of 1024 Hz with a bandwidth of 0 Hz–500 kHz (AB 6216; Nihon Kohden). To determine integrated EMG (iEMG),

signals were fully rectified and integrated (Power Lab Chart 7 software, ADInstruments, Nagoya, Japan). During the experimental session, sEMG was recorded continuously, and each repetition was analyzed individually for iEMG. iEMG values were divided into groups of five successive repetitions, and the average value for each group of five repetitions was represented as a single data point for statistical analysis. To determine the iEMG ratio of agonist muscles, iEMGs during each exercise were normalized to Pre, which were iEMGs without BFR before the first set of each exercise. RPE was measured using the Borg scale immediately after the last set of each exercise (Post) (Borg 1973).

STATISTICAL ANALYSIS

Results are expressed as means \pm standard deviation (SD). A two-way analysis of variance with repeated measures (condition / time) was used to evaluate the training effects for all dependent variables. Post-hoc testing was performed using Tukey's technique when appropriate. All calculations were made with JMP statistical software package v.9.0 (SAS Institute Inc., Tokyo, Japan). Pearson's product correlation was used to determine the relationship between change in iEMG and RPE. Statistical significance was set at *P* < 0.05.

RESULTS

During exercises, muscle activation increased progressively under BFR (approximately 40%, Fig.1, Fig. 2), but not under CON. Muscle activation was significantly higher under BFR than that under CON in all exercises (P < 0.001, Fig. 2). However, during exercises, iEMGs were similar among the last five repetitions of each set under BFR and CON (BFR, 1set, 1.36±0.18, 2set, 1.37±0.28, 3set, 1.44±0.16, 4set, 1.43±0.18, P = NS; CON, 1set, 0.98±0.19, 2set, 0.84±0.12, 3set, 0.86±0.17, 4set, 0.93±0.11, P = NS).

RPE at Post was also higher under BFR than under CON in all exercises (BFR vs. CON; 1set, 12.5 ± 0.5 vs. 11.3 ± 0.8 ; 2set, 13.0 ± 1.1 vs. 11.7 ± 1.0 ; 3set, 13.2 ± 1.0 vs. 11.7 ± 1.0 ; 4set, 13.8 ± 1.3 vs. 11.8 ± 1.0 ; all P < 0.05, Fig. 3). During exercises, RPE significantly increased progressively under BFR (P < 0.005, 4 set vs. 1 set), but not under CON. RPE at Post was significantly correlated with increased iEMG in biceps flexion exercises (r = 0.68, P < 0.001, Fig. 4).

DISCUSSION

These results suggest that low-intensity kaatsu resistance exercises using an elastic band increase muscle activation in patients with cardiovascular diseases. Low-intensity kaatsu resistance training using an elastic band appears to be a promising and useful resistance method for cardiac rehabilitation.

In our previous study, a significant increase of





sEMG

iEMG

Figure 2. Relative integrated EMG data for agonist muscle in the experiments. Data represent the last five repetitions of each set. CON=control, BFR=blood flow restriction (kaatsu training). ***, *P*<0.001 vs. CON; Mean ± SD.



Figure 3. Borg scale after the last set of each exercise. CON=control, BFR=blood flow restriction (kaatsu training). *, *P*<0.05 vs. CON; ¶, *P*<0.005 vs. 1 set; Mean ± SD.



Figure 4. Correlation between relative integrated EMG for agonist muscle in the last five repetitions of each set and Borg scale after the last set of each exercise.

muscle cross-sectional area (CSA) in the quadriceps femoris was observed in patients with ischemic heart disease (Nakajima et al. 2010). Similarly, kaatsu training also increased hamstring and adductor CSA. The estimated thigh CSA in that study increased by approximately 4-10% (P<0.05) following three months of the kaatsu training (2 days per week, 32 total training sessions). Muscle CSA in the quadriceps femoris at 30%, 50% and 70% position of femur length increased 5.1%, 4.6% and 10.4%, respectively. Therefore, the magnitude of the hypertrophic potential (percent increase in muscle CSA divided by total training sessions) in the thigh was about 0.17-0.31% per training session. Recently, Wernborn et al. (2008) reviewed the effects of high-intensity resistance exercise and low-intensity kaatsu resistance exercise

(leg extension) on muscle hypertrophy of the quadriceps femoris in healthy subjects. High-intensity extension exercise two to three times per week, using 80% 1-RM (three to four sets, 6-10 repetitions, interval 60-120 sec), increased the muscle mass by 0.03-0.26% per day and 1-7% per month (3-21% per three months). On the other hand, low-intensity kaatsu training using 20-50% 1-RM (three to four sets, 15-30 repetition, interval 30-60 sec) increased it by 0.04-0.22% per day and 1.2-6.6% per month (3.6-18% per three months). Thus, low-intensity kaatsu resistance training appears to increase muscle mass in a similar way to heavy resistance exercise in healthy subjects. In our previous study, increases of muscle CSA in the quadriceps femoris at mid-thigh were 4.6% per three months (Nakajima et al. 2010), suggesting that even low-intensity kaatsu resistance training may be a useful method for inducing muscle hypertrophy in patients with cardiovascular diseases.

The mechanism by which BFR potentiates the training effect of low-intensity resistance training remains obscure, but it appears to be related, in part, to an increase in muscle activation (Moritani et al. 1992; Takarada et al. 2000; Yasuda et al. 2009). Yasuda et al. (2009) examined muscle activation during lowintensity kaatsu resistance exercise in healthy subjects. Unilateral elbow flexion muscle contractions (20% 1-RM) were performed in their experiment (three to four sets, 15-30 repetitions, interval 30 s) with a moderate restriction of blood flow, complete occlusion of blood flow or unrestricted blood flow (control). In that experiment, the changes in iEMG were greater with moderate restriction of blood flow than in the control but without the apparent contractile metabolic impairment observed with complete occlusion, suggesting that low-intensity muscle contractions with moderate restriction of blood flow leads to more intense activation of the muscle relative to the external load. Meanwhile, recently, the potential mechanisms behind the blood flow restriction stimulus (5 min, 5 repetitions, interval 3 min) in the absence of exercise were investigated (Loenneke et al. 2012). Significant increases in muscle thickness were observed for both the vastus lateralis (6%, P<0.05) and rectus femoris (22%, P=0.001) along with a significant decrease in plasma volume (15%, P=0.001) and the changes in muscle thickness were maintained even after the cuffs had been removed, suggesting that the attenuation of both the muscle atrophy and the decline in strength previously observed with brief applications of blood flow restriction may have been mediated through an acute fluid shift-induced increase in muscle size. Thus, the mechanism by which BFR potentiates the training effect of low-intensity resistance training may in part be related to the acute muscle swelling effects as well as an increase in muscle activation.

Elastic bands/tubing are widely used in rehabilitative

medicine and in health enhancement for resistance training (Zion et al. 2003; Ribeiroet al. 2009; Colado et al. 2010). Elastic bands are also portable, less expensive and easier to use than weight machines/free weights. Elastic resistance training has been shown to be a feasible alternative to high-intensity resistance training (Colado & Triplett 2008; Ribeiro et al. 2009; Andersen et al. 2010). Yasuda et al. (2012) observed that lowintensity, elastic band resistance exercise combined with BFR enhances muscle activation in healthy young subjects. Similar findings were observed in our study using patients with cardiovascular diseases.

Hemostatic and inflammatory responses are major concerns for patients with cardiovascular disease when performing an exercise, because these responses may be related to the cardiovascular events observed during and after strenuous exercise (Womack et al. 2003). However, our previous study showed that applying BFR during low-intensity resistance exercise did not affect exercise-induced changes in markers of hemostasis (D-dimer and FDP) and inflammation (hsCRP) in patients with ischemic heart disease, although the heart rate and plasma noradrenaline concentration were increased (Madarame et al. 2013). Thus, it was suggested that low-intensity kaatsu resistance training using an elastic band was safe as well as effective.

In conclusion, low-intensity kaatsu resistance training using elastic bands enhances muscle activation in patients with cardiovascular diseases and may be an effective method to promote muscle hypertrophy in patients with cardiovascular diseases. Low-intensity kaatsu training using an elastic band may therefore be an effective method to promote muscle hypertrophy in older adults or in patients capable of tolerating only low-load resistance exercise.

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KAATSU training[®] in a case of patients with periventricular leukomalacia (PVL)

H. Iwashita, T. Morita, Y. Sato, T. Nakajima

Int. J. KAATSU Training Res. 2014; 10: 7-11

[Objective] The effectiveness of KAATSU training has been reported in wide-ranging fields from sports medicine to rehabilitation, and KAATSU training has been clinically applied. However, there have been only limited reports on pediatric cases. We performed KAATSU training in a pediatric patient with periventricular leukomalacia (PVL) and examined its effectiveness and safety for cerebral palsy. We report here the results of this examination.

[Methods] KAATSU training was performed on a PVL patient on an outpatient basis. This training was performed once a week for 14 weeks and involved mainly three specified motions of the upper and lower limbs. Evaluation was performed using the Gross Motor Function Measure (Gross Motor Function Classification System: GMFM) and videos. In this report, the effectiveness of short-term KAATSU training was compared with that of intensive physical therapy using data in the literature.

[**Results**] QOL improved due to short-term reduction of muscle tonus and increased acquired movements resulting from KAATSU training.

[Conclusion] The results of this report suggest that KAATSU training can be effective in PVL patients. Further examination is necessary with increased cases and evaluation methods.

Key words: Periventricular leukomalacia, KAATSU training, spastic paralysis, cerebral palsy

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INTRODUCTION

PVL is a disorder reported by Banker et al. in 1962¹). It occurs most commonly in premature infants, mainly those born at less than 32 weeks of gestation. It is a lesion with periventricular necrosis as its major component. Since the corticospinal tracts pass through the periventricular white matter, spastic paraplegia develops that is particularly severe in the lower limbs. The severity of the disability or impairment can vary, including severe impairment such as dragging of feet when walking, inability to sit, inability to walk, and inability to swallow. Intellectual impairment is also seen in many cases. There is currently no effective treatment. We report here a case of PVL in which KAATSU training was effective for spastic quadriplegia. In addition, we compared the effectiveness of shortterm KAATSU training with that of intensive physical therapy using data in the literature.

METHODS

1. Ethical consideration

This study was approved by the Research Ethics Committee of the Graduate School of Medicine and Faculty of Medicine at the University of Tokyo (application number: 3088). Informed consent was obtained from the guardian of the patient before participation in this study.

2. Case report

Patient: A boy aged 7 years and 10 months Diagnosed disease: PVL

Chief complaint: Spastic quadriplegia

Family history: Non-contributory

History of present illness: The patient was born by cesarean section at 28 weeks and 1 day of gestation and had a birth weight of 996g.

The patient and his twin sibling were conceived naturally. His twin sibling was born first and had died at birth.

One- and five-minute Apgar scores were 6 and 7, respectively.

The patient was an extremely low-birth weight infant with apneic events and RDS and was hospitalized in the NICU until 91 weeks after birth.

Age 1 year and 7 months: initiation of physical therapy Age 2 years and 9 months to 3 years and 10 months: initiation of botulinum toxin therapy (total: 4 times)

Age 4 years and 10 months: bilateral muscle release in the hip

The patient continued rehabilitation therapy but began KAATSU training because muscle tonus increased with the growth of the body and acquisition of movements slowed.

3. Methods

a) Motion conditions

KAATSU belts were placed around the upper arms

in the axillary area and around the upper legs in the thigh area. KAATSU MASTER was used for the training. The upper limb training began with a cuff tightness pressure of 40-45 mmHg and a setting pressure of 80-90 mmHg, and the lower limb training with 55-65 mmHg and 110-120 mmHg, respectively. Since the subject was unable to speak, appropriate pressure was estimated using the coloration of the palms and feet, pulse rate, and facial expression. Pressure was applied and released and this process was repeated three times. Passive ROM training was performed, and then KAATSU training was begun. The training involved the following items: three specified motions of the upper and lower limbs, KAASTU walk, and muscle strength training using light weights. One to two sets of the first item were performed once a week for 14 weeks. Generally, this item was performed 10-20 times for the first set and in an all-out manner for the second set. Careful observations were made of changes in the patient's physical conditions during the training. Care was taken to control the vagal reflex by keeping the patient's head below the level of his heart when the pressure was released.

b) Evaluation

1) GMFM

The GMFM evaluates the motor ability of children with cerebral palsy and involves movements that can be performed by children with normal motor ability by age 5 years. These movements are classified into five dimensions of A: lying and rolling, B: sitting, C: crawling and kneeling, D: standing, and E: walking and running. The GMFM contains 88 items each of which is scored on a 0-3 rating scale and can assess in details the motor ability of young children^{2,3}). The GMFM is effective in the evaluation of short-term changes⁴). It was used to evaluate and compare our patient's motor ability before KAATSU training and

after 14 weeks of training.

2) Video

In cerebral palsy associated with PVL, there is a combination of various factors, including delayed motor development, abnormal postural tone such as hypertonia, and sensory, perception, and cognitive immaturity⁵). Therefore, its mechanism of pathogenesis is difficult to evaluate. Thus, in this report, comprehensive evaluation and examination were performed using evaluation of videos.

RESULTS

1. GMFM results

Table 1 shows the GMFM results of the patient. The GMFM was also evaluated before and after botulinum toxin therapy and before and after bilateral muscle release in the hip. These results are also included in the table. The GMFM dimensions of D: standing and E: walking and running were omitted because the patient was unable to perform these motor functions. When the GMFM results were compared before botulinum toxin therapy and at the third therapy session, the motor functions were markedly improved as shown in Table 1. However, when comparison was made for each injection, there was less improvement. There was almost no improvement at the third therapy session. For bilateral muscle release in the hip, Table 1 shows improvements but the scores were decreased before KAATSU therapy began. That is, bilateral muscle release in the hip has limited effectiveness, and it also places a burden on young children.

For KAATSU training, there was one post-training evaluation at three months after training. Thus, longterm evaluation is necessary. In the short-term evaluation, the scores increased to 51/51 for A: lying and rolling and 36/60 for B: sitting. Therefore, KAATSU training showed effectiveness that was comparable to or better than other treatment methods.

	KAATSU	J training		Bilateral muscle release in the hip						
	At the start	After 3 mo	Fi	rst	Second		Third		Before surgery	After 1 mo
			Before injection	After 3 mo	Before injection	After 3 mo	Before injection	After 3 mo		
A: Lying and rolling	41/51 (80.0%)	51/51 (100%)	43 (84.3)	43 (84.3)	45 (88.2)	50 (98.0)	50 (98.0)	50 (98.0)	45 (88.2)	51 (100)
B: Sitting	28/60 (46.6)	36/60 (60.0)	11 (18.3)	20 (33.3)	20 (33.3)	26 (43.3)	26 (43.3)	29 (48.3)	20 (33.3)	29 (48.3)
C: Crawling and kneeling	5/42 (11.9)	8/42 (19.0)	0	3 (7.1)	3 (7.1)	7 (16.6)	7 (16.6)	6 (14.2)	3 (7.1)	9 (21.4)

Table 1. GMFM evaluation of KAATSU training, botulinum toxin therapy, and bilateral muscle release in the hip in our patient

VIDEO EVALUATION

Figure 1 shows images of the patient immediately after beginning KAATSU training. There was severe muscle tonus, and bands were placed at his waist and ankles for immobilization when sitting in the wheelchair (Fig. 1a). Adductor muscle tonus was particularly severe, and three staff members were required to place KAATSU belts on the lower limbs at the first training session. The patient also had difficulty moving the upper limbs only and the lower limbs only. When the patient performed arm curls, his trunk would bend backward (Fig. 1b). Similarly, when the patient kicked a ball, his trunk would bend backward and he needed to be supported from the back (Fig. 1c). In KAATSU walk, his feet would move forward but his knees would not fully extend, showing equinus deformity (Fig. 1d).

Figure 2 shows the conditions on the 14th week of KAATSU training. The patient achieved independent sitting and reduced adductor muscle tonus (Fig. 2a).



Figure 1. Photographs taken immediately after KAATSU training began (explanations for Figures 1a, 1b, 1c, and 1d are in the main text)



Figure 2. Conditions during KAATSU training on the 14th week (explanations for Figures 2a and 2b are in the main text)



Figure 3. Acquired movements after KAATSU training (on the 14th week) (explanations for Figures 3a, 3b, and 3c are in the main text)

In addition, the patient was able to move the upper limbs only and the lower limbs only. Beginning around the tenth week, he was able to sit on a chair on his own to perform his upper limb training (Fig. 2b).

Figure 3 shows the acquired movements after 14 weeks of KAATSU training. Since paper cups are soft and easily crushed, handling such cups is a difficult act for young children with severe spasticity. Our patient was able to use paper cups after KAATSU training (Fig. 3a). He was previously unable to crawl because his trunk area was weak and he could not maintain the posture. However, he was able to successfully crawl after KAATSU training (Fig. 3b). The changes in the lower limbs were not sufficiently large to be observable on images. However, the patient was able to extend his knees to put his heels on the floor while staff supported him in standing training (Fig. 3c). When comparison was made between the video immediately after training began and that after 14 weeks of training, the results suggest that KAATSU training can help reduce muscle tonus, improve muscle control, and increase muscle strength.

DISCUSSION

When the effectiveness of training in young children is examined, it is most important to determine if the positive results are due to children's development or the effects of training. There are several reports on development that is characteristic of young children with cerebral palsy. In the report by Rosenbaum et al., the motor development curve was until 15 years of age. The children reached 90% of their motor function based on the GMFM by approximately age 5 years, and the motor development curve plateaued by approximately 7 years⁶). In addition, the subjects were determined to have level IV severity of cerebral palsy based on a five-level severity scale of the Gross Motor Function Classification System (GMFCS)⁷⁾. Hanna et al. reported that prognosis differed depending on the GMFCS level and that the muscle strength in level IV children decreased starting around 7 years of age⁸⁾. It is also speculated from the results of these reports that the subjects were in a state where acquisition of movements was difficult due to his development. In such a state, acquired movements gained in the short term of 14 weeks can be attributed to the effectiveness of KAATSU training.

The effectiveness of short-term KAATSU training was compared with that of intensive physical therapy. Intensive physical therapy increases the GMFM scores compared with conventional physical therapy and is recommended (grade B) in the second edition of the Guidelines for Rehabilitation of Children with Cerebral Palsy⁹⁾. Comparison was made between our patient and other patients with GMFCS level IV (same as our patient) who were reported in the literature^{10),11),12)} (as shown in Table 2). In the study of Asakai et al.¹³, the mean GMFM percent score of patients at GMFCS level IV was 28.8% at the first examination, and there was an improvement of 2.1 percentage points after a mean of 1.9 months of intensive physical therapy. In our patient, the mean GMFM percent score was 27.7% at the initial examination, and there was an improvement of 8.1 percentage points after 14 weeks of KAATSU training. Asakai et al. used a short training period with a mean of 1.9 months. The frequency was 5 sessions per week (40 minutes per session) for physical therapy, 5 sessions per week (20 minutes per session) for in-ward training (standing and walking training performed by ward personnel), and a mean of 3 sessions per week for occupational therapy. Other services were provided as needed, including speech therapy and psychological evaluation and counseling. Our patient underwent less frequent and shorter training sessions at one session per week and about 40 minutes per session (including time used to place the device). Asakai et al. found that the effectiveness of intensive training was shown by approximately 7-10 years of age, mainly for gross motor functions in young children with spastic cerebral palsy at GMFCS level IV. The effectiveness was particularly marked by age 6. The mean age of these children was 3 years and 8

Study	Study No. of cases GMFM severity		GMFM severity	Mean age	Frequency of training	Training period	Changes in total score
Mal anablin at al	1009	17	71.20/	7.2	171.0 h/m	1 yr	4.2%
McLaughlin et al. 1998		1/	/1.3%	7.2 yr	1/1.8 n/yr	2 yr	7.2%
Wright et al.	1998	12	56.5%	4 yr 10 mo	116 min/wk	1 yr	4.4%
Bower E. et al.	2001	28	GMFM III or below	5.5 yr	1 h/day or more	6 mo	5.1%
	2002	47	GMFM III 52.6%	5 yr 5 mo	40 min/day or	2.1 mo	3.7%
Asakai et al.	2003	55	GMFM IV 28.8%	3 yr 8 mo	more	1.9 mo	2.1%
Our patient	2013	1	GMFM IV 27.7%	7 yr 10 mo	40 min/wk	14 wk	8.1%

 Table 2. Effectiveness of intensive physical therapy and KAATSU training in improvement of GMFM

(partly revised from Asakai et al.¹³⁾

months. Our patient was older at 7 years and 10 months, but there was large improvement of 8.1 percentage points. These results suggest that a certain level of effectiveness can be expected from KAATSU training in young children with cerebral palsy compared with other training methods. In addition, KAATSU training has the advantages of being minimally invasive and easy to perform repeatedly. However, there are many issues, including accuracy in the comparison of our case with cases in other reports because of the small number of cases and non-uniformity of severity, rehabilitation period, and environment. Thus, further studies are necessary.

CONCLUSION

The results of this study suggest that KAATSU training can be an effective treatment method for young children with PVL. Further studies are necessary with increased number of patients and evaluation methods.

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KAATSU training[®] as a new exercise therapy for femoral head avascular necrosis: A case study

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Femoral head avascular necrosis is a condition in which part of the femoral head undergoes necrosis due to decreased blood flow. The femoral head gradually disintegrates causing pain and even today, there are no effective rehabilitation methods other than symptomatic treatment such as decreasing the load on the hip joints with the use of a cane or walker. We herein describe our insights into this condition based on our experience with a case of femoral head avascular necrosis caused by steroid use in which KAATSU training was found to be highly effective. The patient was a 34-year-old woman (154 cm tall and weighing 50 kg, a radiologist). Since the age of 23, this patient had been receiving steroid treatment to control her refractory asthma. She later developed pain in her right hip and aradually suffered hip joint deformation, restricted range of motion, and difficulty in walking. MRI revealed Association Research Circulation Osseous (ARCO) stage IV disease. She suffered marked pain of the right hip joint every time she walked, occasionally falling and required a cane to walk. At the patient's own request, she received KAATSU training including KAATSU walking over a period of 3 months (total 28 sessions). Various assessments were carried out before and after training to determine the effects of KAATSU training. QOL was determined by SF-36v2, and marked improvement of role physical, body pain, general health, vitality, and social functioning were noted. Before training, the Japanese Orthopaedic Association (JOA) hip scores for pain were 10 (right), 40 (left), walking ability 16 (right), 20 (left), while 3 months after training, these scores were markedly improved in the affected side. Furthermore, not only did muscle strength on the affected side show marked improvement, but the MRI also revealed a tendency for improvement of the right femoral head avascular necrosis. DEXA showed signs of a clear increase in bone mineral density. Based on the above, these results suggest that KAATSU training is extremely useful as a rehabilitation method in patients with femoral head avascular necrosis. But, further larger scale investigations should be carried out in the future to support our findings.

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Key words: Femoral head avascuar necrosis, KAATSU training, Bone density, Pain, Rehabilitation

Introduction

Femoral head avascular necrosis is a condition in which part of the femoral head suffers necrosis due to a decrease in blood flow. Although the cause remains unknown, possibilities include thrombosis due to abnormalities in the blood coagulation system, and injuries of the vascular endothelium (Chang et al., 1993). Risk factors include the administration of steroids (Weinstein, 2011). Steroid administration leads to decreases in blood flow due to increases in the internal pressure of the femoral head resulting from hypertrophy and proliferation of adipocytes. Together with decrease in blood flow, it has been reported that direct inhibition of osteoblasts and osteocytes may also be involved (Cruess, 1976; Kerachian et al., 2009; Ding et al., 2015). Pain occurs as the femoral head disintegrates, and other than symptomatic treatment such as using canes and walkers to reduce weight bearing stress upon the hip joint, there are no effective rehabilitation methods (Wang et al., 2014). Muscle

strength training is often used in the rehabilitation exercises but there are cases where pain will make it impossible to exercise while weight-bearing is not possible and so this is often ineffective. Therefore, even today, it is difficult to prevent the progression of disease with conservative treatment alone, and prognosis is poor so that surgery is eventually required.

KAATSU training (Takarada et al., 2000; Sato et al., 2007) allows for muscle growth when blood flow is restricted, even with low stress training, and major increases in muscle mass can be achieved. It is indeed a useful rehabilitation method for patients with various diseases and our aging society (Nakajima, 2010; Abe et al., 2010; Ozaki et al., 2011; Nakajima et al., 2011; Yasuda et al., 2014). Exercise methods include no load, elastic band (Yasuda et al., 2015), and in addition to free weight, walking (Abe et al., 2006), and ergometer (Abe et al., 2010). In this way KAATSU training is being featured as a method of rehabilitation in cases where high exercise loads are to be avoided.

In addition, KAATSU training with restricted blood flow causes decreases in the intramuscular oxygen partial pressure during exercise and hypoxemia. With active refilling of vasculature in response to belt release, reactive hyperemia develops and vascular endothelial growth factor (VEGF) and nitric oxide (NO) production are enhanced (Takano et al., 2005; Horiuchi and Okita, 2012) and this may lead to improvements in both endothelial function and circulation. In this way, KAATSU training appears to be effective as a rehabilitation method in patients with femoral head avascular necrosis, but there are few reports regarding the effects of KAATSU training in these patients until now.

We experienced a case of femoral head avascular necrosis in one patient and would like to report our findings.

1. Case introduction

Case report: A 34-year-old woman (height 154 cm, weight 50 kg, profession radiologist)

Diagnosis: femoral head avascular necrosis

Chief complaint: pain of the hip joint during ambulation, gait disturbance.

Family history: no notable findings.

History of present illness: bronchial asthma with an onset during youth. Since experiencing a severe attack of aspirin-induced asthma at age 15, the patient became dependent on steroids and repeatedly required hospitalization because of poor control and had continued to take a large dose of steroids. As of age 23, HOT was introduced, and since age 25, as soon as her clinical rotations started, pain in the right hip joint began to manifest. Thereafter, deformation of the right hip joint progressed until at age 33, and she developed femoral head avascular necrosis (OA). Gradually, the hip joint pain worsened with the limitations in her range of motion (ROM), and abduction external rotation contracture appeared. The patient suffered extreme pain in the right pelvis during walking and in order to reduce the joint load during walking had been using a cane. She presented at our hospital hoping to receive exercise treatment with KAATSU training. The patient had been taking prednisolone 3 mg/day, theophylline, montelukast sodium, and salmeterol/fluticasone.

This research was approved by the Institutional Review Board at the University of Tokyo and performed after receiving patient consent.

2. KAATSU training protocol

A KAATSU belt is wrapped around the base of the thigh and a KAATSU Master device (KAATSU Japan, Co., Ltd) was used. KAATSU training was provided for a total of 28 sessions over 3 months. Base pressure was 45 – 50 SKU. The optimal pressure started at 100

- 140 SKU and while repeatedly pressurizing and depressurizing, it gradually increased in 20 SKU increments, until an optimal pressure was reached. The training menu was as follows. 1) 3 point set: Toe curls, ankle dorsiflexion, and ankle plantar flexion. 2) KAATSU walk: gradually increased distance to approximately 150 to 300m during each session until a comfortable walking speed was achieved. 3) Non-KAATSU walk: belts were removed and the patient was allowed to walk for 75m to check for symptoms. In late stage intervention, calf-raises and squats were added, and the patient extended her comfortable walking distance to approximately 300m. Calf raises and squats were performed standing and bearing one's own weight. The KAATSU side in the early stage of intervention was the affected leg only, starting with a KAATSU pressure of 300-320 SKU. In the latter stages, in addition to the affected right thigh, the healthy left thigh (160-300 SKU) exercise therapy under KAATSU condition was also conducted.

3. Evaluation

The following evaluations were conducted before and after training.

- 1) Magnetic resonance imaging (MRI)
- 2) Life functioning: 36-item short form health survey (SF–36v2).
 - The effect of training on life functioning was investigated using the SF-36v2 evaluation survey. Role physical, body pain, general health, vitality, social functioning, role emotional, and mental health were assessed.
- 3) Muscle strength evaluation using Cybex
- Measurements were taken with the subject seated in a chair with the trunk and thighs immobilized. Exercise involved bending the knee joint 90°, from a flexed position to extension and flexion. Bilateral lower limb muscle strength (isomeric knee extension strength: knee joint 75°, isokinetic knee extension and flexion muscle strength: 30°/sec, 90°/sec, 180°/ sec) was determined.
- 4) Functional analysis of the hip joint by Japanese Orthopaedic Association (JOA) hip scores as shown in Table 1 (Takatori et al., 2010)
- 5) Dual energy x-ray absorbed absorptiometry (DEXA) bone mineral density measurement device.

4. Results and clinical course

MRI findings before training are shown in Fig. 1A (left). T1 and T2 weighted images of the right femoral head weight-bearing areas revealed areas of low intensity signals. The surrounding areas showed high signals in the T2 weighted image, suggesting bone necrosis and an edematous change of the surrounding marrow. Her right hip joint showed joint space narrowing and formation of bone spurs, while the femoral head showed signs of flattening leading to

<u>I. Pain</u>			point					
No complaints regarding the hip joint								
Indefinite complaints (feeling strange, fatigue) present but no pain								
No pain during walking (however at the start of walking or after walking for long distances there is sometimes pain)								
No spontaneous pain. Pain during walking, but disappears with short rests								
Spontaneous pain is sometimes present. Pain during walking but alleviates with rest								
Continuous spontaneous pain or nocturnal pain			0					
II. Range of motion assessment								
Flexion: Joint angle counted in 10° intervals, one point per 10°. However, all points bey	ond 120° are	counted						
as 12 points (if joint contracture is present this is subtracted and evaluations based on m	ovable range)	•						
Abduction: Joint angles are measured in 10° increments and each 10° is counted as 2 pc	oints.							
However any angle over 30° is counted as 8 points								
III. Walking ability								
Absolutely no complaints regarding the joint								
Indefinite complaint (feeling strange, fatigue), no pain								
No pain during walks (however there may be pain at the start of the walk or after walki	ng long distar	ices)	30					
No spontaneous pain. Pain during walks disappears after short rests.			20					
Spontaneous pain is sometimes present. Pain present during walks but is relieved after r	est.		10					
Continuous or spontaneous pain or nocturnal pain.			0					
IV. Activities of daily living (ADL)								
Evaluation	easy	difficult	impossible					
Knee blankets	4	2	0					
Standing work (includes housework) (continues for about 30 minutes).	4	2	0					
If rests are required, it is considered "difficult". In cases where exercise								
can only be continued for 5 minutes, it is considered "impossible".								
Squatting and standing (those who require assistance: consider it "difficult")	4	2	0					
Climbing up and down stairs (those who require a railing: consider it "difficult")	4	2	0					
Getting on and off cars and buses	4	2	0					



Figure 1. A: MRI findings before and after KAATSU training. Before training (left), after training (right) B: Dual-energy x-ray absorptiometry (DEXA) scan bone mineral density findings before and after KAATSU training. After training, bone mineral density in the right leg (affected side) was clearly increased, compared with the left leg (healthy side).



Figure 2. Efficacy of KAATSU training in a patient with femoral head avascular necrosis. A: Effect on JOA score. a: JOA score (total, comprehensive), b: Pain, c: Walking, d: ROM (range of motion), B: SF-36v2. Role physical, Body pain, General health, Vitality, Social functioning, Role emotional, Mental health

a case of secondary arthrosis. Surrounding areas included a small high-signal intensity subchondral bone cyst. The other hip joint showed no signs of abnormally high intensity areas. Based on these MRI findings, the patient was diagnosed with Association Research Circulation Osseous (ARCO) stage IV right femoral head avascular necrosis and secondary hip arthrosis.

Before and after training, several evaluations were carried out. Fig. 2A shows the effectiveness of KAATSU training on JOA hip scores. JOA (pain, b) scores were 10 (right), 40 (left); while the JOA (walk, c) scores were 16 (right), 20 (left); and JOA (score, comprehensive, a) was 55 (right), 84 (left). After training, all scores showed marked improvement in the right affected leg compared to the left healthy leg. JOA (ROM (range of motion), d) revealed no signs of improvement on the affected side. In the latter half of training, pain of the hip joint during walking dissipated so that the patient no longer required a cane to walk. In addition, there was a clear improvement in the patient's walking style.

Fig. 2B shows the effect on SF-36v2 before and

after KAATSU training. Role physical, body pain, general health, vitality, social functioning, role emotional, and mental health were evaluated. Physical function, role physical, body pain, general health, vitality, social functioning all improved after KAATSU training

Figure 3 shows the effects of KAATSU training on lower limb muscle strength (extension and flexion). Muscle strength during extension and flexion was markedly improved in the right leg on the affected side, compared to the healthy left leg.

Fig. 1B shows the efficacy of using KAATSU training on bone mineral density measured by DEXA. There was a clear increase in right affected leg bone mineral density from 0.798 to 0.836. On the other hand, the left healthy leg went from 0.857 to 0.868 with no clear signs of improvement. Furthermore, MRI findings after KAATSU training shown in Fig. 1A (right) showed a tendency for the right femoral head necrosis to improve.



Figure 3. Effect of KAATSU training on lower limb muscle strength (knee extension, knee flexion) measured by Cybex.

5. Discussion

We treated a patient with femoral head avascular necrosis for 3 months with KAATSU training and noted the following improvements. 1) After KAATSU training, the JOA score of the hip joint and the JOA pain score both improved markedly. 2) Together with an apparent improvement in gait, femoral muscle strength improvement was noted while bone mineral density of the lower limbs had clearly increased in the affected side. 3) MRIs showed a tendency for improvement of the right femoral head necrotic site.

In this patient, SF–36v2 was evaluated. Of the life functions, physical function, role physical, general health, vitality, social functioning, and walking ability based on the Japan Orthopedic Association hip score (JOA hip score) (Takatori et al., 2010) had all improved. Furthermore, muscle strength on the affected side had increased, proving that muscle strength improvement with KAATSU training can lead to improved walking ability and have a major impact on enhancing QOL. This meant that in the latter half of the training, the patient became able to walk without a cane. In addition, the affected limb could support weight without use of a cane, and it is believed that there was a clear increase in bone mineral density on the affected side.

The mechanism for this effect of KAATSU training includes a characteristic of KAATSU training (Takarada et al., 2000; Sato et al., 2007) which is that exercise under restricted muscle blood flow conditions leads to increased muscle strength and muscle growth after only a short period of low stress exercise. In this case, we observed a clear increase in muscle strength with only 3 months of KAATSU training. KAATSU training is just the right rehabilitation method for patients with various diseases and our aging society (Nakajima, 2010; Abe et al., 2010; Ozaki et al., 2011; Nakajima et al., 2011; Yasuda et al., 2014). Loading included nonweight bearing, elastic band (Yasuda et al., 2015), a dumbbell, walking (Abe et al., 2006), and ergometer (Abe et al., 2010) loads. In this way, KAATSU training is an extremely useful rehabilitation method for use in patients in whom excessive stress would be inappropriate. In our case, we added squats and calf raises to the KAATSU walking schedule, carrying out various forms of exercise in a way that the femoral head would not have to bear any weight. As a result, patient ADLs improved markedly. However, the ROM did not improve. Therefore, in the future we believe a longer investigation is warranted.

Furthermore, after KAATSU training, pain scores based on the SF-36v2 and JOA hip scores showed clear signs of improvement. With improvement of her pain, the patient no longer required a cane and we believe her quality of life has been greatly improved because she regained the ability to walk. The mechanism responsible for improving pain in KAATSU training is still unknown, but increased muscle strength, muscle growth and alleviation of the weight-bearing on the hip joints are believed to play a role. On the other hand, KAATSU training leads to a decrease in intramuscular partial O2 pressure and hypoxemia during exercise under restricted blood flow. Reactive hyperemia develops and vascular endothelial growth factor (VEGF), and nitric oxide (NO) production is enhanced (Takano et al., 2005; Horiuchi and Okita, 2012) in response to vascular refilling after the belts are released, leading to improvements in endothelial function and blood flow. We propose these improvements in blood flow helped to heal the necrosis in the femoral head. Animal models of femoral head avascular necrosis caused by steroids have actually shown that as blood flow into the femoral head decreases, both VEGF protein and mRNA decrease (Wang et al., 2010). However, the improvement of MRIs in the present study for 3 months was minor so the effects should be further confirmed in a long-term study. In addition,

investigations into elucidating the mechanism behind pain relief with KAATSU training are warranted.

Conservative treatment for femoral head avascular necrosis should be instituted carefully to avoid crushing the femoral head, and there are currently no effective rehabilitation methods other than symptomatic treatment such as using a cane or walker to reduce placing weight on the affected hip joint. From the present study, KAATSU training may be a useful rehabilitation method to treat femoral head avascular necrosis.

Summary

We reported our experience with a patient who developed femoral head avascular necrosis while on steroid therapy who was successfully treated with KAATSU training. We believe further clinical research will be necessary.

<Nakajima T, Yasuda T, Fukumura K, and Morita T have participated in seminars until September 2014 donated by KAATSU Japan.>

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Effects of low-intensity "KAATSU" resistance exercise on hemodynamic and growth hormone responses

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Growth hormone (GH) is secreted in a pulsatile fashion during exercise, which promotes skeletal muscle growth and muscle strength. We compared the effects of different types of short-term lowintensity resistance exercise (STLIRE) on the hemodynamic and GH responses of men aged 20 to 45 years. Eleven untrained men performed 30 repetitions for 2 to 4 sets (mean 61 \pm 7 repetitions) until fatigue for bilateral leg extension-flexion exercise (20% of 1 RM -Proteus Multi Exercise Machine) under the conditions of reduced muscle blood flow by applied pressure at the proximal ends of both legs by a specially-designed belt (Kaatsu Training). In the controlled exercise condition, without Kaatsu (n=9), subjects again performed the same exercise protocol as described above. Finally, a group of 5 men performed 3 sets of 10 repetitions using the Power Rehabilitation machine. Hemodynamic parameters were measured by using the impedance cardiography. Serum concentrations of GH, noradrenaline (NOR), and lactate were also measured. STLIRE with Kaatsu significantly increased GH, compared to STLIRE without Kaatsu. Maximal heart rate (HR) and blood pressure (BP) in STLIRE with Kaatsu were higher when compared to the control condition, however, stroke volume (SV) was lower compared to the controlled condition due to a decreased venous return induced by Kaatsu training. Total peripheral resistance (TPR) did not change significantly. The increase in NOR and lactate in STLIRE with Kaatsu was also significantly higher than without Kaatsu. These results suggest that "Kaatsu" leg resistance exercise caused a significant exercise-induced GH response even in STLIRE, with a reduction of cardiac preload during exercise. The results of the study indicate that Kaatsu training may become a unique method for rehabilitation in patients with cardiac diseases or low physical fitness.

Key words: resistance exercise, growth hormone, hemodynamics, cardiac output, rehabilitation

INTRODUCTION

Heavy resistance training has been known to be a potent stimulus for muscle cell growth and hypertrophy (MacDougall et al., 1977; Staron et al., 1984), resulting in improvement of muscle strength and increased exercise capacity. This is due, in part, to the exercise-induced increase of endogenous anabolic hormones and growth factors such as GH and IGF-1 (Kraemer et al., 1990). GH has also been well established as a regulator of cardiac growth, structure and function (Lombardi et al., 1997; Khan et al., 2002), and GH has been used in the treatment of congestive heart failure in patients with dilated cardiomyopathy and myocardial infarction (Fazio et al., 1996; Genth-Zots et al., 1999). However, exerciseinduced GH secretion depends on specific exercise characteristics, and only certain heavy resistance exercise protocols are required to induce significant elevations in serum GH (Lukaszewska et al., 1976; VanHelder et al., 1984). Therefore, it is difficult to get a significant GH response for patients with cardiovascular diseases in rehabilitation programs and

the aged because of the heavy loads that are required. Alternatively, a variety of other factors such as increased metabolic demand and hypoxia may exert an influence on the exercise-induced GH response. Low resistance exercise with tourniquet ischemia has been shown to be an useful method for strength training (Shinohara et al., 1998; Takarada et al., 2000a), where the potent secretion of GH may play a part in obtaining muscle hypertrophy (Takarada et al., 2000b). High-intensity resistance exercises (~80 % of 1 repetition maximum (1-RM)) produce a 100-fold increase in plasma concentration of GH (Kraemer et al., 1990), on the other hand, short-term lowintensity "Kaatsu" resistance exercise produces a 290fold increase (Takarada et al., 2000b), resulting in muscle hypertrophy and strength gains even after short-term training in healthy subjects (Abe et al., 2004). These results strongly suggest that short-term low-intensity resistance exercise (STLIRE) with Kaatsu may be a novel method for training cardiac patients, but the influence of the "Kaatsu" exercise on hemodynamic responses has not been investigated.

Therefore, the purpose of the present study was to compare the hemodynamic and exercise-induced GH responses (EIGHR) during STLIRE with/without Kaatsu in healthy non-trained males.

METHODS

Subject

Sixteen normal healthy adult males, 20-45 yr (32 \pm 5 yr), participated in this study. All were non-trained volunteers, and informed consent was obtained prior to the study. Mean height was 175 \pm 4 cm, and mean weight was 66 \pm 4 kg. None of the subjects had any diseases, nor took any medications. The study protocol was approved by the ethics committee of the University of Tokyo.

Exercise protocols

All the studies were performed in the afternoon at least 4 h after the last lunch. An indwelling heparinlock catheter was inserted into the superficial vein of left arm. After 30 min of supine rest, control blood samples were collected and hemodynamic parameters were recorded for 3 min by using an impedance method (see below). Subjects were then seated against a backrest, with both arms resting on a table throughout the protocol. Resting hemodynamic parameters were again measured in this sitting position for 3 min. Blood flow to both legs was then restricted by an applied pressure using a specially designed belt (Kaatsu), as described below. Subsequently, under the condition with Kaatsu, eleven subjects performed bilateral leg extensionflexion exercise (Proteus Multi Exercise Machine) with the lower extremity positioned at $\sim 90^{\circ}$ flexion. The intensity of STLIRE was about 20% of 1 RM, which was obtained at least one week prior to testing. Subjects performed 30 repetitions, and after a 20seconds rest, they performed repeated knee extensions and flexions until exhaustion. Following completion of the exercise protocol, i.e., muscular fatigue was achieved, unrestricted blood flow was returned to the lower limbs and blood samples were obtained immediately after exercise, 10 and 30 min after the exercise. All blood samples were stored at -20°C until analyzed. For the controlled exercise condition, nine of the initial 11 subjects performed the same exercise protocol but this time, without Kaatsu, with both sessions being separated by at least 2 to 4 weeks.

For the exercise condition, 5 healthy males performed the same bilateral leg extension-flexion resistance exercise (20 % of 1-RM) utilizing a different type of exercise equipment (POWER Rehabilitation machine, Compass series, Proxomed Co., Germany). These subjects performed 10 repetitions, and after a 20-seconds rest, they performed three additional sets.

Induction of the reduction of muscle blood flow by Kaatsu

The method for inducing the reduction of muscle blood flow has been previously described (Takarada et al., 2000b). Local application of external pressure over both legs (a banding pressure of 1.3 times higher than resting systolic blood pressure, 160-180 mmHg) was used to reduce exercise blood flow. Briefly, both thighs had pressure applied at the proximal ends of the lower limbs by means of specially designed belts (33 mm in width, 880 mm in length) just before the start of the exercise, and the pressure was released immediately after the exercise.

Measurement of hemodynamic parameters

To evaluate hemodynamic parameters, a Task Force Monitor (CNSystems Medizintechnik, Graz, Austria) (Gratze et al., 1998; Fortin et al., 1998), which includes surface electrocardiograms (ECG), impedance cardiography (ICG), beat-to-beat blood pressure by the vascular unloading technique (Penaz, 1973) and oscillometric blood pressure recording for the upper arm was used. The ECG, impedance signal and beat-to-beat blood pressure were sampled with 1000 Hz. These data were then used to calculate all hemodynamic parameters which included heart rate (HR), blood pressure (systolic (sBP), diastolic (dBP) and mean (mBP)), left ventricular ejection time (LVET), stroke volume (SV), stroke index (SI), cardiac output (CO), cardiac index (CI), total peripheral resistance (TPR) and total peripheral pressure index (TPRI). The calculation of SI, CO, CI, TPR and TPRI was as follows.

> SI=SV/BSA CO=SV·HR CI=CO/BSA TPR=mBP·80/CO TPRI=mBP·80/CI, where BSA was body surface area.

Biochemical analyses

Plasma levels of lactate were measured by the use of an enzyme system employing lactate oxidase combined with N-ethyl-N-(3-methylphenyl)-N'acetyl ethylenediamine and an auto-analyzer, HITACHI Type 7170. Growth hormone (GH) was measured with radioimmunoassay. Plasma noradrenaline (NOR) levels were measured by highperformance liquid chromatography.

Data analyses

All values are expressed as means \pm SE. Student's paired t-test was used to compare two sets of data from the same subjects. Comparisons of time courses of parameters were analyzed by one-way ANOVA for repeated measures. When differences were indicated, a Bonferroni/Dunnett's comparison was used to
determine significance. Spearman rank correlation coefficients (r) were used to examine the relationships between the individual exercise-induced changes. Differences were considered significant if P value was less than 0.05.

RESULTS

Hemodynamic changes in short-term low-intensity leg extension-flexion exercise with and without Kaatsu

Fig. 1 shows a typical recording of hemodynamics (HR, BP and SI, CI, and TPRI) before, during and after exercise. This subject performed 4 sets of exercise until fatigue under the conditions with Kaatsu (total 100 repetitions) with a 9 kg load. Resting, seated HR increased from 69 bpm to 113 bpm at the peak of exercise, sBP increased from 150 mmHg to 210 mmHg, CI increased from 2.8 L/min to 3.3 L/min, and TPRI increased from 3324 dyn*sec/cm5 to 3767 dyn*sec/cm5. Eleven men performed for 2 to 4 sets (total 61 \pm 7 repetitions) until fatigue

Fig. 2 shows the effects of STLIRE on HR and BP. HR increased from 73 \pm 3 bpm at rest to 109 \pm 5 bpm (Fig. 2A, n=9, P<0.01) at peak exercise with Kaatsu, which corresponds to 55 \pm 4 % of target heart rate as calculated from age and gender. SBP, dBP, and mBP significantly increased from 127 \pm 4 mmHg (Fig. 2B), 86 \pm 5 mmHg (Fig. 2C), and 98 \pm 6 mmHg (Fig. 2D) to 182 \pm 6 mmHg, 105 \pm 6 mmHg, and 127 \pm 4 mmHg, respectively. On the other hand, the increase in HR and BP was lower in STLIRE without Kaatsu or



Figure 1. Hemodynamic changes in a single bout of shortterm low-intensity resistance exercise with Kaatsu. Hemodynamic parameters (heart rate (HR), continuous blood pressure (ContBP), stroke index (SI), cardiac index (CI), and total peripheral resistance (TPRI) were measured at rest (supine and sitting position), during the short-term lowintensity resistance exercise with Kaatsu and after the exercise. The proximal portions of both thighs were banded by a specially designed-belt (denoted as lines).

control STLIRE (10 repetitions x 3 sets, n=5) than in STLIRE with Kaatsu.

Fig. 3 shows the effects of STLIRE on the hemodynamic parameters (CO, SV, TRP and LVET). In STLIRE with Kaatsu, CO increased from 5.1 ± 0.5 l/min at rest to 6.2 ± 0.5 l/min (Fig. 3A, n=9, P<0.01) at the peak of exercise, while STLIRE without Kaatsu, CO increased from 5.2 ± 0.3 l/min to 6.9 ± 0.5 l/min (P<0.01). In STLIRE with Kaatsu, SV significantly decreased from 71 ± 9 ml to 62 ± 7 ml (Fig. 3B, P<0.05), while it did not significantly change in STLIRE without Kaatsu. TRP did not change



Figure 2. Effects of STLIRE with/without Kaatsu on HR and BP. HR and BP (sBP, dBP, and mBP) at rest in sitting position and at the peak exercise are shown in STLIRE with (n=9, closed circles) and without Kaatsu (open circles), and control STLIRE (10 repetitions x 3 sets; n=5, open squares). Values are means \pm S.E.. Significant difference between rest and exercise and between the 2 different types of exercise are shown. *P<0.05, **P<0.01



Figure 3. Effects of STLIRE with/without Kaatsu on the hemodynamic parameters (SV, CI, TRP and LVEF). The hemodynamic parameters at rest in sitting position and at the peak exercise are shown in STLIRE with (n=9, closed circles), and without Kaatsu (open circles), and control STLIRE (10 repetitions x 3 sets; n=5, open squares). Values are means \pm S.E.. Significant difference between rest and exercise and that between 2 types of exercise are shown. *P<0.05, **P<0.01

significantly (1650 \pm 205 dyn*sec/cm5 at rest and 1554 \pm 214 dyn*sec/cm5 (Fig. 3C, P=N.S.) at the peak exercise of STLIRE with Kaatsu), similar to the 1473 \pm 109 dyn*sec/cm5 at rest and 1471 \pm 209 dyn*sec/cm5 (P=N.S.) at the peak of exercise of STLIRE without Kaatsu. LVET was decreased in both STLIRE with and without Kaatsu (Fig. 3D, P<0.05).

Changes in serum concentrations of NOR and lactate

Fig.4A and B show the time courses of the changes in serum NOR (A) and lactate (B) concentrations during STLIRE with and without Kaatsu. In STLIRE with Kaatsu, NOR increased from 0.2 ± 0.06 ng/ml at rest to 0.54 ± 0.14 ng/ml (Fig. 4A, P<0.01) immediately after the exercise, and gradually decreased after the exercise. Serum lactate level increased from 11.2 ± 1.5 mg/dl at rest to $24.1 \pm$ $2.6, 30.9 \pm 4.3$ and 16.8 ± 2.4 mg/dl (P<0.01) immediately after exercise, at 10, and 30 min after the exercise with Kaatsu, respectively (Fig. 4B). On the other hand, the increase in NOR and lactate was lower in STLIRE without Kaatsu or control STLIRE (10 repetitions x 3 sets, n=5) than in STLIRE with Kaatsu.

Effects of STLIRE with Kaatsu on plasma concentrations of GH

Fig. 4C shows the time course of plasma concentrations of GH. After the exercise with Kaatsu, GH significantly increased, first increasing gradually after the exercise, then reaching a peak at 30 min after the exercise (Fig. 4C) $(0.11 \pm 0.03 \text{ ng/ml} \text{ to } 8.6 \text{ ms})$



Figure 4. Time course of changes in plasma concentrations of norepinephrine (NOR), lactate, and GH in STLIRE with and without Kaatsu. The concentrations of NOR, lactate and GH at rest in sitting position and after the exercise are shown in STLIRE with (n=9, closed circles), and without (open circles), and control STLIRE (10 repetitions x 3 sets; n=5, open squares). Significant difference between rest and exercise and that between 2 types of exercise are shown. *P<0.05, **P<0.01

 \pm 1.1 ng/ml; n=9; P<0.01). On the other hand, in STLIRE without Kaatsu, GH only slightly increased from 0.16 \pm 0.08 ng/ml to 0.48 \pm 0.26 ng/ml (P<0.05) following the exercise session, then returned back to resting values following 30 minutes of rest. Similarly, GH elevation in control STLIRE (10 repetitions x 3 sets, n=5) was much less than that in STLIRE with Kaatsu (P<0.01) and essentially did not change over the entire time period.

Figures 5A and B show the relationships between the changes of serum lactate/NOR concentrations and those of GH. There were no statistical differences between the changes of serum lactate (n=11, Fig. 5A) or NOR (Fig. 5B) concentration and those of GH.

DISCUSSION

The major findings of the present study are as follows; (1) STLIRE when combined with the reduction of muscle blood flow to both legs (Kaatsu) resulted in a significant exercise-induced GH response (EIGHR) which was much higher than experienced without Kaatsu. (2) The increase in HR and BP during STLIRE with Kaatsu was larger than without Kaatsu. (3) During STLIRE with Kaatsu, the increase in CO depended on the increase of HR, but not SV, since SV declined probably due to the inhibition of venous return by banding both legs. (4) The increase in NOR and lactate concentrations attained in STLIRE with Kaatsu was higher than without Kaatsu, but the increase in GH was related to neither NOR nor lactate. These results suggest that the short-term lowintensity "Kaatsu" leg resistance exercise significantly induces a growth hormone response, with the reduction of a cardiac preload during exercise. This may suggest that Kaatus resistance exercise may become a unique method for rehabilitation in patients with cardiac diseases or low physical fitness.



GH is secreted in a pulsatile fashion mainly during

Figure 5. Relation between the changes of serum GH and those of serum lactate (A) or NOR (B) induced by STRILE with Kaatsu. Relationship between individual exercise-induced changes (peak level of postexercise - preexercise level) (Δ lactate, Δ GH, Δ NOR). (A, C) Relation between Δ GH and Δ lactate (B, D) Relation between Δ GH and Δ NOR. *P<0.05, **P<0.01 vs. control

exercise and sleep. The exercise-induced pulsatile release of GH is more effective than continuous administration to induce a GH response at the target organs such as muscle and bone (Isgaard et al., 1988; Materson and Preston, 1999; Jaffe et al., 2002), which can ultimately result in improved muscle strength and an increased exercise capacity. GH has also been well established as a regulator of cardiac growth, structure, and function (Lombardi et al., 1997; Khan et al., 2002), and the subcutaneous or intravenous application of GH has been used for the treatment of congestive heart failure in patients with dilated cardiomyopathy and myocardial infarction (Fazio et al., 1996; Genth-Zots et al., 1999). In general, EIGHR is dependent on specific exercise characteristics, those usually involving certain heavy resistance exercise protocols (i.e. 60% of 1 RM) (Lukaszewska et al., 1976; VanHelder et al., 1984). Therefore, it has been difficult to apply heavy exercise protocols for aged people and patients with cardiovascular diseases in rehabilitation programs, who cannot tolerate high mechanical stress placed on muscle, tendon, and joints. However, other factors may influence on the exercise-induced GH response, such as increased metabolic demand or hypoxia. In previous studies, hypoxia has been induced by tourniquet ischemia in combination with low resistance exercise and has been reported as a successful method for strength training (Shinohara et al., 1998; Takarada et al., 2000a). In our study, we used STLIRE (20% of 1 RM and 30 repetitions x 2-4, total 61 ± 7 repetitions) with Kaatsu. The maximal heart rate attained during the exercise with Kaatsu only reached 109 ± 5 bpm, which equals approximately 55 ± 4 % of estimated maximal heart rate when adjusted for gender and age. The ratepressure product, which is accepted as a non-invasive estimate of myocardial oxygen demand during physical stress, achieved a final value of 198x102 mmHg.bpm. Even in spite of such mild strength exercises, the increased plasma GH concentrations after the exercise were approximately 100-times as high as that at rest. The level of the increased GH concentration reached levels previously described for high-intensity exercise (~80% 1 RM, 10 repetitions x 6) (Kraemer et al., 1990). Takarada et al. (2000b) reported similar results for STLIRE (20% of 1 RM and 14 repetitions x 5 sets, total 70 repetitions) with young male athletes aged 20-22 yr. Their plasma GH concentration increased ~290 times compared to resting vales. They also reported much larger increases in the concentrations of NOR and lactate compared to the values obtained in our study, suggesting that the differences between the two studies may depend on the total exercise volume. Baically, STLIRE when combined with Kaatsu appears to be a useful method to induce significant

EIGHR, while exercise at the same intensity but

without Kaatsu is ineffective for inducing a GH response, however, it is unclear whether the pulsatile EIGHR can improve cardiac function in a similar manner to GH therapy (Fazio et al., 1996; Genth-Zots et al., 1999). On the other GH has been well known to increase serum IGF-1 stability, which may also improve cardiac function, but further clinical trials are needed to clarify this possibility.

The several mechanisms underlying GH release during STLIRE with Kaatsu may be proposed. Under the conditions with Kaatsu, serum lactate and NOR concentrations increased following the exercise protocol compared to the controlled exercise protocol without Kaatsu, suggesting that exercise with Kaatsu produces a greater demands on anaerobic glycolysis which may stimulates serum GH elevation. However, since no consistent systematic relationships between GH and lactate/NOR were observed, a combination of anaerobic factors such as local ischemia and/or local accumulation of lactate in the legs induced by the restriction of muscle blood supply may then stimulate peripheral afferent nerves, resulting in enhancing GH-releasing hormone secretion and/or inhibition of somatostatin (Glustina et al., 1998). Kaatsu by itself, in the absence of exercise, failed to induce any significant GH secretion in healthy males (data not shown), suggesting that exercise is also required. The greater GH secretion during Kaatsu exercise may be from afferents originating in fast twitch skeletal muscle fibers (Gosselink et al., 1998) since fast twitch fibers have been reported to be recruited preferentially during Kaatsu resistance exercise (Yasuda et al., 2004) or under dynamic ischemic training (Nygren et al., 2000). These mechanisms are still speculative and further studies are needed to clarify the basic mechanisms of GH release induced by the Kaatsu exercise.

It has been known that moderate to heavy resistance exercise markedly increases BP (Kilbom and Brundin, 1976; Bosisio et al., 1980; Bezucha et al., 1982; Lewis et al., 1983). Even though heavy leg extension exercise appears to be a predominately dynamic in nature, it still has a substantial static component (Miles et al., 1987). During static exercise, there is a rise in BP caused by an increase in CO, which in turn is due to an increase in HR (Helfant et al., 1971; Perez Gonzalez, 1981). In STLIRE with Kaatsu, CO increased without significant changes in TPR, and a decrease in SV (about 12 %) when compared to control exercise, which was probably due to the reduced venous return during venous restriction. Thus, the increase in BP was largely dependent on the increase in CO due to a significant increase in HR, not SV. Therefore, the increase in BP observed in our exercise was more typical of an exercise with a static component. The inhibition of

venous return during STLIRE with Kaatsu can reduce cardiac preload during exercise, which may be useful in rehabilitating patients with cardiac diseases.

In conclusion, the short-term low-intensity "Kaatsu" leg resistance exercise significantly induced GH responses with a reduction in cardiac preload during exercise, which may provide to be a promising method for rehabilitation in patients with cardiac diseases or low physical fitness.

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Electromyographic responses of arm and chest muscle during bench press exercise with and without KAATSU

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The purpose of this study was to compare the EMG activity of blood flow restricted (limb) and nonrestricted (trunk) muscles during multi-joint exercise with and without KAATSU. Twelve (6 women and 6 men) healthy college students [means (SD) age: 24.1 (3.5) yrs] performed 4 sets (30, 15, 15, and 15 reps) of flat bench press exercise (30% of a predetermined one repetition maximum, 1-RM) during two different conditions [with KAATSU and without KAATSU (Control)]. In the KAATSU condition, a specially designed elastic cuff belt (30 mm wide) was placed at the most proximal position of the upper arm and inflated to a pressure of 100% of individual's resting systolic blood pressure. Surface EMG was recorded from the muscle belly of the triceps brachii (TB) and pectoralis major (PM) muscles, and mean integrated EMG (iEMG) was analyzed. During 4 sets of the exercise, gradual increases in iEMG were observed in both TB and PM muscles for the KAATSU condition. The magnitude of the increases in iEMG in the TB and PM muscles were higher (P<0.05) with KAATSU compared to the Control condition. In the first set, the mean exercise intensity from normalized iEMG was approximately 40% of 1-RM in both Control and KAATSU conditions. However, the mean exercise intensity of both muscles were 60-70% of 1-RM for the KAATSU condition and only about 50% of 1-RM for the Control condition, respectively, during the fourth set. We concluded that increases in iEMG in the trunk muscle during KAATSU might be an important factor for KAATSU training-induced trunk muscle hypertrophy.

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INTRODUCTION

A number of publications have reported that lowintensity exercise training combined with KAATSU training (restriction of muscular venous blood flow from the limb muscles) can result in significant and rapid increases in muscle size and strength in the arm and thigh muscles (Burgomaster et al., 2003; Kawada and Ishii, 2005; Shinohara et al., 1998; Takarada et al., 2000; 2002). To the best of our knowledge, only one study has focused on KAATSU training-induced trunk muscle hypertrophy. They reported that muscle hypertrophy occurred not only in the limb muscle but also in the trunk muscle following KAATSU resistance training (Abe et al., 2005). The results of previous studies indicate that muscle hypertrophy can occur in blood flow restricted limb muscle, and also in non-restricted trunk muscle following multi-joint KAATSU resistance training, however, the mechanism for this adaptation is not fully understood.

Resistance exercise with restricted muscular blood flow has been shown to result in an increase in the integrated electromyogram (EMG) of the active limb muscle (Moritani et al., 1992; Takarada et al., 2000; 2002), and this response is an important factor for KAATSU training-induced limb muscle hypertrophy (Takarada et al., 2000; 2002). Under these conditions, synergistic action between limb and trunk muscles may occur during the multi-joint KAATSU resistance exercise. Therefore, we hypothesized that increases in EMG activity during KAATSU resistance exercise could be achieved not only in the limb muscle but also in the trunk muscle. Thus, the purpose of this study was to compare the EMG activity of the blood flow restricted (limb) and nonrestricted (trunk) muscles during multi-joint exercise with and without KAATSU.

METHODS

Subjects

Twelve (6 women and 6 men) healthy college students [age, 24.1 (3.5) yrs; height, 167.5 (8.4) cm; weight, 61.8 (11.2) kg, means (SD)] with no previous resistance training experience volunteered for the study. All subjects were informed of the procedures, risks, and benefits, and signed an informed consent document before participation. The study conformed to all standards for the use of human subjects in research as outlined in the Helsinki declaration and was approved by the Tokyo Metropolitan University Ethics Committee for Human Experiments.

Experimental design and exercise protocols

The subjects participated in two conditions [with

KAATSU and without KAATSU (Control)] of flat bench press exercise in random order (about 5 hours interval between the conditions). A supinated grip at the standard grip position (160% of biacromial distance) was used (Lehman 2005). The subjects performed 30 repetitions (approximately constant velocity in 2.4 sec, 1.2 sec for the eccentric phase and 1.2 sec for the concentric phase) of bench press exercise at 30% of a pre-determined one repetition maximum (1-RM). Following a 30 sec rest period, the subjects then performed three sets of 15 repetitions, with each set separated by a 30 sec rest period (Abe et al., 2005; Sato et al., 2005).

Restriction of muscular blood flow by KAATSU

A specially designed elastic belt (30 mm wide) was placed around the most proximal portion of each upper arm during KAATSU testing only. The belt contained a pneumatic bag along its inner surface that was connected to an electronic air pressure control system that monitored the restriction pressure (Kaatsu-Mater, Sato Sports Plaza Ltd., Tokyo, Japan). Prior to the KAATSU testing, the subjects were seated on a chair, the belt air pressure was set at 60 mmHg for 30 s, and the air pressure was released. The air pressure was increased by 20 mmHg and held for 30 s, then released for 10 s between occlusive stimulations. This process was repeated until a final restriction pressure of 100% of an individual's resting systolic blood pressure was reached. This pressure was then maintained for the entire exercise session, including the rest periods. The belt pressure was released immediately upon completion of the session. Resting systolic blood pressure of the arm (at heart level) was measured using an automatic sphygmomanometer (Fit Cuff, Omron, Tokyo, Japan)



Figure 1. Typical examples of EMG signals recorded from triceps brachii (TB) and pectoralis major (PM) muscles during low-intensity flat bench press exercise with and without KAATSU.

before KAATSU testing.

EMG measurement and analysis

EMG activities of the triceps brachii (TB) and pectoralis major (PM) muscles were measured with bipolar surface electrodes (Vitrode F, Ag/AgCl, 1-cm diameter, Nihon Kohden, Tokyo, Japan) placed on each muscle with a fixed 2-cm inter-electrode distance (center to center). Prior to electrode placement, the skin area was polished with skin preparation gel and cleaned with ethanol in order to reduce skin impedance and to ensure good adhesion of the electrodes. The EMG signals were sampled via differential amplifiers (Nihon Kohden AB-621G, Tokyo, Japan) at 1000 Hz for each muscle (Figure 1). Raw EMG signals were digitized and stored on hard disk in a computer by the Chart software program, version 4.2 (AD Instruments Pty Ltd, Australia). Digital rectification and integration of EMG (iEMG) data were performed with the same computer.

Normalized iEMG and relative exercise intensity determination

To determine the relative exercise intensity during bench press exercise with and without KAATSU for each set, a correlation between relative lifting load (20, 30, 40, 50, 60, and 70% of 1-RM, 5 repetitions for each load) and corresponding iEMG was measured for each subject before testing. Using the regression line between lifting load and iEMG (Figure 2), the relative intensity was calculated from iEMG data during bench press exercise with and without KAATSU.

Statistical analyses

All values are expressed as means (standard



Figure 2. Relationship between relative lifting load and mean iEMG during bench press exercise (subject SS). The relative exercise intensity during bench press with and without KAATSU was calculated using the regression line.

deviations) for all variables. Statistical analyses were performed by a two-way analysis of variance (ANOVA) with repeated measures [Condition (Kaatsu and Control) x Time (sets)]. Post-hoc testing was performed by a student's paired t-test. Statistical significance was set at P < 0.05.

RESULTS

A significant condition x time interaction was observed for the normalized mean iEMG in TB and PM muscles (P<0.01, Figure 3). Post hoc analyses indicated that KAATSU bench press resulted in a significantly greater (P<0.05 or P<0.01) increase in mean iEMG activity when compared to the Control bench press. During the first 30 reps of bench press, mean exercise intensity from the normalized iEMG was approximately 40% of 1-RM in both Control and KAATSU conditions. In the third set of 15 reps, the mean exercise intensity was 60-70% of 1-RM in both TB and PM muscles for the KAATSU condition and only about 50% of 1-RM in both muscles for the Control condition. There was no gender difference in



Figure 3. Changes in exercise intensity determined by normalized iEMG during 4 sets of bench press exercise with and without KAATSU. *P<0.05, **P<0.01, KAATSU vs. Control

iEMG activity during bench press exercise with and without KAATSU (data not shown).

DISCUSSION

It is known that low-intensity resistance exercise with restricted venous blood flow (KAATSU) (Takarada et al., 2000; 2002) or occluded blood flow (Moritani et al., 1992) has been shown to result in an increase in the integrated EMG of the active limb muscle. However, it is unknown whether the increase in EMG activity during multi-joint exercise with KAATSU or blood flow occlusion could be achieved, not only in the limb muscle, but also in trunk muscles. Our findings demonstrated that increases in iEMG activity was observed in both limb (triceps brachii) and trunk (pectoralis major) muscles during low-intensity KAATSU bench press exercise. The results of this study support our hypothesis that EMG activity is increased by the synergistic action of blood flow restriction in both limb muscles and nonrestricted trunk muscles in the shoulder joint. KAATSU exercise-induced increases in EMG activity in the trunk muscle may be one of the important factors for the muscle hypertrophy seen in the trunk muscle following low-intensity KAATSU resistance training in a previous study (Abe et al. 2005).

Blood flow restriction pressure (belt air pressure) is an important variable for determining the exercise intensity during KAATSU exercise (Abe et al., 2006). A previous study reported that increase in EMG activity depends on blood flow restriction pressure during a constant load of KAATSU exercise (Yasuda et al. 2006). In the present study, the restriction pressure was set at 100% of individual's resting systolic blood pressure (approximately 110 mmHg), resulting in blood lactate concentrations measured immediate after the KAATSU exercise to increase to about 4 mmol/L (data not shown). Previous KAATSU-training studies have used similar or even higher restriction pressures compared to the present study which resulted in blood lactate concentrations to rise to about 10 mmol/L after low-intensity KAATSU exercise (Sato et al., 2005; Takarada et al., 2000). During exercise with highly restricted blood flow from the working muscle, the EMG activity may be increased to a greater extent than exercise alone, which would recruit more fast-twitch fibers and their higher threshold motor units in both arm and trunk muscles during milt-joint KAATSU exercise. Therefore, our results support the previous findings of muscle hypertrophy seen in the trunk muscle following multi-joint KAATSU exercise training (Abe et al. 2005).

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ORIGINAL ARTICLE

Effects of KAATSU training on haemostasis in healthy subjects

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Purposes: The KAATSU training is performed under the reduction of muscle blood flow by a speciallydesigned belt (KAATSU belt), which induces blood pooling in capacitance vessels by restricting venous return. However, no prior studies have examined the effects of KAATSU training on haemostasis. The purpose of the present study was to investigate acute effects of KAATSU training on haemostasis including fibrinolytic responses in healthy subjects. Methods: Two protocols have been performed. (1) 6 healthy men (mean age= 48 ± 5 yr) performed KAATSU (160 mmHg) of both thighs for 15 minutes and then KAASTU training combined with low-intensity leg and foot aerobic exercises for ~10 minutes in hypobaric chamber, which mimics 8000 feet in airflight. (2) Another 7 men (mean age=30 \pm 4 yr) performed leg press exercises (30 % 1 RM) with and without KAATSU of both thighs 24 h after bed rest. Blood samples were taken at rest, immediately after KAATSU, and exercises with or without KAATSU, and after exercise. For the investigation of blood fibrinolysis, determinations of tissue-type plasminogen activator (tPA) activity or antigen, plasminogen activator inhibitor (PAI)-1 activity or antigen, fibrin degradation product (FDP) and D-dimer were used. Prothrombin time (PT) and platelet counts were also measured. Results: (1) In hypobaric chamber, KAATSU by itself significantly increased tPA activity, while PAI-1 activity was unchanged. Furthermore, immediately after the exercise, tPA activity increased significantly. (2) During the exercises combined with KAATSU 24 h after bed rest, tPA antigen significantly increased, compared with control exercises, but PAI-1 antigen was unchanged. In both cases, KAATSU training did not induce fibrin formation as assessed by fibrin D-dimer and FDP. Conclusions: This study indicates that potentially favorable changes occur in fibrinolytic factors after KAATSU and KAATSU training in healthy subjects.

Key words: KAATSU training; fibrinolytic activity; tissue-type plasminogen activator (tPA); plasminogen activator inhibitor (PAI)-1; bed rest; airflight; exercise

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INTRODUCTION

The KAATSU training is a novel method for muscle training performed under the reduction of muscle blood flow by a specially-designed belt (KAATSU belt). Under the conditions of restricted muscle blood flow, even short-term, low-intensity exercise such as walk training can induce muscle strength, and increased muscle mass (Takarada et al. 2000a; b; c; Takarada et al., 2002a,b; Abe et al. 2006). In addition, KAATSU femoral blood flow restriction induces lower-body venous pooling and reduces venous return (Takano et al., 2006), resulting in stimulating cardiovascular effects of orthostasis in 1G like lower body negative pressure (LBNP) (Iida et al., 2007). Since the most effective countermeasure regimen to prevent cardiovascular deconditioning in space flight would be a gravitation-like stress combined with exercises, the use of KAATSU in combination with low-intensity resistance exercise would provide a significant countermeasure like LBNP for space flight countering both cardiovascular and musculoskeletal

decline. Thus, the KAATSU training may be a promising training in astronauts to prevent cardiovascular deconditioning and muscle atrophy in space flights (Iida et al., 2006) as well as athletes and healthy persons as described previously (Nakajima et al., 2006).

Haemostasis is achieved through a delicate equilibrium between the coagulation and fibrinolytic cascades (Wu and Thiagarajan, 1996). Exercises have been shown to affect activation of both the coagulation and fibrinolytic cascades (Davis et al., 1976; Andrew et al., 1986; Boman et al., 1994; Weiss et al., 1998; El-Sayed et al., 2000; Ribeiro et al., 2006). Regular exercise preferentially activates fibrinolysis, which is generally associated with favorable alterations in risk from cardiovascular morbidity, while strenuous exercise may increase blood coagulation, and promote thrombus formation (Hilberg et al., 2002), resulting in exertion-related ischemic events (Giri et al., 1999). Thus, it is likely that effects of exercise on the haemostatis are dependent upon exercises intensity and duration (Rosing et al., 1970; Weiss et al., 1998; Molz et al., 1993; Rankinen et al., 1995). The KAATSU training is an exercise performed under the restriction of venous blood flow. Therefore, occlusion of blood vessels may affect the haemostasis, and cause the formation of thrombus, though serious side effects of KAATSU training such as pulmonary embolism have not been reported until recently (Nakajima et al. 2006). However, no prior studies have examined the effects of KAATSU training on haemostasis.

The coagulation cascade may be enhanced in various conditions such as airflight and bed rest. Economy class syndrome is a serious problem in airflight, where the activation of the coagulation cascade and subsequently thrombus formation may occur. Since the KAATSU training is a novel exercise performed under the restriction of venous blood flow, it is interesting to know the effects of the KAATSU training on the haemostasis under the airflight and bed rest that are known to increase the risk for coagulation.

Therefore, we investigated acute effects of the KAATSU training on haemostasis including fibrinolytic responses under the conditions in hypobaric chamber and 24 hours after bed rest in healthy subjects.

METHODS Subjects

This study consisted of two protocols as shown in Fig.1. Protocol 1 was approved by the ethics committee of the University of Tokyo. Protocol 2 was approved by the institutional review board (IRB) of human research of Japan Aerospace Explosion Agency (JAXA) and the ethics committee of the University of Tokyo. All were non-trained volunteers, and informed consent was obtained prior to the study. None of the subjects had any diseases and took any medications. (1) In protocol 1, 6 healthy adult males, aged 48 ± 5 years (mean height, 1.71 ± 0.2 metres; mean weight, 70.1 ± 4.3 kg), performed KAATSU only and KAATSU training in hypobaric chamber (8000 feet). (2) Another 7 men, aged 31.6 ± 1.1 years (mean height, 1.76 ± 1.6 metres; mean weight, 75.3 ± 3.9 kg), performed leg press exercises (30 % 1 RM) with and without KAATSU after 24 hours bed rest.

Protocol 1

Aircraft cabins are pressurized so that the cabin pressure is maintained at the equivalent of around 5,000-8,000 feet altitude irrespective of the cruising altitude. Therefore, subjects entered the 8,000 feet hypobaric chamber in JAXA (Fig.1A). The protocol is shown in Fig. 1B. After 30 minutes of rest in the seated position, a sample of blood was taken to represent 0 feet. The pressure of the chamber was decreased to the pressure of 8000 feet in airflight. The rest sample of blood at 8,000 feet was taken after 30 minutes of rest in the seated position. Subsequently, KAATSU at the pressure of 160 mmHg was applied to both thighs by using KAATSU master belt for 15 minutes. Immediately after the release of KAATSU, the blood sample was again obtained. After 15 minutes rest of sitting position, the subjects performed 5 lower extremity exercises (~10 minutes total exercise time) while seated and under a KAATSU pressure of 160 mmHg. The exercises consisted of 1) toe flexion and extension (20 reps, 2 sets), 2) ankle dorsi flexion (20 reps, 2 sets), 3) ankle plantar flexion (20 reps, 2 sets), 4) unilateral knee



Figure 1. Experimental design. (A) The model of hypobaric chamber in JAXA (B) Protocol 1 (C) Protocol 2

extension (20 reps each), and 5) unilateral leg press motion (20 reps each) Immediately after the exercises, the pressure was released. Blood samples were also taken immediately after the release of KAATSU and 15 minutes after the exercises. O₂ saturation was monitored from right finger by Onyx (Nonin Medical, Inc. M.N. U.S.A).

Heart rate (HR) and blood pressure (BP) were obtained by the Task Force Monitor (CNSystmes Medizintechnik, Graz, Austria) (Gratze et al. 1998; Fortin et al. 1998; Takano et al., 2005).

Protocol 2

Subjects maintained 6° head-down tilt position during the bed rest period, where the coagulation cascade may be enhanced. The protocol is shown in Fig. 1C. After 24 h -6° bed rest, the control blood samples were collected. After that, each subject was divided into two groups randomly; One group performed leg press exercises without KAATSU first, and 2 hours later, the similar resistance exercises were performed under the application of KAATSU at the pressure determined automatically (auto) as described below. The other group performed leg press exercises with KAATSU and 2 hours later, the similar resistance exercises without KAATSU were done. In these exercises, HR and BP were continuously recorded for 5 minutes before exercises (Pre) and during exercises with and without KAATSU, by using Task-Force monitor. All exercises were performed on the leg press exercises in the 6° head-down tilt position. The resistance protocol involved performing 4 sets (1 set of 30 repetitions followed by 3 sets of 15 repetitions) at an intensity of 30% 1RM (repetition maximum). The speed of the movement during each repetition was held constant at approximately 1 repetition per 3 seconds. 1RM was determined in advance. Blood samples were taken immediately before and 1, 10, and 60 minutes into the recovery period after exercise with or without KAATSU. Blood samples were obtained using an indwelling heparinlock catheter inserted into the superficial antebrachial vein of left arm. All blood samples were processed to serum or plasma before storage at -20°C until analysis.

Reduction of femoral muscle blood flow by KAATSU

A method for inducing the reduction of muscle blood flow is similar as previously reported (Takarada et al. 2000a; b; c; Takarada et al. 2002a,b; Takano et al. 2005; Abe et al., 2006). Both sides of their thighs had pressure applied at the proximal ends by KAATSU Master belt (protocol 1) or belts developed for space (protocol 2). In space, it is convenient that KAATSU can be applied automatically to astronauts. To obtain it, the apparatus developed for space was used. It can apply KAATSU at the pressure, where the pulse wave of leg becomes maximal, named as auto. The level of auto was 172.5 ± 6.75 mmHg and 1.42 ± 0.10 times larger than systolic blood pressure (sBP) at the sitting position, and it was 158.8 ± 2.95 mmHg and 1.36 ± 0.07 times larger than sBP at 6° head down tilt position after 24 h bed rest.

Measurement of hemoglobin, lactate, noradrenaline, and haemostasis parameters

Blood samples for measurement of blood hematocrit (Hct), and hemoglobin (Hb, 2 ml) and hormone determination (7 ml) were collected in preheparinised syringes. For Hb, blood was drawn into test tubes containing EDTA-2Na. Blood Hb (g/100 ml) was measured by the cyanomethemoglobin method (Coulter hemoglobinometer). Hct (%) was measured by micro-hematocrit using ultra centrifugation. For hormone determination, blood was drawn into test tubes containing 10.5 mg of EDTA-2Na. All samples were kept in ice-cold water and centrifuged (3000 rpm) for 10 minutes and the plasma stored at -20°C until the assays were performed. Plasma concentrations of noradrenaline (NOR) were measured using high performance liquid chromatography (HPLC) method. The lower limit of detection of the assay was 6 pg/ml. Ten milliliters of blood were collected into 3.2% sodium citrate for tissue type plasminogen activator (tPA) activity or antigen assay, plasminogen activator inhibitor (PAI)-1 activity or antigen assays, prothrombin time (PT), thrombin time (TT), D-dimer (D-D), fibrin degradation products (FDP), fibrinogen, factor 8 and factor 10. The following assays were performed by using ELISA: tPA antigen (Technoclone GmbH, Vienna, Austria); tPA activity (Molecular Innovations, Inc., M.I., U.S.A.); PAI-1 antigen (Technoclone GmbH, Vienna, Austria); PAI-1 activity (HYPHEN BioMed., Neuville-Sur-Oise, France). The other variables were measured at commercially available laboratories (SRL Inc., Tokyo, Japan).

The change of blood volume (BV) (%) and plasma volume (PV) (%) was derived from the following equation;

 $BV_B/BV_A = Hb_A/Hb_B$

 $\Delta PV = 100*((Hb_B/Hb_A)*((1-Hct_A*10^{-2})/(1-Hct_B*10^{-2}))) = 100$

where A is the value at rest (pre), and B is the value at the corresponding time.

Data analysis

All values are expressed as means \pm S.E.M. Student's paired t-test was used to compare two sets of data from the same subjects. Comparison of time courses of parameters was analyzed by one-way ANOVA for repeated measures. When differences were indicated, a Bonferroni's comparison was used to determine significance. Differences were considered significant if P value was less than 0.05.

RESULTS

Effects of KAATSU training on haemostasis in hypobaric chamber

In hypobaric chamber (8000 feet), oxygen saturation by pulse oximetry (SpO₂) markedly decreased from 99 \pm 0.3 % to 91 \pm 1.1 % (n=6, P<0.01), but brain natiuretic peptide (BNP) did not change significantly as shown in table 1 and Fig.2. The pressurization of KAATSU (160 mmHg) to both thighs by itself did not affect SpO₂, but a series of short-term and low-intensity aerobic exercises combined with KAATSU increased SpO₂. HR increased to 81.1 ± 8.0 bpm at the peak of the exercises (Table 1). KAATSU by itself significantly increased NOR from 394 ± 67 pg/ml to 525 ± 96 pg/ml (n=6, P<0.01, table 1), and KAATSU combined with exercises induced a further increase in NOR $(738 \pm 78 \text{ pg/ml}, P < 0.01)$. The serum concentration of lactate increased from 14.2 ± 1.5 mg/dl at rest 8000 feet to $26.3 \pm 1.9 \text{ mg/dl}$ (n=6, P<0.01) after the exercises. PV and BV tended to decrease during KAATSU, but statistically not significant. On the other hand, PV and BV significantly decreased during the exercises combined with KAATSU. PV significantly decreased during the exercises combined with KAATSU, compared with rest (0 feet).

Under these hypobaric conditions, where SpO₂ markedly decreased, we investigated the effects of KAATSU and KAATSU combined with the exercises on haemostatis as shown in table 2 and Fig. 3. As

shown in table 2, PT, TT, fibrinogen, factor 8 and Plt did not change significantly. Both D-D and FDP also did not significantly change during KAATSU and KAATSU combined with the exercises. TPA activity resting at 8000 feet altitude was not statistically different from that resting at 0 feet altitude (0.24 ± 0.01 vs. 0.26 ± 0.02). On the other hand, tPA activity was significantly increased from 0.26 ± 0.016 U/ml resting at 8000 feet altitude to 0.32 ± 0.04 U/ml resting at 8000 feet with KAATSU (Fig. 3A, n=6, P<0.05). TPA activity increased more when exercises were combined with KAATSU (0.34 ± 0.03 U/ml, n=6). In contrast, PAI-1 activity (Fig. 3B) did not significantly change during KAATSU and exercises combined with KAATSU.



Figure 2. Time courses of SpO₂ under the hypobaric conditions (8000 feet) during KAATSU and a series of short-term and low-intensity aerobic exercises combined with KAATSU. All values are means \pm S.E.M. obtained from 6 subjects. ** p<0.01 vs. rest (0 feet)

Parameter	0 feet	8000 feet	KAATSU 15 min	KAATSU + Exercises	Recovery 15 min
HR (bpm)	71.0 ± 8.2	70.3 ± 8.5	72.6 ± 9.1	81.1 ± 8.0**	71.7 ± 8.7
SBP (mmHg)	113.9 ± 7.1	116.5 ± 4.9	125.1 ± 6.2	128.2 ± 8.1**	115.8 ± 3.0
HT (%)	46.0 ± 1.1	45.4 ± 1.2	47.6±1.1	50.8 ± 1.0**	48.0 ± 1.0
BV (%△)	-	1.46 ± 3.1	-3 ± 1.2	-8±1.0**	-3.1 ± 1.1
PV ((%△)	-	3.09 ± 5.9	-5.9 ± 2.2	$-16 \pm 1.5^{**}$	-6.7 ± 1.9
NOR (pg/ml)	336 ± 26	394 ± 67	525 ± 96**	738 ± 78**	492 ± 84**
Lactate (mg/dl)	16.9 ± 1.2	14.2 ± 1.5	14.3 ± 1.7	26.3 ± 1.9**	24.0 ± 1.0**
BNP (pg/ml)	14.5 ± 7.0	13.2 ± 6.1	19.5 ± 8.2	14.5 ± 7.8	14.6 ± 7.9

Table 1. Effects of KAATSU and exercises combined with KAATSU on various parametersunder the conditions of 8000 feet in airflight

Parameter	0 feet	8000 feet	KAATSU 15 min	KAATSU + Exercises	Recovery 15 min
PT (%)	30.4 ± 1.7	31 ± 1.5	31.9 ± 1.0	33.7 ± 3.1	29.9 ± 1.4
TT (sec)	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
Fibrinogen (mg/dl)	260 ± 31	243 ± 27	227 ± 24	230 ± 47	254 ± 33
Factor 8 (%)	117 ± 21	121 ± 24	105 ± 19	138 ± 27	138 ± 20
FDP (µg/ml)	2.17 ± 0.17	2.00 ± 0.00	2.00 ± 0.00	3.33 ± 0.95	2.50 ± 0.34
D-D (µg/ml)	0.47 ± 0.15	0.42 ± 0.14	0.41 ± 0.14	0.43 ± 0.14	0.39 ± 0.13
Plt (x10000/µl)	16.5 ± 2.7	16.0 ± 3.6	17.2 ± 3.1	18.3 ± 2.9	16.4 ±3.0

 Table 2. Effects of KAATSU and exercises combined with KAATSU on haemostatic

 parameters under the conditions of 8000 feet in airflight



Figure 3. Effects of KAATSU only and low-intensity aerobic exercises combined with KAATSU on tPA activity (A) and PAI-1 activity (B) under the hypobaric condition (8000 feet). All values are means \pm S.E.M. obtained from 6 subjects. * p<0.05, ** p<0.01 vs. rest (0 feet)

Effects of leg press exercises combined with KAATSU on haemostasis parameters at -6° bed rest for 24 h

Next, we examined the effects of leg press exercises (30% 1 RM) combined with and without KAATSU at 24 h -6° bed rest. Tables 3 and 4 summarize the effects of leg press exercises with and without KAATSU on hemodynamic and neurohumoral parameters. HR at the peak exercises with KAATSU was larger than that without KAATSU. The peak exercise HR with KAATSU increased from 61.9 \pm 4.9 bpm at rest to 107.1 \pm 9.4 bpm (n=7, P<0.01) during exercise. SBP increased during KAATSU exercise, and

reached to 154.2 ± 8.2 mmHg. Table 3 also shows the time courses of the changes in serum lactate and NOR concentration during the leg press exercises with and without KAATSU. The increase in lactate concentration after exercise with KAATSU was much higher than that without KAATSU. In leg press exercises with KAATSU, NOR increased from 140 \pm 20 pg/ml at rest to 514 ± 110 pg/ml (n=7, P<0.01) immediately after the exercise, and gradually decreased after the exercise. On the other hand, it increased from 131 ± 16 pg/ml to 239 ± 47 pg/ml (n=7, P<0.01) in the control exercise. Thus, the increase in NOR concentration attained in the leg press exercises with KAATSU was significantly higher than that without KAATSU. PV and BV decreased during exercises with and without KAATSU. But, the exercises combined with KAATSU showed a larger decrease in PV and BV, compared with the control exercise as shown in table 3.

The effects of exercises combined with KAATSU on haemostatis were investigated at -6° bed rest for 24 h as shown in Fig. 4 and table 4. As shown in table 4, PT, fibrinogen, factor 10, and Plt did not change significantly. Both D-D and FDP also did not significantly change during leg press exercises combined with KAATSU. TPA antigen did not change significantly during the leg press exercises without KAATSU (2.2 \pm 0.1 g/ml at rest and 2.3 \pm 0.1 ng/ml immediately after the exercise). On the other hand, tPA antigen increased from 2.1 \pm 0.1 ng/ml to 2.7 \pm 0.2 ng/ml (n=7, P<0.05) immediately after the exercises combined with KAATSU as shown in Fig. 4A. The increased tPA antigen returned to the control level within 10-30 minutes after the exercise. In contrast, PAI-1 antigen (Fig. 4B) did not significantly change during KAATSU and the exercises combined with KAATSU.

Parameter	Pre	EX			
HR (-)	61.3 ± 4.9	85.6±5.7**			
(bpm) (+)	61.9 ± 4.9	107.1 ± 9.4**	^**		
sBP (-)	124.8 ± 6.0	139.6±8.6**			
(mmHg) (+)	127.5 ± 4.9	154.2 ± 8.2**			
Parameter	Pre	0–1 min		10 min	30 min
Hct (-)	48.8 ± 0.3	50.2 ± 0.6**		48.8±0.5	48.1 ± 0.6
(%) (+)	48.5 ± 0.6	51.6±0.5**	**	49.6±0.5**	48.1 ± 0.5
BV (-)	-	-2.9. ± 0.3**		-0.4 ± 0.4	1.3±0.3*
(%△) (+)	-	-5.9 ± 0.4**	**	$-2.3 \pm 0.7 **$	0.2 ± 0.9
PV (-)	-	-5.6 ± 1.0**	**	-0.5± 0.8	$2.7 \pm 0.8*$
(%△) (+)	-	-11.6± 0.8**		-4.4 ± 1.1**	0.8 ± 1.7
NOR (-)	131 ± 16	239 ± 47**		161 ± 23	126 ± 17
(pg/ml) (+)	140 ± 20	514 ± 110**	**	224 ± 42	144 ± 23
lactate (-)	8.9 ± 0.7	20.9 ± 2.6**		15.0±1.9*	9.2 ± 0.6
(mg/dl) (+)	9.7 ± 0.7	27.4 ± 3.8**	**	27.1 ± 4.1**	15.6±2.4

Table 3. Effects of leg press exercises (EX) with (+) and without (-) KAATSU compared to resting values (Pre) on various parameters 24 h after -6° bed rest

* p<0.05 vs. Pre *p<0.05 KAATSU(-) vs. KAATSU(+)

** p<0.01 vs. Pre

**p<0.05 KAATSU(-) vs. KAATSU(+)

Table 4. Effects of leg press exercises (EX) with (+) and without (-) KAATSU compared to resting values (Pre) on haemostatic parameters 24 h after -6° bed rest

Parame	ter	Pre	0–1 min	10 min	30 min
PT (%)	(-)	83.6±3.3	85.3 ± 3.0	83.6 ± 2.6	82.9 ± 3.0
	(+)	82.3 ± 2.7	86.4 ± 3.0	83.7 ± 3.1	83.6 ± 3.6
Fibrinogen	(-)	239 ± 28	251 ± 29	232 ± 22	238 ± 27
(mg/dl)	(+)	237 ± 25	264 ± 31	239 ± 28	235 ± 26
FDP	(-)	2 ± 0	2 ± 0	2 ± 0	2 ± 0
(µg/ml)	(+)	2 ± 0	2.14 ± 0.14	2.57 ± 0.30	2.71 ± 0.57
D-D	(-)	0.21 ± 0.04	0.23 ± 0.03	0.20 ± 0.03	0.21 ± 0.05
(µg/ml)	(+)	0.21 ± 0.06	0.27 ± 0.08	0.25 ± 0.05	0.26 ± 0.05
Plt	(-)	25.1 ± 1.7	26.0 ± 2.1	25.5 ± 1.5	25.0 ± 1.9
(x10000/µl)	(+)	25.0 ± 1.8	27.2 ± 2.4	26.1 ± 2.1	24.9 ± 1.0
Factor 10	(-)	105 ± 6	111±7	107 ± 5	105 ± 5
(%)	(+)	105 ± 5	116 ± 6	108 ± 5	103 ± 3
1		1		1	1



Figure 4. Effects of leg press exercises combined with and without KAATSU on tPA antigen (A) and PAI-1 antigen (B) after 24 h at -6° bed rest. The serum concentration of tPA and PAI-1 antigen in control rest (24 h bed rest), immediately after exercises (0-1 minute), 10 and 30 minutes after exercises are shown in leg press exercises with KAATSU and without. All values are means \pm S.E.M. obtained from 7 subjects. * p<0.05 vs. control (Pre).

DISCUSSION

The present study shows that the KAATSU training did not induce fibrin formation as assessed by fibrin D-dimer and FDP, while potentially favorable changes occur in fibrinolytic factors after KAATSU and KAATSU training in healthy subjects. Thus, this enhanced fibrinolytic activity may be an important mechanism mediating cardioprotective effect provide by the KAATSU training.

Haemostasis is achieved through a delicate equilibrium between the coagulation and fibrinolytic cascades (Wu and Thiagarajan, 1996). Exercises have been shown to affect activation of both the coagulation and fibrinolytic cascades (Smith, 2003). Regular moderate aerobic exercise preferentially activates fibrinolysis (Davis et al., 1976; Andrew et al., 1986; Weiss et al., 1998; El-Sayed et al., 2000; Hilberg et al., 2003a,b), subsequently generally associated with favorable alterations in risk from cardiovascular morbidity. In contrast, strenuous exercise may increase blood coagulation, and promote thrombus formation (Hilberg et al., 2002), resulting in exertionrelated ischemic events (Giri et al., 1999). Thus, the effects of exercise on the haemostatis may be strongly dependent upon exercises intensity and duration (Rosing et al., 1970; Weiss et al., 1998; Molz et al., 1993; Rankinen et al., 1995). The KAATSU training is a novel training performed under the restriction of venous blood flow, but occlusion of blood vessels may affect the haemostasis and then cause the formation of thrombus. Therefore, we examined the effects of KAATSU training on haemostasis under the conditions in hypobaric chamber and 24 h after bed rest in healthy subject, where the activation of the coagulation cascade and subsequently thrombus formation may be occurred. Under the training combined with KAATSU 24 h after bed rest, plasma volume (PV) significantly decreased, as observed in heavy resistance exercises. Similarly, a decrease in PV was observed in aerobic exercises combined with KAATSU under a hypobaric chamber, where SpO₂ markedly decreased to approximately 90%. In some types of exercise, the shortening of PT, a measure of the activity of extrinsic and common coagulation pathways, and TT, a measure of common coagulation pathway, has been reported due to the enhanced coagulation cascade (El-Sayed et al., 1995; Smith, 2003). However, under our conditions, PT and TT did not change. Changes in individual components of the coagulation cascade as a result of exercise have also been reported. Factor 8 has been reported to increase, dependent on volume and intensity of exercise (Andrew et al., 1986). In the present study, factor 8 did not significantly change. Fibrinogen level also did not change in both protocols used in the present study, suggesting that fibrinogen is not consumed in our experimental conditions. The end products of fibrinolysis, fibrin degradation products (FDP), and D-D, products of the breakdown of activated factor 8 (fibrin stabilizing factor) have been reported to increase after heavy endurance exercises of different types (Arai et al., 1990; Molz et al., 1993; Prisco et al., 1998). However, in the present study, FDP and D-D did not significantly change. From these observations, it is likely that the KAATSU training used in the present study did not activate coagulation cascade, induce fibrin formation and subsequently thrombosis as summarized in table 5.

Generally, during physical heavy exertion, potential for blood coagulation increases, and typically balanced by a corresponding increase in fibrinolytic activity, defined as the capacity to lyse inappropriate or excessive clot. The capacity to thrombolize fibrin clots is influenced by many factors of the fibrinolytic system, but particularly tPA and PAI-1. The increased fibrinolytic potential occurs due to an increase of tPA activity or antigen, which catalizes the conversion of plasminogen into plasmin, and/or a decrease in PAI-1 (Collen et al., 1980; Molz et al., 1993; Rankinen et al., 1995; Weiss et al., 1998; El-Sayed et al., 2000; Huber, 2001; Ivey et al., 2003; Cooper et al., 2004; Womack et al., 2006). Under basal conditions, tPA circulates mainly as a complex with PAI-1 with low levels of free tPA, and PAI-1 inhibits tPA by binding to it and

Table 5. Summary of effects of exercises combined withKAATSU on markers of coagulation and fibrinolysis

Parameters	Types of exe	rcises
	low-intensity aerobic exercises	leg press exercises
	(8000 feet)	(24 h bed rest)
Coagulation		
PT	unchanged	unchanged
TT	unchanged	not done
Factor 8	unchanged	unchanged
Factor 10	unchanged	unchanged
Fibrinolysis		
Fibrinogen	unchanged	unchanged
tPA	increased	increased
PAI-1	unchanged	unchanged
FDP	unchanged	unchanged
D-D	unchanged	unchanged
Platelet aggregation		
Plt account	unchanged	unchanged

forming an inactive complex. Synthesis of both tPA and PAI-1 occurs in the vascular endothelial cells (Wu and Thiagarajan, 1996). Thus, fibrinolytic responses to acute exercises play an important role in preventing exertion-related ischemic events (Giri et al., 1999) due to the thrombosis. The present study showed that larger fibrinolytic responses of tPA activity or antigen to the KAATSU training were observed, compared with the control exercises. The similar findings were observed in KAATSU training under hypobaric chamber, where SpO₂ decreased to approximately 90%. Therefore, the enhanced fibrinolytic activity observed in the KAATSU training may be an important mechanism mediating cardioprotective effect provide by the KAATSU training.

Acute hypoxemia may affect fibrinolytic activity. Under a hypobaric chamber of 8000 feet, SpO₂ markedly decreased to approximately 90%. However, tPA activity at rest (8000 feet) was not statistically different from rest (0 feet) (0.24 \pm 0.01 vs. 0.26 \pm 0.02). PAI-1 activity also did not change significantly. Therefore, acute hypoxia did not affect blood fibrinolytic activity, which was compatible with the previous paper (Stegnar et al., 1987). On the other hand, during the conditions used in the present study, where the coagulation cascade may be enhanced, the KAATSU training increased tPA activity, but did not activate coagulation cascade, induce fibrin formation and subsequently thrombosis. Several mechanisms may be involved in enhancement of tPA activity observed during the KAATSU training. During KAATSU only for 15 minutes, tPA activity

significantly increased. Venous occlusion has been reported to enhance the fibrinolytic activity (Szymanski et al., 1994; Nikfardjam et al., 1999; Monagle et al., 2003), where tPA activity or antigen increases. Since KAATSU training restricts venous flow, and then pooling of blood, the similar mechanism may be involved in the enhancement of tPA activity or antigen observed during KAATSU. However, the further increase in tPA activity or antigen was observed in the exercises combined with KAATSU. It has been reported that the fibrinolytic responses to exercise are related to changes in plasma lactate and /or NOR (Davis et al., 1976; Wheeler et al., 1986; Weltman et al., 1994; Hilberg et al., 2003b). The underlying mechanism has not been clarified, but it may depend on exercise intensity and duration exercise. Some papers showed that lactate threshold or sympathetic drive may be a critical intensity to elicit acute fibrinolytic changes in tPA (Davis et al., 1976; Wheeler et al., 1986; Weltman et al., 1994). In the present study, the increase in lactate and NOR concentration after exercise with KAATSU was much higher than that without KAATSU. Thus, the increase in lactate and NOR may also contribute to the enhanced fibrinolytic activity during the KAATSU training.

The KAATSU training is an exercise performed under the restriction of venous blood flow. Therefore, occlusion of blood vessels may affect the haemostasis, and cause the formation of thrombus, though serious side effects of KAATSU training such as pulmonary embolism have not been reported until recently (Nakajima et al. 2006). In this report, one patient was suspected of pulmonary embolism. However, this patient was not admitted the hospital, and had no serious problems. He might have had acute bronchitis, judging from the hearing of the instructor. In addition, pulmonary embolism has not occurred among approximately 200,000 subjects, who have received KAATSU training (Sato Y, personal communication). It may be compatible with the present findings that the KAATSU training, even under the conditions of airflight and bed rest, where the coagulation cascade may be enhanced, did not activate coagulation cascade, induce fibrin formation and subsequently thrombosis, but rather enhanced fibrinolytic activity.

In conclusion, the KAATSU training did not induce fibrin formation as assessed by fibrin D-dimer and FDP, while potentially favorable changes occur in fibrinolytic factors after the KAATSU training even under the conditions where the coagulation cascade may be enhanced.

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Hemodynamic responses to simulated weightlessness of 24-h head-down bed rest and KAATSU blood flow restriction

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Abstract The KAATSU training is a unique method of muscle training with restricting venous blood flow, which might be applied to prevent muscle atrophy during space flight, but the effects of KAATSU in microgravity remain unknown. We investigated the hemodynamic responses to KAATSU during actually simulated weightlessness (6° head-down tilt for 24 h, n = 8), and compared those to KAATSU in the seated position before bed rest. KAATSU was applied to the proximal ends of both the thighs. In the seated position before bed rest, sequential incrementing of KAATSU cuff pressure and altering the level of blood flow restriction resulted in a decrease in stroke volume (SV) with an increase in heart rate (HR). KAATSU (150–200 mmHg) decreased SV comparable to standing.

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Department of Human and Engineered Environmental Studies, Graduate School of Frontier Science, The University of Tokyo, Chiba, Japan Following 24-h bed rest, body mass, blood volume (BV), plasma volume (PV), and diameter of the inferior vena cava (IVC) were significantly reduced. Norepinephrine (NOR), vasopressin (ADH), and plasma renin activity (PRA) tend to be reduced. A decrease in SV and CO induced by KAATSU during the simulated weightlessness was larger than that in the seated position before bed rest, and one of eight subjects developed presyncope due to hypotension during 100 mmHg KAATSU. High-frequency power (HF_{RR}) decreased during KAATSU and standing, while low-frequency/high-frequency power (LF_{RR}/HF_{RR}) increased significantly. NOR, ADH and PRA also increased during KAATSU. These results indicate that KAATSU blood flow restriction reproduces the effects of standing on HR, SV, NOR, ADH, PRA, etc., thus stimulating a gravity-like stress during simulated weightlessness. However, syncope due to lower extremity blood pooling and subsequent reduction of venous return may be induced during KAATSU in microgravity as reported in cases of lower-body negative pressure.

Keywords KAATSU training · Autonomic function · Space flight · Cardiovascular deconditioning · 6° head-down tilt bed rest · Sympathetic activity

Introduction

The weightless environment of space flight causes serious adaptive changes in cardiovascular function as well as muscle atrophy. A shift in blood volume from lower-body capacitance vessels toward the head and elevation of tissue capillary perfusion pressure in the head causes facial and intracranial edema and headache, which distresses astronauts. And, nearly all crew members develop cardiovascular deconditioning characterized by orthostatic intolerance and reduced upright exercise capacity, which is manifest after space flight (Nicogossian et al. 1995; Buckey et al. 1996; Fritsch-Yelle et al. 1996). The mechanisms involved in this deconditioning include hypovolemia, decreased baroreflex responsiveness, and decreased skeletal muscle stiffness. Therefore, effective countermeasures during spaceflight are needed to maintain the cardiovascular system, as well as the musculoskeletal structure–function to ensure the wellbeing and safety of crew members during space flight and upon return to Earth.

An elastic thigh cuff, called "bracelets", has been reported to be an effective passive countermeasure for reducing edema and venous stasis in the cephalic region by pooling blood in the vascular and extravascular compartments of the legs, easing the stress of zero gravity (Lindgren et al. 1998; Arbeille et al. 1999; Millet et al. 2000). However, the effectiveness of bracelets to prevent cardiovascular deconditioning is incomplete, providing only partial compensation for the cardiovascular changes (Herault et al. 2000). The most effective countermeasure for preventing cardiovascular deconditioning appears to be imposition of a gravity-like stress, such as lower-body negative pressure (LBNP) (Güell et al. 1990, 1992; Lathers and Charles 1993). When combined with intensive exercise, LBNP is a potent orthostatic stimulus, which presumably provides an effective prevention of orthostatic intolerance following space flight (Lee et al. 1997; Watenpaugh et al. 2000). However, a large-scale apparatus combined with exercise machine is required. In addition, LBNP can be combined with treadmill (Murthy et al. 1994), but not resistance-type machines. The use of resistance exercises may be essential to prevent muscle atrophy during space flight as resistance training specifically promotes muscle enlargement and muscular strength, which are negatively impacted by weightlessness. Thus, if a method of exercise with a potent orthostatic stimulus like LBNP exists, it may provide an effective countermeasure for cardiovascular deconditioning as well as muscle atrophy in weightlessness.

The key physiological features of LBNP are lower extremity blood pooling, reduction of venous return to the heart, and subsequent hemodynamic changes including increased autonomic nervous system activation (Stevens and Lamb 1965; Tomaselli et al. 1987; Lathers and Charles 1993). The KAATSU training is a unique technique of performing low-load exercises such as resistance exercises and treadmill with restricted muscle blood flow that results in an increase in muscle mass and muscular strength comparable to high-intensity training (Takarada et al. 2000; Abe et al. 2006; Fujita et al. 2007). Since KAATSU femoral blood flow restriction induces the retention of blood flow in lower extremities, it reduces venous return, and induces

subsequent hemodynamic changes such as decreased SV and CO and increased TPR like LBNP (Stevens and Lamb 1965; Güell et al. 1990, 1992; Melchior et al. 1994; Murthy et al. 1994; Lee et al. 1997; Watenpaugh et al. 2000; Iida et al. 2007). Thus, KAATSU may partly provide an orthostatic stimulus, and an effective countermeasure for cardiovascular deconditioning in weightlessness like LBNP. However, the potency of KAATSU for inducing pooling of venous blood and then hemodynamic changes in microgravity has not been investigated. In addition, the occurrence of syncopal attack has been reported in cases of LBNP, especially when using high pressure more than -40 mmHg (Stevens and Lamb 1965), but the safety of KAATSU in microgravity remain unclear.

Therefore, the aim of this study is to examine the hemodynamic responses to KAATSU during actually simulated weightlessness (6° head-down tilt for 24 h), and to compare those to KAATSU in the seated position before bed rest. In addition, the potency for inducing pooling of venous blood and then hemodynamic changes during KAATSU is compared to those reported during LBNP.

Materials and methods

Subjects

Eight males (age 32.8 ± 1.0 years; height 176 ± 16 cm; weight 75.3 ± 3.9 kg) participated in the following experiments. All subjects were healthy, free of neuromuscular or cardiovascular disease, were not on any medication, and none had a specific history of physical exercise training. This investigation was approved by the institutional review board (IRB) of human research of Japan Aerospace Explosion Agency (JAXA) and the ethics committee of the University of Tokyo, and all subjects gave their informed consent prior to inclusion.

Experimental protocol

This study consisted of two experiments as summarized in Fig. 1. All experiments were separated by 1–2 weeks, and were performed at the Japanese Aerospace Explosion Agency between 25 September and 26 October in 2006.

Experiment A

As shown in Fig. 1a, to mimic KAATSU training on Earth, the effects of sequential incrementing of KAATSU cuff pressure (50 mmHg step) on hemodynamic parameters in the seated position were examined in the morning. After a 30-min rest in the seated position, we took rest measurements of hemodynamic parameters at this position for

A KAATSU (sitting)

rest 30 min	KAATSU 10 min	rest 10 min	rest 30 min	
Hemodynamic	1. 100 mm 2. 150 mm 3. 200 mm	nHg x 3 nHg nHg	standing 5	min

B KAATSU (24 h bed rest)



Fig. 1 Experimental design. **a** KAATSU in the seated position (mimic KAATSU on Earth) **b** simulated weightlessness and KAATSU (mimic KAATSU on space)

5 min by using an impedance cardiography. Then, both the proximal thighs were pressure-applied with the specially designed belt developed for spaceflight (see below). After recording the hemodynamic parameters for 10 min under 100 mmHg KAATSU, the banding pressure was released and the hemodynamic parameters were continuously taken during a 5–10 min recover time. After the additional 30 min rest in the seated position, the effects of 150 mmHg KAATSU on hemodynamic parameters were investigated. Similarly, we repeated the experiments of KAATSU (200 mmHg). Finally, the effects of standing on hemodynamic parameters were also investigated. During the first experiment, nobody complained of any symptoms including syncopal attack.

Experiment B

This experiment was designed to investigate the effects of KAATSU on hemodynamic parameters during the weightlessness, which was simulated for 24 h using bed rest with a 6° head-down tilt. Subjects maintained 6° head-down tilt position during the entire bed rest period. Transportation and toilet procedures were restricted to the head-down recumbent position. Subjects were allowed to rest on their elbows during meals and could move volitionally but remained horizontal to the bed. To ensure compliance, the volunteers were monitored at all times by video-camera surveillance. Subject's diet, fluid intake, and urine volume were also monitored.

The control echocardiographic data, and blood samples were collected after 10 min (0-h bed rest) after bed rest. After 24 h after bed rest (24-h bed rest), the effects of KAATSU on hemodynamic parameters were examined while maintaining the head-down tilt and bed rest position. Control hemodynamic parameters after 24-h bed rest were monitored continuously for 5 min, followed by echocardiography and collection of blood samples. Then, hemodynamic responses to KAATSU were studied at two levels of KAATSU belt pressure (50 and 100 mmHg). The following timing pattern was employed: apply KAATSU, 10 min continuous hemodynamic measurement, remove KAATSU, and record an additional 10 min of continuous hemodynamic response, then rest without KAATSU for 30 min. The KAATSU pressure was first set to 100 mmHg, but the first subject complained of presyncopal attack and was excluded from the following experiments and data analysis. Therefore, the remaining subjects (n = 7) began with a lower KAATSU cuff pressure (50 mmHg) prior to 100 mmHg. After completing the two KAATSU pressure trials in bed rest position, subject stood up and hemodynamic response was recorded continuously for 5 min in standing position. We had not examined 150 mmHg due to the marked decrease in SV during 100 mmHg. With each pressure perturbation, blood samples were collected immediately following release of KAATSU (0-1 min), and 30 min after the release. The data obtained from seven subjects who completely finished both experiments were shown.

Methods

KAATSU blood flow restriction

Femoral blood flow was impaired using the KAATSU technique, which restricts venous blood flow and causes pooling of blood in capacitance vessels distal to the cuff (Takarada et al. 2000; Takano et al. 2005; Abe et al. 2006; Fujita et al. 2007; Iida et al. 2007). KAATSU was applied to the proximal end of both the thighs as near to the hip joint as possible by using KAATSU belts (65 mm in width and 650 mm in length). The cuff pressure was controlled by the KAATSU apparatus as previously described (Iida et al. 2007).

Cardiovascular hemodynamics

Hemodynamic parameters were determined using the Task Force Monitor (CNSystmes Medizintechnik, Graz, Austria) as previously described (Takano et al. 2005; Iida et al. 2007). Analysis included electrocardiograms (ECG), impedance cardiography, beat-to-beat blood pressure by vascular unloading technique and oscillometric blood pressure. Data were obtained for every beat with a 1,000 Hz sampling rate and used to calculate all hemodynamic parameters in real time. Data included heart rate (HR; bpm), mean arterial blood pressure (mAP; mmHg), systolic blood pressure (sBP), diastolic blood pressure (dBP), stroke volume (SV; ml), cardiac output (CO; l/min) and total peripheral resistance (TPR; dyne s cm⁻⁵). TPR was calculated in relative units as MAP·CO⁻¹, and the calculation of CO and TPR was as follows.

$$CO = SV \times HR$$

 $TPR = MAP \times 80 \times CO^{-1}$

Histograms of RR intervals were computed and pseudodigitized at ten samples per second. Auto-regressive modeling (Burg method) was used to construct frequency domain spectrograms of the heart rate variability (HRV). Parameters extracted from the variability spectra were lowfrequency power (LF_{RR}, 0.03–0.15 Hz) and high-frequency power (HF_{RR}, 0.16–0.50 Hz), normalized to total power over the range from 0.01 to 0.50 Hz.

Cardiac dimensions

Trans-thoracic echocardiography was performed using Aplio80. Left ventricular end-systolic dimension (LVDs; mm), left ventricular end-diastolic dimension (LVDd; mm) and the diameter of inferior vena cava (IVC; cm) were determined using the M-mode recording in the parasternal long-axis view with the pulsed wave as described previously (Iida et al. 2007).

Hormone-metabolite levels

Venous blood samples were collected and analyzed for hematocrit, hemoglobin, noradrenaline, plasma renin activity and vasopressin. Blood sample was accomplished with an indwelling catheter inserted into the superficial antebrachial vein of left arm. For measurement of hemoglobin and hematocrit, 2 ml of blood was placed into test tubes containing EDTA-2Na. For hormone determination, blood (7 ml) was placed in test tubes containing 10.5 mg of EDTA-2Na. All samples were kept in ice-cold water and centrifuged (3,000 rpm) for 10 min and the plasma was stored at -20° C until the assays were performed. Blood hemoglobin (Hb, $g dl^{-1}$) was determined by the cyanomethemoglobin method (Coulter hemoglobinometer) and hematocrit (Hct, %) by the micro-hematocrit ultra centrifugation technique. Plasma concentrations of noradrenaline (NOR; lower limit of detection 6 pg ml⁻¹) were measured using high performance liquid chromatography. Plasma renin activity (PRA; lower limit of detection 0.1 ng ml⁻¹ h⁻¹) and vasopressin (ADH; lower limit of detection 0.2 pg ml^{-1}) were determined by radioimmunoassay. These assays were completed at commercially available laboratories (SRL Inc., Tokyo, Japan).

Changes in blood and plasma volume (%) were derived from the following equation;

$$\begin{split} BV_B \times BV_A^{-1} &= Hb_A \times Hb_B^{-1} \\ \% \Delta PV &= 100 \times \left(Hb_B \times Hb_A^{-1}\right) \\ &\times \left(\left(1 - Hct_A \times 10^{-2}\right) / \left(1 - Hct_B \times 10^{-2}\right)\right) \\ &- 100 \end{split}$$

where A is the initial value and B is the value at the corresponding time.

Data analysis

All values are expressed as means \pm SEM Student's paired *t*-test was used to compare two sets of data from the same subjects. Comparison of time courses of parameters was analyzed by one-way ANOVA for repeated measures. When differences were indicated, a Bonferroni's comparison was used to determine significance. Differences were considered significant if P < 0.05.

Results

Table 1 shows the hemodynamic changes during KAATSU (100-200 mmHg) in the seated position and the standing position before bed rest. The pressurization of 100-200 mmHg significantly increased HR, and decreased SV, which depended on the pressure. 200 mmHg KAATSU increased HR from 64.8 ± 3.2 to 74.0 ± 4.0 bpm (n = 7, P < 0.01). After the release of pressure, HR promptly returned to the pre test level. The pressurization of 200 mmHg decreased SV from 73.6 \pm 3.5 to 60.2 \pm 2.2 ml (n = 7, P < 0.01). After an orthostatic stress (standing), HR increased with decreasing SV. The decrease in SV observed during 150-200 mmHg was lower than that in the standing position (66.9 \pm 2.4 ml). CO did not significantly change during KAATSU in the seated position before bed rest. TPR, sBP, mBP, and dBP were also not significantly altered during KAATSU.

Following 24 h of 6° head-down tilt bed rest, there was a 2.0 kg decease in body mass (75.3 \pm 3.9 to 73.3 \pm 3.8 kg, n = 7, P < 0.01) associated with a significant urine output (2052 \pm 249 ml d⁻¹) that markedly exceeded water intake (1320 \pm 67 ml d⁻¹). After 24 h of 6° head-down tilt bed rest, blood (4.4 \pm 1.4%) and plasma (7.9 \pm 2.5%) volume decreased as Hct (46.4 \pm 1.2% to 48.5 \pm 0.8%, P < 0.01) and Hb (15.0 \pm 0.3 to 15.7 \pm 0.3 mg/dl, P < 0.01) significantly increased.

Table 1 Hemodynamic responses during KAATSU in the seated position

	HR (bpm)	sBP (mmHg)	mBP (mmHg)	dBP (mmHg)	SV (ml)	$CO (1 min^{-1})$	TPR (dyne s cm^{-5})
Pre	68.8 ± 3.1	122.9 ± 6.8	93.8 ± 4.6	81.5 ± 4.0	74.8 ± 3.1	5.1 ± 0.3	1459 ± 103
100	$70.2 \pm 3.7^{**}$	118.5 ± 6.4	93.0 ± 5.2	82.5 ± 4.6	67.7 ± 2.9**	4.7 ± 0.3	1570 ± 128
Post	69.7 ± 3.7	124.4 ± 5.7	97.4 ± 4.5	85.4 ± 3.7	67.5 ± 3.6**	4.7 ± 0.3	1679 ± 135
Pre	65.5 ± 3.2	122.0 ± 6.5	96.0 ± 4.9	83.3 ± 4.4	71.0 ± 3.8	4.6 ± 0.3	1689 ± 168
150	$72.3 \pm 3.6^{**}$	123.9 ± 4.5	95.4 ± 3.8	84.4 ± 3.5	$63.5 \pm 2.5^{**}$	4.6 ± 0.3	1664 ± 123
Post	67.4 ± 3.5	125.4 ± 4.5	94.6 ± 3.2	82.1 ± 3.8	69.9 ± 3.8	4.7 ± 0.3	1638 ± 120
Pre	64.8 ± 3.2	124.1 ± 4.7	93.9 ± 4.1	82.6 ± 3.9	73.6 ± 3.5	4.8 ± 0.3	1576 ± 114
200	$74.0 \pm 4.0^{**}$	124.2 ± 5.2	97.6 ± 3.9	86.6 ± 3.5	$60.2 \pm 2.2^{**}$	4.4 ± 0.3	1758 ± 126
Post	67.3 ± 3.2	124.4 ± 4.5	99.3 ± 3.8	86.2 ± 3.2	68.2 ± 3.2**	4.5 ± 0.3	1758 ± 111
Pre	66.7 ± 2.8	125.9 ± 6.0	98.8 ± 4.8	85.1 ± 4.1	68.2 ± 2.2	4.5 ± 0.3	1723 ± 109
Stand	$71.4 \pm 3.4^{**}$	$129.6 \pm 5.7*$	$104.5 \pm 4.5*$	$91.6 \pm 4.2*$	$66.9 \pm 2.4^{**}$	4.7 ± 0.2	1753 ± 109

* P < 0.05 versus Pre

** P < 0.01 versus Pre

There was a significant decrease in the diameter of IVC $(1.71 \pm 0.13 \text{ cm to } 1.34 \pm 0.12 \text{ cm}, P < 0.05, n = 7)$ following 24 h of head-down tilt bed rest (P < 0.05) that was associated with the reduced plasma volume and then venous return. The diameter of LVDd (51.6 ± 1.3 to 51.2 ± 1.1 mm) was not significantly altered by 24 h of head-down tilt bed rest. HR (59.2 ± 4.0 to 58.9 ± 3.7 bpm), and sBP (121.9 ± 5.1 to 126.0 ± 5.6 mmHg) were also not altered by 24 h of head-down bed rest.

The serum concentration of PRA $(1.51 \pm 0.48 \text{ to} 0.86 \pm 0.18 \text{ ng ml}^{-1} \text{ h}^{-1}$, n = 7, P = 0.08), ADH $(1.81 \pm 0.26 \text{ to} 1.50 \pm 0.12 \text{ pg ml}^{-1}$, P = 0.08), NOR $(201 \pm 45 \text{ to} 157 \pm 24 \text{ pg ml}^{-1}$, P = 0.09), and DOP $(7.1 \pm 1.5 \text{ to} 5.4 \pm 0.4 \text{ pg ml}^{-1}$, P = 0.09) tended to decrease following 24 h of 6° head-down tilt bed rest.

During experiment B, one subject complained of dizziness and developed neurocirculatory presyncope because of hypotension, ~ 5 min after 100 mmHg KAATSU. No other symptoms were articulated or observed in the remaining seven subjects for the duration of the experiment.

IVC was significantly reduced by KAATSU at 50 mmHg (1.34 \pm 0.12 to 1.09 \pm 0.09 cm, n = 7, P < 0.05), and 100 mmHg (to 1.05 \pm 0.15 cm, n = 7, P < 0.05). LVDd was reduced with both 50 mmHg KAATSU (51.2 \pm 1.1 to 46.4 \pm 1.3 mm, P < 0.01) and 100 mmHg KAATSU (to 45.44 \pm 1.63 mm, P < 0.01).

SV (Table 2) was significantly reduced by KAATSU at 50 mmHg (88.0 \pm 5.3 to 68.5 \pm 3.9 ml, P < 0.01) and 100 mmHg (84.8 \pm 4.5 to 58.3 \pm 3.3 ml, P < 0.01). Although there was a significant increase in HR, CO was significantly reduced at each level of KAATSU. SV, HR, and CO returned to pre-KAATSU levels immediately after the release of KAATSU (data not shown). There were no changes in Hct, Hb, BV, or PV associated with KAATSU blood flow restriction (50, 100 mmHg; Table 3). Upon standing, SV decreased (82.7 \pm 4.7 to 54.4 \pm 2.1 ml, P < 0.01); a decrement comparable to 100 mmHg KAATSU. The significant increase in HR upon standing was greater than that observed during KAATSU, and thus, CO was maintained during standing (Table 2). TPR increased at each level of KAATSU, but was significantly less than

Table 2 Hemodynamic responses during KAATSU following 24-h bed rest

	HR (bpm)	sBP (mmHg)	mBP (mmHg)	dBP (mmHg)	SV (ml)	$CO (l min^{-1})$	TPR (dyne s cm^{-5})
Pre	58.9 ± 3.7	126.0 ± 5.6	93.3 ± 3.2	79.4 ± 2.5	88.0 ± 5.3	5.2 ± 0.6	1480 ± 145
50	$62.8 \pm 4.0^{**}$	122.8 ± 5.2	89.0 ± 3.6	75.7 ± 3.1	68.5 ± 3.9**	$4.3 \pm 0.5^{**}$	$1732 \pm 157^{**}$
Post	58.0 ± 4.0	122.8 ± 5.9	91.1 ± 3.8	76.0 ± 3.0	87.2 ± 5.4	5.1 ± 0.6	1485 ± 143
Pre	56.3 ± 4.2	123.7 ± 4.3	91.6 ± 3.5	79.0 ± 3.0	84.8 ± 4.5	4.8 ± 0.6	1605 ± 211
100	67.3 ± 3.3**	119.7 ± 3.3	89.2 ± 2.1	76.7 ± 1.9	58.3 ± 3.3**	$3.9 \pm 0.4^{**}$	$1878 \pm 166^{*}$
Post	58.6 ± 3.0	124.2 ± 6.8	92.4 ± 5.2	77.6 ± 4.0	79.3 ± 5.8	4.7 ± 0.6	1668 ± 211
Pre	58.5 ± 3.1	124.7 ± 5.3	95.6 ± 3.7	82.8 ± 3.9	82.7 ± 4.7	4.8 ± 0.6	1657 ± 186
Stand	$77.9 \pm 3.9^{**}$	129.0 ± 7.3	$104.5 \pm 6.5*$	91.4 ± 7.0	$54.4 \pm 2.1^{**}$	4.3 ± 0.3	$1996 \pm 189^{*}$

* P < 0.05 versus Pre

** P < 0.01 versus Pre

	Hb (mg dl^{-1})	Hct (%)	BV (%Δ)	PV (%Δ)	NOR (pg ml ⁻¹)	PRA (ng ml h ⁻¹)	ADH (pg ml ⁻¹)
Pre	15.7 ± 0.25	48.5 ± 0.75	-4.4 ± 1.4	-7.9 ± 2.5	157 ± 24	0.86 ± 0.18	1.5 ± 0.12
50	15.7 ± 0.25	48.2 ± 0.77	-3.9 ± 1.5	-6.4 ± 2.5	$211 \pm 35^{**}$	0.94 ± 0.14	1.9 ± 0.18
Pre	15.6 ± 0.26	47.9 ± 0.81	-3.6 ± 1.3	-5.5 ± 2.4	148 ± 20	0.77 ± 0.13	1.39 ± 0.17
100	15.8 ± 0.21	48.4 ± 0.63	-4.7 ± 1.7	-7.5 ± 2.9	$235 \pm 41^{**}$	$1.09 \pm 0.20^{**}$	$2.2 \pm 0.62*$
Post	15.6 ± 0.25	48.0 ± 0.74	-3.6 ± 1.4	-5.8 ± 2.5	174 ± 23	0.69 ± 0.12	1.7 ± 0.20

Table 3 Hemostatic responses during KAATSU following 24-h bed rest

* P < 0.05 versus Pre

** P < 0.01 versus Pre

The data of BV and PV show the percentage changes (% Δ), compared with the volume at 0-h bed rest

the increase observed upon standing. BP did not change significantly. Upon standing, mBP was significantly increased (Table 2).

Figure 2 summarizes comparative effects of 100 mmHg KAATSU on hemodynamic parameters in the seated position before bed rest and at 6° head-down tilt bed rest during the simulated weightlessness. HR (Fig. 2a) and sBP

(Fig. 2b) during KAATSU in the simulated weightlessness were not significantly different from those in the seated position before bed rest. On the other hand, KAATSU markedly reduced SV (Fig. 2c) and CO (Fig. 2d) with an increase in TPR (Fig. 2e) during the simulated weightlessness, compared to the seated position before bed rest.

Fig. 2 Comparative effects of KAATSU (100 mmHg) on the hemodynamic parameters in the seated position before bed rest and 6° head-down position 24 h after bed rest. Effects of KAATSU (100 mmHg) on HR (a), sBP (b), SV (c), CO (d) and TPR (e) are illustrated. All values are means \pm SEM obtained from seven subjects. * P < 0.05, ** P < 0.01



NOR, PRA, and ADH were all significantly increased during 100-mmHg KAATSU, but NOR only significantly increased with 50 mmHg KAATSU (Table 3). All values returned to pre-KAATSU levels during the recovery period.

HF_{RR} (Fig. 3b), determined from the power spectra of HR variability (HRV; Fig. 3a), was reduced with 100 mmHg KAATSU (P < 0.05) and tended to decrease with 50 mmHg KAATSU (P = 0.17). In contrast, the LF_{RR}·HF_{RR}⁻¹ component was increased during each KAATSU trial (Fig. 3c). All changes returned to pre-KAATSU levels during the recovery period. Upon standing, HF_{RR} decreased significantly and equivalently to the changes with KAATSU (Fig. 3b), however, the increase in LF_{RR}/HF_{RR} was greater than that observed during KAA-TSU (Fig. 3c).

Discussion

The present study shows that following 6° head-down tilt bed rest for 24 h, a model simulating microgravity effects on the cardiovascular system, KAATSU blood flow restriction reproduces the effects of standing on HR, SV, NOR, ADH, PRA, etc., thus stimulating a gravity-like stress during simulated weightlessness. However, syncope due to lower extremity blood pooling and subsequent reduction of venous return may be induced during KAA-TSU in microgravity as reported in cases of LBNP.

During 6° head-down tilt bed rest, a model to simulate zero G eliminates the normal downward hydrostatic pressure gradients and causes an immediate central fluid shift from lower extremities toward the thoracic–cephalic region (Norsk et al. 1993). The central hypervolemia affects hormonal regulation of fluid excretion and stimulates central cardiac volume receptors, resulting in a loss of plasma volume (Norsk et al. 1993; Duranteau et al. 1995). In the present study, 24 h 6° head-down tilt bed rest resulted in a total urine volume (2.052 \pm 249 ml d⁻¹) that was in excess of fluid intake $(1,320 \pm 67 \text{ ml d}^{-1})$. Then, blood and plasma volume was decreased by a mean value of 4.4 and 7.9%, respectively. This fluid volume loss was reflected in the significant decrease in body mass and IVC diameter, which was compatible with the previous papers (Nixon et al. 1979; Gaffney et al. 1985). Furthermore, head-down bed rest induces an initial cephalad-fluid shift with an inhibition of the renin-angiotensin system and ADH. Hughson et al. (1995) showed a 40% decrease in PRA after 10-h head-down tilt. In the present study, concentration of PRA and ADH tended to decrease following 24 h of 6° head-down tilt bed rest, but not statistically NOR $(201 \pm 45 \text{ to } 157 \pm 24 \text{ pg ml}^{-1})$ different. P = 0.09) also tended to decrease as reported throughout space flight missions and bed rest (Leach et al. 1983). Thus, it is likely that physiological alterations caused by 6° headdown tilt bed rest condition observed here mimicked the weightless condition of space flight.

During an orthostatic stress, part of the blood and plasma volume can pool in the capillary bed of the legs, and subsequently, SV decreases because of reduced venous return. KAATSU applied to both the legs also decreased venous return by pooling blood into the vascular and extracellular compartment of the legs and ultimately induced the hemodynamic alterations comparable to standing as previously reported (Iida et al. 2007). A decrease in SV induced by KAATSU (150–200 mmHg) in the seated position was larger than that induced by standing. Similarly, during the simulated weightlessness, the

Fig. 3 Effects of KAATSU on heart rate variability (HRV) after 24-h 6° bed rest. a The time courses of HRV during KAATSU. Note that during KAATSU (100 mmHg), LF_{RR} increased, compared with the control, while HF_{RR} decreased. **b** Effects of orthostatic stress (standing) and KAATSU on HF_{RR}. c Effects of orthostatic stress (standing) and KAATSU on $HF_{RR} \cdot LF_{RR}^{-1}$. All values are means \pm SEM obtained from seven subjects. * P < 0.05, ** P < 0.01 versus control (pre)



decrease in SV induced by 100 mmHg KAATSU was approximately equivalent to that induced by standing. The effects of 50 mmHg KAATSU on SV were less than 100 mmHg KAATSU. Thus, the magnitude of venous pooling, reduced venous return, and the decrement of SV is greatly dependent upon the level of the KAATSU (Iida et al. 2007). While orthostatic stress decreases SV, and induces hypotensive stimuli, CO and BP are maintained by an increased HR and TRP via arterial and cardiopulmonary baroreceptor control of circulation. Under the hypovolemic condition of 24-h bed rest, KAATSU (50 and 100 mmHg) induced a larger decrease in SV with an increase in HR and TRP. Thus, during KAATSU, arterial baroreceptors as well as cardiopulmonary baroreceptors were unloaded (Furlan et al. 2001; Brown et al. 2003), which mimicked orthostatic stress. During actual space flight, the Russian physicians have already used a unique method for a countermeasure, called "bracelets" to reduce edema and venous stasis in the cephalic region by pooling blood in the vascular and extravascular compartments of the legs (Lindgren et al. 1998; Arbeille et al. 1999; Millet et al. 2000). This method applies a pressure of ~ 30 mmHg, proposing that the degree of venous pooling induced by the bracelets is much lower than that observed in KAATSU, where the cuff pressure of 100-250 mmHg is used to restrict muscle blood flow and increase muscle mass. Regarding autonomic nervous responses to KAATSU, HF_{RR}·LF_{RR}⁻¹, a marker of sympathetic activity, and the serum concentration of NOR, a well-known neurotransmitter released from sympathetic nerve, increased, which depended on the pressure of KAATSU. On the other hand, HF_{RR}, a marker of parasympathetic activity, decreased with KAATSU. Overall, it also indicates that during KAATSU, the arterial baroreceptor unloading is the dominant phenomenon leading to sympathetic excitation during the simulated weightlessness. The renin-angiotensin system is activated by an orthostatic stress (Duranteau et al. 1995). And, orthostatic intolerance after bed rest or spaceflight may partly result from impaired vasoconstriction, possibly due to a decreased secretion of renin-angiotensin (Fortney et al. 1991). During 6° head-down bed rest, the secretion of PRA and ADH was increased during KAATSU, depending on the degree of the pressure. From these observations, it is likely that the application of KAATSU, when using a proper pressure, on both the thighs partly simulates hemodynamic, systemic cardiovascular, autonomic nervous and hormonal effects of orthostasis during the simulated weightlessness.

Both LBNP and KAATSU induce the retention of blood flow in lower extremities, and induce subsequent hemodynamic changes such as decreased SV and CO and increased TPR (Stevens and Lamb 1965; Güell et al. 1990, 1992; Melchior et al. 1994; Murthy et al. 1994; Lee et al.

1997: Watenpaugh et al. 2000: Iida et al. 2007). The mechanism triggered by these methods (negative pressure vs. KAATSU) is quite different. However, the key physiological features of both the methods are lower extremity blood pooling, reduction of venous return to the heart, and subsequent hemodynamic changes including increased autonomic nervous system activation (Stevens and Lamb 1965; Tomaselli et al. 1987; Lathers and Charles 1993). Therefore, the effects of KAATSU and LBNP on hemodynamic parameters previously reported (Frey et al. 1986; Sandler et al. 1988; Tomaselli et al. 1990; Lathers and Charles 1993; Melchior et al. 1994; Iida et al. 2007) are compared and summarized in Table 4. Application of LBNP (-30, -40 and -50 mmHg) decreases SV in a pressure-dependent manner. 50 mmHg LBNP simulates systemic cardiovascular effects of orthostasis in 1 G (Wolthuis et al. 1974). On the other hand, KAATSU (150-200 mmHg) in the supine position (Iida et al. 2007) decreases SV, which is comparable to standing and LBNP of -30 to -40 mmHg as shown in Table 4. The present study showed that during actually simulated weightlessness (6° head-down tilt for 24 h), KAATSU 100 mmHg on both the thighs produced 32% reduction of SV, which was equal to that observed in LBNP (-40 mmHg). Thus, KAATSU appears to effectively induce pressure-dependent retention of blood flow, and induce subsequent hemodynamic changes like LBNP.

Syncopal attack has been reported to occur in LBNP, especially when using high pressure more than -40 mmHg(Stevens and Lamb 1965). The mechanisms in the occurrence of syncope remain unsettled, but several factors such as blood pooling in the extremities and splanchnic territory, and the deterioration of distal leg arterial and venous compliance have been proposed. The present study also showed that during actually simulated weightlessness, one subject had presyncope due to a drop of blood pressure during 100 mmHg KAATSU. This subject had no signs or verbal complaints associated with KAATSU (100-200 mmHg) in the seated position before bed rest. And, KAATSU markedly reduced SV and CO with an increase in TPR during the simulated weightlessness, compared with the seated position before bed rest, proposing that blood pooling in the extremities under hypovolemia developed during bed rest may be partly involved in the induction of presyncope. Thus, the occurrence of syncope should be taken into account during the actual space flight, when the KAATSU training is used during space flight. But, the KAATSU training is usually combined with exercises, and during exercise, the skeletal muscle pumping mechanism partially counteracts accumulation of blood in hyperemic lower extremities like LBNP (Eiken et al. 1986; Watenpaugh et al. 1994), where dynamic leg exercise combined with LBNP has been reported to double

Control		LBNP 30	mmHg		LBNP 40 mi	nHg		LBNP 50 m	mHg		References
HR SV(CO(I) 	HR	SV(I)	CO(I)	HR	SV(I)	CO(I)	HR	SV(I)	CO(I)	
64 79	5.0	69 (+8)	61 (-21)	4.0 (-20)	78 (+21)	51 (-35)	3.7 (-26)	89 (+39)	38 (-51)	3.3 (-34)	Frey et al. (1986)
59 47	2.7	61 (+3)	35 (-25)	2.1 (-20)	67 (+7.9)	32 (-31)	2.1 (-20)				Sandler et al. (1988)
57 86	5.0	62 (+9)	63 (-27)	3.8 (-24)	66 (+16)	59 (-31)	3.5 (-30)	74 (+30)	45 (-48)	3.2 (-36)	Tomaselli et al. (1990)
		(+5.7)	(-24)	(-23)	(+10)	(-36)	(-30)				Melchior et al. (1994)
					(+4)	(-20)	(-20)	(+8)	(-39)	(-27)	Lathers and Charles (1993)
Control		KAATSU 15	0 mmHg		KAATSU	200 mmHg		KAAI	rsu 250 mmł	Ig	
58 90	5.3	64 (+10)	66 (-27)	4.0 (-25)	66 (+14)	62 (-31)) 4.0 (-25	5) 71 (+	22) 56 ((-37) 3.9	(-26) Iida et al. (2007)
Control		KAATS	U 50 mmHg			KAATSU 100) mmHg				
59 8.	8 5.2	63 (+22	2) 69 (-	-21) 4.3	3 (-17)	67 (37)	58 (-32)	3.9 (-19)			Present study (bed rest)
The data are	shown as m	iean value repor	ted in the refer	tences							

LBNP tolerance (Watenpaugh et al. 1994). In a similar way, leg exercise combined with KAATSU may increase the tolerance for developing syncope and then prevent its occurrence.

Currently, astronauts practice 2-3 h of intensive exercise using treadmill, ergometer and resistance machines. These time-consuming countermeasures decrease plasma volume loss, and partly prevent muscle atrophy and bone loss. But, they cannot prevent astronauts from cardiovascular deconditioning. Therefore, alternate countermeasure strategies are necessary. Now, the most effective countermeasure regimen appears to be a gravitation-like stress combined with exercises. LBNP combined with treadmill has been shown to be a useful method to prevent orthostatic intolerance after space flight or during bed rest periods, probably through its effect as orthostatic stimulus (Güell et al. 1992; Murthy et al. 1994; Buckey et al. 1996; Lee et al. 1997, 2007; Watenpaugh et al. 2000; Schneider et al. 2002). It has been reported to maintain sub-maximal exercise responses such as maximal heart rate, respiratory exchange ratio, and ventilation, aerobic fitness and sprint speed, then preventing orthostatic intolerance during bed rest (Lee et al. 1997; Watenpaugh et al. 2000). In the present study, KAATSU appears to effectively induce pressure-dependent retention of blood flow, and induce subsequent hemodynamic changes like LBNP. The KAA-TSU training has been reported to result in an increase in muscle mass and muscular strength without any complications (Takarada et al. 2000; Abe et al. 2006; Fujita et al. 2007). And, it can be applied to all types of exercises including treadmill, ergometer, and resistance machines, which can be easily used by astronauts. Thus, the KAA-TSU training may provide an appropriate countermeasure for cardiovascular deconditioning as well as musculoskeletal decline associated with weightlessness. However, further studies are needed to clarify this interesting possibility as well as the safety during the long-term weightlessness.

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Watenpaugh DE, Ballard RE, Schneider SM, Lee SM, Ertl AC, William JM et al (2000) Supine lower body negative pressure exercise during bed rest maintains upright exercise capacity. J Appl Physiol 89:218–227 Wolthuis RA, Bergman SA, Nicogossian AE (1974) Physiological effects of locally applied reduced pressure in man. Physiol Rev 54:566–595 ORIGINAL ARTICLE

Pentraxin3 and high-sensitive C-reactive protein are independent inflammatory markers released during high-intensity exercise

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Abstract High-intensity exercise shares similarities with acute phase responses of inflammatory diseases. We investigated the influences of acute exercise on inflammatory markers, plasma pentraxin3 (PTX3) and serum high-sensitive C-reactive protein (CRP) (hsCRP). Nine healthy male subjects (41 ± 3 years old) participated. Each subject performed three types of exercise; ergometer exercise at 70% workload of anaerobic threshold (AT) for 30 min (70% AT exercise), peak ergometer exercise (peak EX, 20 watt increase/min until fatigue) and resistance exercises of 70% 1 RM (70% RE) until exhaustion. We measured plasma PTX3, serum hsCRP, lactate, noradrenaline (NOR), white blood cells (WBC), interleukin-6 (IL-6) and

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Department of Otolaryngology and Head and Neck Surgery, Faculty of Medicine, The University of Tokyo, Tokyo, Japan myeloperoxidase (MPO), a marker of neutrophil degranulation. The effects of exercise on intracellular PTX3 and MPO in neutrophils were also investigated, by using flow cytometry analysis. Circulating PTX3 and hsCRP significantly increased immediately after 70% RE and peak EX, while they did not increase after 70% AT exercise. The exercise-induced fold increase in PTX3 and hsCRP relative to the resting level was positively correlated with the changes in WBC, NOR, lactate and MPO. The exerciseinduced fold increase in IL-6 was positively correlated with that in NOR, but not with that in PTX3 and hsCRP. Neutrophils isolated immediately after 70% RE, but not 70% AT exercise, exhibited lower mean fluorescence for PTX3 and MPO than those from pre-exercise blood. These results provide the evidence that high-intensity exercises significantly increase circulatory PTX3 as well as hsCRP. The release from peripheral neutrophils is suggested to be involved in the exercise-induced plasma PTX3 increase.

Keywords Pentraxin3 · High-sensitive CRP · Inflammation · Neutrophil · Interleukin-6 · Resistance exercise · High-intensity exercise · Myeloperoxidase · Flow cytometry

Introduction

Pentraxins, a family of multimeric pattern-recognition proteins highly conserved in evolution (Mantovani et al. 2008), are divided into two groups (short pentraxins and long pentraxins), based on the primary structure of the subunit. C-reactive protein (CRP), a classic short pentraxin, is mainly produced in the liver and operates as a major acute phase reactant with a pronounced rise in plasma concentration in response to a variety of inflammatory diseases or tissue injury (Volanakis 2001; Pepys and Hirschfield 2003). On the other hand, PTX3 is a newly identified long pentraxin and produced by various cell types including skeletal muscle, monocytes/macrophages, endothelial cells, and vascular smooth muscle cells at the site of inflammation in response to primary inflammation or tissue injury (see review, Presta et al. 2007; Mantovani et al. 2008). PTX3 has also been reported to exist in neutrophils, suggesting that neutrophils are one of the sources of PTX3 (Jaillon et al. 2007). Thus, PTX3 is thought to be a useful serological marker reflecting tissue inflammation and damage under diverse clinical conditions, but it is completely distinct from CRP (Presta et al. 2007; Mantovani et al. 2008). Elevated plasma PTX3 levels have also been described in acute myocardial infarction, chronic renal failure, cardiac surgery, immediately after coronary stenting (Latini et al. 2004; Boehme et al. 2007; Kunes et al. 2007; Malaponte et al. 2007; Presta et al. 2007; Kotooka et al. 2008) and in systemic or localized inflammation such as sepsis (Muller et al. 2001). A general characteristic emerging from these studies about PTX3 blood levels in human pathology is considered to be the rapid rate of increase compared with CRP, together with a lack of correlation between CRP and PTX3 levels.

Daily regular exercise is known to induce anti-inflammatory effects and protect against the risk of cardiovascular diseases associated with chronic low-grade systemic inflammation such as atherosclerosis (Petersen and Pedersen 2005; Plaisance and Grandjean 2006), resulting in a decrease in serum CRP (Kasapis and Thompson 2005). Recently, we have reported that cardiac rehabilitation using aerobic exercise in patients with cardiovascular diseases decreases plasma PTX3 level as well as hsCRP (Nakajima et al. 2009). However, in contrast with anti-inflammatory effect of exercise, acute high-intensity and prolonged exercise by itself causes production of stress hormones and alterations in the circulating quantity and function of various immune cells, including leukocyte mobilization and leukocytosis (Hoffman-Goetz and Pedersen 1994; Pedersen and Hoffman-Goetz 2000; Peake et al. 2004; 2005a; Büttner et al. 2007). As such, strenuous exercise activates acute phase inflammatory response, the release of inflammatory mediators and cytokines such as interleukin (IL)-1, IL-6 and granulocyte colony-stimulating factor (G-CSF) (McCarthy et al. 1992; Ostrowski et al. 2000; Yamada et al. 2002; Natale et al. 2003; Peake et al. 2005a, b; Mooren et al. 2006; Zaldivar et al. 2006), alterations in gene expression profiles of neutrophils (Fehrenbach et al. 2003; Büttner et al. 2007) and causes substantial tissue injury (Shephard 2001). Thus, high-intensity exercise elicits inflammatory responses similar to many clinical physical stressors including surgery, trauma and sepsis, indicating that exercise may be a good model for the inflammatory response (Hoffman-Goetz and Pedersen 1994; Shephard 2001). Until now, there have been several studies on the effects of acute exercise on serum CRP. Long-lasting strenuous exercise such as a marathon run has been reported to increase serum CRP, measured 16 h after activity (Castell et al. 1997). Risøy et al. (2003) reported a rapid increase in CRP with 1 h running, and an increase of CRP level has been also reported after eccentric exercise (Phillips et al. 2003). Thus, high-intensity exercise elicits inflammatory response and then increase serum CRP. On the other hand, there is no information regarding the changes in plasma PTX3 levels in exercise-induced inflammatory responses.

Therefore, the purpose of the present study is to investigate the influences of various types of acute exercise on inflammatory markers, plasma pentraxin3 (PTX3) and serum CRP, and the characteristics of these markers in acute exercise-induced inflammation. Serum levels of highsensitive CRP (hsCRP), a sensitive marker of CRP, and plasma levels of PTX3 were measured concurrently in three types of exercise. We also measured serum myeloperoxidase (MPO), a marker of neutrophil degranulation, and compared these with the increase in hsCRP and PTX3. In addition, neutrophils isolated from the peripheral blood were assessed for the effects of exercise on intracellular PTX3 and MPO in neutrophils by using flow cytometry analysis. Here, we provide the evidence that high-intensity exercises significantly increase circulatory PTX3 as well as hsCRP. The release from peripheral neutrophils is suggested to be involved in the exercise-induced plasma PTX3 increase.

Methods

Subjects and experimental designs

Nine healthy males [age, 41 ± 3 years old; height, 169 ± 8 cm; weight, 70 ± 7 kg, body mass index (weight/ height², 24 ± 2 kg/m²)] participated in this study. This study was approved by the Ethics Committee of the University of Tokyo. All subjects were informed of the methods, procedures and risks, and signed an informed consent document before participation. None of the subjects had any diseases or took any medications such as anti-inflammatory drugs and steroids. They were non-smokers, and sedentary, and none of the subjects had participated in strength/resistance-type training for a minimum of 2 years prior to the start of the study.

Each subject visited the laboratory on a total of five occasions within this study of 4 weeks. On visits 1 and 2, the peak oxygen uptake (VO₂ peak) and one-repetition maximal (1 RM) voluntary force were measured. Perbreath gas exchange was measured, and VO₂ peak was

determined using a standard increment cycle ergometer protocol, using a ramp pulse (20 watt increase/min) with AE 300S (Minato Medical Science CO., LTD., Tokyo, Japan). The peak VO₂ value in this study was 30 ± 5 ml/ min/kg (n = 9). One RM was measured at three different stations of a resistance exercise circuit: leg press, leg curl and leg extension. Subsequently, on visits 3-5, subjects were assigned to a sequence of three experimental conditions, using a randomized block design described as follows: (1) prolonged ergometer exercise at 70% workload of anaerobic threshold (AT) for 30 min (70% AT exercise); (2) Peak ergometer exercise (Peak EX; 20 watt 4 min and then 20 watt ramp; 20 watt increase/min exercise) until fatigue; (3) resistance exercises (RE) using 70% 1RM (leg press, leg extension and leg curl) (70% RE). Subjects performed four sets of each exercise with 1-min interval until exhaustion. The total mean repetitions were 52 ± 4 (leg press), 52 ± 3 (leg curl) and 53 ± 2 (leg extension). Each of the three experimental exercise trials was separated at least by 5 days.

In flow cytometry analysis using peripheral neutrophils, three subjects performed two types of exercise (70% RE and 70% AT exercise) within 2 weeks again.

Blood sampling and analysis

On each exercise visit (visits 3-5), blood samples were obtained at 9:00-10:00 a.m. using an indwelling heparinlock catheter, which was inserted into the superficial antebrachial vein of the left arm. The subjects were instructed to sit quietly before taking blood sample. The baseline (resting/pre-exercise) blood sample was taken before the start of exercise. Further blood samples were obtained immediately after each exercise and after a 1-h recovery period. The subjects were instructed to sit quietly during the recovery phase. All samples were kept in ice-cold water and centrifuged (1,500g) for 10 min and the plasma was stored at -20° C until the assays were performed. Blood samples for measurement of blood hemoglobin (Hb), hematocrit (Htc) and white blood cell count (WBC) (2 ml) were drawn into test tubes containing EDTA-2 K. Blood hemoglobin (Hb, $g dl^{-1}$) was measured by the cyanomethemoglobin method (Coulter hemoglobinometer). Htc was measured by a micro-hematocrit using ultracentrifugation. For hormone determinations, blood (7 ml) was drawn into test tubes containing 10.5 mg EDTA-2Na. The plasma noradrenaline (NOR) concentration was measured using a high-performance liquid chromatography (HPLC) method. The lower limit of detection of the assay was 6 pg/ml. The plasma lactate level was measured using an enzyme system based on lactate oxidase combined with N-ethyl-N-(3-methylphenyl)-N-acetyl ethylenediamine and an auto-analyzer, HITACHI Type 7170.

For immunostaining, blood samples (5 ml) were collected in pre-heparinized syringes from three subjects at rest. For flow cytometry analysis, whole blood (300 μ l) from pre-exercise and immediately after each exercise was drawn into test tubes containing EDTA-2K.

Serum IL-6 and G-CSF levels were measured using the Cytometric Bead Array (CBA) from BD Biosciences (CA, USA). Serum myeloperoxidase (MPO) level was measured by an ELISA with Immundiagnostik AG (Benshein, Germany). The limit of detection for the human IL-6, G-CSF and MPO assays was 0.09 pg/ml, 0.1 pg/ml and 1.9 ng/ml, respectively.

High-sensitive CRP was measured at SRL Co. (Tokyo, Japan) in 500 μ l serum samples, using a method of latex turbidimetric immunoassay (LTIA). The limit of detection of hsCRP was 50 ng/ml. Plasma PTX3 levels were measured at Perseus Proteomics Inc. (Tokyo, Japan), using a sandwich ELISA technique as previously reported (Inoue et al. 2007; Kotooka et al. 2008). The limit of detection of the PTX3 assay was 0.1 ng/ml.

Neutrophils isolation and immunostaining

Whole blood (5 ml) from peripheral blood was layered over 5 ml of Polymorphprep (Axis-Shield, Oslo, Norway) and spun for 35 min at 690 g at 20°C. Neutrophils (polymorphonuclear leukocytes, PMNs) were harvested from the second interface, washed with phosphate-buffered saline (PBS, Wako Pure Chemical Industries, Ltd., Osaka) and spun for 10 min at 270 g at room temperature. Neutrophils were resuspended in the buffer (3% FBS and 0.05% NaN₃ in PBS), mixed with ice-cold 70% ethanol for 30 min and spun for 10 min at 270 g at room temperature. Subsequently, neutrophils were resuspended in 1 ml 70% ethanol, dropped on a slide glass and dried. For immunostaining, the fixed cells were washed in PBS, blocked for 10 min with 2% horse serum in PBS and incubated with the primary antibody in a humid chamber overnight at 4°C. The primary antibodies were the rabbit polyclonal antihuman myeloperoxidase (Novus Biological, Littleton, CO) and rat monoclonal antibody against human PTX3 (clone MNB4, Alexis Biochemicals, San Diego, CA). After the preparations were washed in PBS, the secondary antibody was incubated for 60 min at room temperature. Alexa Fluor 488-conjugated donkey anti-rabbit IgG (H + L) antibody (Molecular Probes, Molecular Probes, Eugene, OR), and Cy3-conjugated goat anti-rat IgG (Jackson ImmunoResearch, West Grove, PA) were used to visualize MPO and PTX3 expression. For negative controls, cells were treated without antibody. The cells were also stained with Hoechist 33258 (Sigma-Aldrich) to visualize nuclei. A confocal laser scanning microscope (Olympus FluoView FV300, Olympus Co., Tokyo, Japan) was used for observations.

Flow cytometry

For flow cytometry analysis using neutrophils, whole blood (300 µl) was lysed with Versalyse reagent (Beckman Coulter, Fullerton, CA) and spun for 5 min at 270 g. Then, cells were resuspended in 100 µl of 4% paraformaldehyde for 15 min. Then, 1 ml of the buffer (see above) was added and spun for 5 min at 270 g. The cells were resuspended in 100 µl buffer containing 0.1% saponin for 5 min and incubated for 30 min at room temperature with a mixture of primary antibody (the rabbit polyclonal anti-human myeloperoxidase (Novus Biological, Littleton, CO) and rat monoclonal antibody against human PTX3 (clone MNB1, Alexis Biochemicals, San Diego, CA) for 30 min. Then, the cells were added with 1 ml buffer containing 0.1% saponin, spun at 270g and incubated with secondary antibody (Alexa Fluor 488-conjugated donkey anti-rabbit IgG (H + L) antibody (Molecular Probes Inc., Eugene, OR) and PE-conjugated goat anti-rat IgG (H + L) antibody (Beckman Coulter, Fullerton, CA). Finally, the cells were again resuspended in the buffer. Flow cytometry was performed on a flow cytometer (EPICS XL-MCL, Beckman Coulter, Fullerton, CA).

Data analysis

All values are expressed as the mean \pm SE. Statistical analyses were performed by a two-way analysis of variance (ANOVA) with repeated measures [Time (pre- and posttesting) × group (Peak EX, 70% AT exercise and RE)]. When differences were indicated, Bonferroni's comparison was used to determine significance. Student's paired *t* test was used to compare two sets of data from the same subjects. Spearman's rank correlation coefficient (*r*) was used to examine the relationship between individual exerciseinduced changes. Differences were considered significant if *p* value was less than 0.05. The intra- and inter-individual coefficients of variation (CV) were determined as the ratio of the standard deviation to the mean value.

Results

We first investigated the daily variation of plasma PTX3 and serum CRP level obtained from nine healthy male subjects under resting conditions and the relationships between them. Blood samples were obtained three times under resting condition for each subject at the same time on different days before three exercise trials were performed. The daily variability of plasma PTX3 (A) and serum hsCRP (B) level is shown for each subject (Fig. 1a, b). The mean value, and inter- and intra-individual coefficient of variation (CV) for PTX3 and hsCRP are shown in Table 1. As shown in Fig. 1 and Table 1, the inter- and intra-individual CV was lower for the resting plasma PTX3 level than for the resting serum hsCRP level, suggesting that plasma PTX3 is relatively stable compared with serum hsCRP. Figure 1c shows the absence of a significant correlation between resting plasma PTX3 and serum hsCRP level.

Figure 2a–c shows the effects of three types of exercise on WBC (Fig. 2a), lactate (Fig. 2b), and NOR (Fig. 2c). All three types of exercise induced a significant increase in WBC (Fig. 2a, p < 0.01) immediately after exercise, but baseline values were restored by 1 h after exercise. The peak value of WBC was greater for peak EX and 70% RE than 70% AT exercise (p < 0.01). Both lactate and NOR concentration were significantly increased immediately after peak EX and 70% RE (p < 0.01), while it did not change significantly after 70% AT exercise (Fig. 2b, c).



Fig. 1 Diurnal variation of resting plasma PTX3 (a) and serum hsCRP level (b). Blood samples were obtained three times under resting condition for each subject at 9:00-10:00 a.m. before three exercise trials were performed. Plasma PTX3 and serum hsCRP were measured, and the data were plotted in Rest 1, Rest 2 and Rest 3. c Correlations between resting plasma PTX3 and serum hsCRP level obtained from nine healthy subjects

 Table 1
 Mean value, intra- and inter-individual coefficients of variation for plasma PTX3 and serum hsCRP levels in nine healthy subjects

	Mean	Inter-individual CV	Intra-individual CV
PTX3 (ng/ml)	1.83	0.38	0.13
hsCRP (ng/ml)	368	0.98	0.37

PTX3 pentraxin3, hsCRP high-sensitive CRP, CV coefficient of variation

Fig. 2 White blood cell count (WBC, a), lactate (b), noradrenaline (NOR, c), hematocrit (Htc, d), CPK (e) and myeloperoxidase (MPO, f) before exercise (Pre EX), immediately after (Post EX) and 1 h after EX. Peak ergometer exercise until fatigue (Peak EX); ergometer exercise at 70% workload of anaerobic threshold (AT) for 30 min (70% AT exercise); resistance exercises (leg press, leg curl and leg extension) of 70% 1 RM until exhaustion (70% RE). Values are means \pm S.E. for nine subjects. **Significantly different from Pre EX, p < 0.01. *Significantly different from Pre EX, p < 0.05





Fig. 3 Correlations between the fold increase in serum MPO level and that in lactate (a), WBC (b) and noradrenaline (NOR, c)

Figure 2d, e shows the effects of three types of exercise on Htc and CPK. The significant rise in Htc was observed immediately after both peak EX and 70% RE (p < 0.01). But, Htc did not significantly increase in 70% AT exercise. CPK (Fig. 2e) increased from pre-exercise to post-exercise (time effect, p < 0.01); however, no significant group differences were observed (p > 0.05).

Figure 2f shows the effects of three types of exercise on serum MPO concentration, a marker of neutrophil degranulation. A rise in serum MPO was observed immediately after both peak EX and 70% RE (p < 0.01), but not after 70% AT exercise. Figure 3 shows the correlation between the fold increase in serum MPO and that in lactate, WBC and NOR. The fold increase in MPO was positively correlated with WBC, NOR and lactate.

Figure 4 shows the effects of three types of exercise on serum hsCRP (Fig. 4a) and plasma PTX3 (Fig. 4b). As shown in Fig. 4, both peak EX and 70% RE significantly increased hsCRP and PTX3 (p < 0.01) immediately after exercise. The increased hsCRP and PTX3 level returned to baseline levels within 1 h. Figure 4c shows the correlation between the fold increase in hsCRP and that in PTX3 during the exercise. The fold increase in PTX3 was positively correlated with that in hsCRP. On the other hand, neither hsCRP nor PTX3 were significantly affected by 70% AT exercise (Fig. 4a, b).

We compared the fold increase in lactate, NOR, WBC or MPO with that in serum hsCRP or plasma PTX3 as shown in Table 2. The fold increase in hsCRP and PTX3



Fig. 4 Serum hsCRP (a) and plasma PTX3 (b) level before (Pre EX), immediately after (Post EX), and 1 h after EX. Peak EX, 70% AT exercise, and 70% RE. Values are means \pm SE for nine subjects. **Significantly different from Pre EX, p < 0.01. c Correlations between the fold increase in hsCRP and that in PTX3 during exercises

relative to the resting level was positively correlated with that in WBC, NOR, lactate and MPO.

It is well known that the inflammatory markers hsCRP and PTX3 are induced by cytokines such as IL-6 and IL-1 (Li and Goldman 1996; Mantovani et al. 2008). Therefore, we investigated the effects of exercise on serum IL-6 and G-CSF level. Peak EX significantly increased IL-6 from 0.89 ± 0.48 pg/ml at rest to 1.40 ± 0.60 pg/ml immediately after exercise (p < 0.01). The increased IL-6 level returned to 0.99 ± 0.47 pg/ml at 1 h after exercise; 70% RE did not significantly increase IL-6 (0.66 \pm 0.28 pg/ml at rest, 1.03 ± 0.44 pg/ml immediately after exercise and 1.06 ± 0.35 pg/ml at 1 h after exercise); 70% AT exercise also did not change IL-6. On the other hand, G-CSF was not significantly affected by these exercises (data not shown). The fold increase in IL-6 or G-CSF was not correlated with that in PTX3 or hsCRP (Table 2).

Finally, we investigated the existence of PTX3 and MPO in human neutrophils. The flow cytometry analysis showed the existence of both PTX3 (Figs. 5a, 6) and MPO (Fig. 6) using specific antibody. Immunostaining studies also demonstrated the presence of MPO (Fig. 5c) and PTX3 (Fig. 5d) in human neutrophils. Similar data were

obtained from three subjects examined. The nucleus staining is shown in Fig. 5b. Figure 6 shows the effects of exercise (70% AT exercise and 70% RE) on PTX3 and MPO in neutrophils using flow cytometry. The typical data are shown in Fig. 6a, b. The mean fluorescence density of PTX3 and MPO in neutrophils isolated from pre-exercise blood is considered as 100%, and the relative value of the mean fluorescence density of neutrophils isolated from blood immediately after exercises is shown in Fig. 6c. The data were obtained from three subjects. Neutrophils isolated from blood immediately after 70% RE exhibited lower mean fluorescence for PTX3 and MPO than those from pre-exercise blood (Fig. 6a, c). On the other hand, neutrophils isolated from blood immediately after 70% AT exercise exhibited no significant changes of the mean fluorescence for PTX3 and MPO (Fig. 6b, c), compared with those isolated from pre-exercise blood.

Discussion

The major findings of the present study are as follows. (1) The short-term acute high-intensity exercise (peak EX and 70% RE) until exhaustion increased plasma PTX3 and serum MPO as well as serum hsCRP in untrained healthy male subjects. (2) The increase in PTX3 and hsCRP was positively correlated with that in NOR, lactate, WBC and MPO, but not with IL-6 or G-CSF. (3) Neutrophils isolated from blood immediately after 70% RE, but not after 70% AT exercise, exhibited lower mean fluorescence for PTX3 and MPO than those from resting blood. These results provide the evidence that highintensity exercises significantly increase circulatory PTX3 as well as hsCRP. The release from peripheral neutrophils is suggested to be involved in the exercise-induced plasma PTX3 increase.

Acute exercise is an important stressor to the body, leading to an activation of immune responses (Hoffman-Goetz and Pedersen 1994; Pedersen and Hoffman-Goetz 2000; Peake et al. 2004; 2005a; Büttner et al. 2007). Such an exercise-induced acute phase response consists of typical changes including leukocytosis and release of cytokines and acute phase proteins, similar to acute phase

Table 2 Correlation between inflammatory markers (fold		Lactate	NOR	WBC	MPO	G-CSF	IL-6
increase in hsCRP or PTX3) and neurohumoral responses (fold increase in lactate, NOR, WBC, MPO, G-CSF or IL-6)	hsCRP r value (p value) PTX3	0.75 (6 × 10 ⁻⁶)	0.71 (5 × 10 ⁻⁵)	0.55 (2×10^{-4})	0.47 (7 × 10 ⁻³)	0.14 (0.2)	0.32 (0.06)
	<i>r</i> value (<i>p</i> value)	0.56 (1 × 10 ⁻³)	0.5 (6 × 10 ⁻³)	0.67 (8 × 10 ⁻⁵)	0.48 (6 × 10 ⁻³)	0.22 (0.12)	0.1 (0.28)



Fig. 5 Expression of MPO and PTX3 in human neutrophils isolated from peripheral blood. **a** Flow cytometry analysis of PTX3 expression in human neutrophils isolated from peripheral blood. The neutrophils were counterstained with Hoechst33258 to visualize nuclei in **b**. **c**, **d** Immunostaining of MPO (**c**) and PTX3 (**d**) in neutrophils

responses evoked by other stressors such as trauma and sepsis (McCarthy et al. 1992; Ostrowski et al. 2000; Yamada et al. 2002; Natale et al. 2003; Peake et al. 2005b; Mooren et al. 2006; Zaldivar et al. 2006). In the present study, peak EX and 70% RE markedly increased NOR, lactate and WBC, compared with 70% AT exercise. These high-intensity exercises also increased serum MPO concentration, a marker of neutrophil degranulation, which is consistent with previous papers showing that changes in serum MPO concentration depends on exercise intensity (Peake et al. 2004). In addition, flow cytometry analysis showed that neutrophils isolated from blood immediately after RE, but not after 70% AT exercise, exhibited lower mean fluorescence for MPO than those from resting blood, suggesting that high-intensity exercise released MPO from peripheral neutrophils. Serum CPK level increased from pre-exercise to post-exercise independently of exercise programs. The mean fold increase in serum CPK value at 70% RE was 1.15. These values are almost equal to the rise in Htc, indicating that the increase in serum CPK might be partly explained by the hemoconcentration with the relatively high-intensity exercise compared with the lowintensity exercise of 70% AT exercise. However, we cannot exclude the possibility that some muscle damage might have occurred during exercise.

Cytokines are produced by a wide variety of cell types, including skeletal muscle, when exposed to various inflammatory stimuli such as acute high-intensity exercise.



Fig. 6 Effects of 70% RE and 70% AT exercise on MPO and PTX3 in neutrophils using flow cytometry. The typical data are shown at 70% RE (**a**) and 70% AT exercise (**b**). Note that neutrophils isolated from blood immediately after 70% RE, but not 70% AT exercise, exhibit lower mean fluorescence for PTX3 and MPO than those from pre-exercise blood. The mean fluorescence density of MPO and PTX3 in neutrophils isolated from pre-exercise blood is considered as 100%, and the relative value of the mean fluorescence density of neutrophils isolated from blood immediately after exercises is shown in **c**. Data were obtained from three subjects. Note that the mean fluorescence density of MPO and PTX3 in neutrophils isolated from blood immediately after 70% AT exercise, significantly decreased, compared with the pre-exercise level (100%). *p < 0.05 versus pre-exercise level

Production of IL-6 in contracting human skeletal muscles can account for the exercise-induced increase in plasma IL-6 (Steensberg et al. 2000). The present study also showed that IL-6 significantly increased in peak EX. On the other hand, G-CSF plays essential roles in bone marrow release of cells from a neutrophil lineage and activates neutrophils. Intensive exercise has been reported to increase serum G-CSF level (Smith et al. 2000; Suzuki et al. 2000; Yamada et al. 2002). In our study, however, G-CSF was not significantly increased after peak EX and 70% RE, which might be
due to differences in exercise protocols (intensity and duration) and the ages of subjects studied.

Long-lasting strenuous exercise, such as a marathon run, has been reported to increase serum CRP, measured 16 h after the activity (Castell et al. 1997). Risøy et al. (2003) also reported a rapid blood increase in CRP in healthy untrained subjects after a single 1-1.5 h run (from 2.6 to 5.1 ng/ml) on measuring hsCRP. However, until now, the effects of various types of acute exercise on CRP have not been fully investigated. The present study using hsCRP showed that the short-term acute high-intensity exercise (70% RE and peak EX) increased serum hsCRP in healthy male subjects. CRP is produced mainly in the liver under the regulatory control of IL-6 (Li and Goldman 1996). However, it is unlikely that IL-6 may be involved in the rapid increase of serum CRP in our exercise protocol, since we used short-term exercise within 30 min, and the increase in serum CRP was not correlated with that in serum IL-6. Alternatively, the rapid increase in serum CRP may be caused by release of the preformed protein from the liver. Actually, it has been shown that the rate of CRP secretion is greatly accelerated during the acute phase response (Macintyre et al. 1985). However, the additional sites of CRP production such as kidney and vascular wall could not be excluded (Volanakis 2001; Sun et al. 2005). As compared with hsCRP, PTX3 has been shown to be a stable and high-sensitive marker for inflammation under the various pathophysiological conditions (Presta et al. 2007; Mantovani et al. 2008). In the present study, no correlations were found between the resting plasma PTX3 and serum hsCRP levels, proposing that CRP and PTX3 are independent markers of inflammation, as previously described (Presta et al. 2007; Mantovani et al. 2008). We also showed that the inter- and intra-individual CVs for the resting plasma PTX3 level were less than that for the resting serum hsCRP levels, suggesting that plasma PTX3 may be a relatively stable marker compared with hsCRP. In addition, we provided the evidence that plasma PTX3 increased with high-intensity exercise (peak EX and 70% RE), but not with 70% AT exercise.

The exercise-induced increase in hsCRP was strongly correlated with that in NOR and lactate, WBC and MPO, while the exercise-induced increase in PTX3 was strongly correlated with that in WBC and MPO. Thus, it is likely that the trigger mechanisms underlying the acute exercise-induced CRP and PTX3 may be different. PTX3 as well as CRP are known to be induced by cytokines such as IL-1 (Li and Goldman 1996; Mantovani et al. 2008). However, in a similar fashion to CRP, the increase in PTX3 was not correlated with that in serum IL-6 or G-CSF investigated in the present study. Thus, a rapid increase in PTX3 may be due to the release of the preformed protein. Jaillon et al. (2007) reported that PTX3 exists in neutrophils and is

stored in granules, suggesting that neutrophils could be one of the sources of PTX3 released into the blood stream. The present study also showed the presence of PTX3 as well as MPO in neutrophils. Recently, Kotooka et al. (2008) reported that rapid changes in plasma PTX3 occurred across the stented vascular bed within 15 min, and the relationship between the response of plasma PTX3 and activated macrophage antigen-1 suggests that local leukocytes recruited on the stented lesion in response to stentinduced vascular injury may be the major cellular source responsible for the increased PTX3. The present study showed that 70% RE increased serum MPO as well as plasma PTX3, and neutrophils isolated from blood immediately after 70% RE exhibited lower mean fluorescence for PTX3 and MPO than those from resting blood. These results suggest that high-intensity exercise releases both PTX3 and MPO from peripheral neutrophils, which may be involved in the exercise-induced PTX3 and MPO increase. Similarly, changes in neutrophil surface receptor expression including CD16 and degranulation have also been reported immediately after high-intensity exercise (a treadmill for 1 h at 80% VO₂max) (Peake et al. 2004). Thus, under high-intensity exercise, the rapid release of stored PTX3 by activated neutrophils appears to play a role in the early phase of its elevation preceding gene expression-dependent production, as described for acute exerciseinduced gene alterations (Büttner et al. 2007).

In conclusion, high-intensity exercises significantly increase circulatory PTX3 as well as hsCRP. The release from peripheral neutrophils is suggested to be involved in the exercise-induced plasma PTX3 increase.

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ORIGINAL ARTICLE



Repetitive restriction of muscle blood flow enhances mTOR signaling pathways in a rat model

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Abstract Skeletal muscle is a plastic organ that adapts its mass to various stresses by affecting pathways that regulate protein synthesis and degradation. This study investigated the effects of repetitive restriction of muscle blood flow (RRMBF) on microvascular oxygen pressure (PmvO₂), mammalian target of rapamycin (mTOR) signaling pathways, and transcripts associated with proteolysis in rat skeletal muscle. Eleven-week-old male Wistar rats under anesthesia underwent six RRMBF consisting of an external compressive force of 100 mmHg for 5 min applied to the proximal portion of the right thigh, each followed by 3 min rest. During RRMBF, PmvO₂ was measured by phosphorescence quenching techniques. The total RNA and protein of the tibialis anterior muscle were obtained from control rats, and rats treated with RRMBF 0-6 h after the stimuli. The protein expression and phosphorylation of various signaling proteins were determined by western blotting. The mRNA expression level was measured by real-time RT-PCR analysis. The total

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muscle weight increased in rats 0 h after RRMBF, but not in rats 1–6 h. During RRMBF, $PmvO_2$ significantly decreased (36.1 ± 5.7 to 5.9 ± 1.7 torr), and recovered at rest period. RRMBF significantly increased phosphorylation of p70 S6-kinase (p70S6k), a downstream target of mTOR, and ribosomal protein S6 1 h after the stimuli. The protein level of REDD1 and phosphorylation of AMPK and MAPKs did not change. The mRNA expression levels of FOXO3a, MuRF-1, and myostatin were not significantly altered. These results suggested that RRMBF significantly decreased PmvO₂, and enhanced mTOR signaling pathways in skeletal muscle using a rat model, which may play a role in diminishing muscle atrophy under various conditions in human studies.

Keywords Blood flow restriction \cdot Mammalian target of rapamycin (mTOR) \cdot Microvascular pO₂ \cdot Muscle atrophy \cdot Myostatin \cdot p70 S6-kinase \cdot Rat skeletal muscle \cdot Hypoxia

Abbreviations

RMBF	Restriction of muscle blood flow
RRMBF	Repetitive restriction of muscle blood flow
PmvO ₂	Microvascular oxygen pressure
HIF-1α	Hypoxia-inducible factor-1α
mTOR	Mammalian target of rapamycin
p70S6k	p70 S6-kinase
ERK1/2	Extracellular signal-regulated kinase 1/2
FOXO3a	Forkhead box O3A
MuRF-1	Muscle ring finger-1
VEGF	Vascular endothelial growth factor

Introduction

It has been well known that muscle size is mainly determined by the balance between muscle protein synthesis and degradation [1, 2]. Muscle hypertrophy occurs when the protein synthesis exceeds the protein degradation, while muscle atrophy occurs when the protein degradation overcomes the protein synthesis. After muscle disuse, for example during long-term bed rest, spaceflight, and simulated models of nobearing activity, skeletal muscle atrophy develops due to the altered protein metabolism, leading to decreased muscle contractile protein content. To prevent it and increase muscle protein synthesis, resistance exercise, an established potent stimulus for enhancing muscle protein synthesis and subsequent muscle hypertrophy, is usually used. The increased muscle protein synthesis is associated with enhanced phosphorylation events on numerous kinases to regulate mRNA translation, including mammalian target of rapamycin (mTOR1)/ p70 S6-kinase (p70S6k) signaling pathways [3, 4].

The conventional strength training pursued at intensity lower than 60-70 % 1RM usually does not increase muscle size, compared with high-intensity exercise. However, highintensity loading entails a risk of injury to the moving organs or circulatory system in elderly individuals and patients with various diseases. On the other hand, several human studies have shown that low-intensity (20-30 % 1RM) resistance exercise under restriction of muscle blood flow (RMBF), also known as KAATSU training [5, 6], is a new strength training method which can stimulate numerous muscle fibers and produce increased muscle size and strength even with low-intensity loading [6, 7]. Fujita et al. [8] reported that the protein synthesis increased in healthy young adults 3 h after a brief session of RMBF knee extension exercise. Additionally, they showed that phosphorylation of p70 S6-kinase, a key regulator of the mTOR pathway, was enhanced after RMBF exercise, compared with control exercise. Moreover, reduced expression of the proteolysis-related genes such as forkhead box O3A (FOXO3a), atrogin-1 and muscle ring finger-1 (MuRF-1) as well as myostatin, a negative regulator of muscle mass, has been reported after acute RMBF exercise [9, 10]. These mechanisms may play a role on hypertrophic effects of RMBF exercise, even by using low-intensity resistance exercise. On the other hand, skeletal muscle is a plastic organ that adapts its mass to various stresses by affecting pathways that regulate protein and cellular turnover. Takarada et al. [11] reported that repetitive restriction of muscle blood flow (RRMBF) even without exercise effectively diminished postoperative disuse atrophy of knee extensors in patients who underwent reconstructive surgery of the anterior cruciate ligament. They used RRMBF stimulus, each consisting of five repetitions of RMBF (mean maximal pressure, 238 mmHg) for 5 min, and release of pressure for 3 min. Kubota et al. [12] also reported that the application of RRMBF to the lower extremity prevented disuse muscular weakness induced by immobilization without weight bearing. They used RRMBF at an external compressive force of 200 mmHg for 5 min, followed by 3 min rest, a regimen repeated five times in a single session. Thus, RRMBF appears to be a novel stimulus of skeletal muscle to induce a net positive protein balance and prevent atrophy. However, the underlying mechanisms of RRMBF have not been investigated.

Therefore, we investigated the effects of RRMBF on mTOR signaling pathways involved in initiating skeletal muscle translation, and transcripts associated with proteolysis as well as microvascular pO_2 (PmvO₂) in skeletal muscle using a rat model. Here we documented for the first time that RRMBF significantly decreased PmvO₂, and enhanced mTOR signaling pathways in skeletal muscle using a rat model.

Materials and methods

Animals

Male Wistar rats (n = 34, age 11 weeks) were used in our study (Japan SLC, Inc., Shizuoka Lab. Animal Center). All rats were housed in a temperature-controlled room at 22 ± 2 °C with a light–dark cycle of 12 h, and were maintained on rat feed and water ad libitum as previously described [13]. All procedures for microvascular PO₂ (PmvO₂) measurement and blood flow restriction experiments were performed under isoflurane anesthesia (50 mg/ kg body wt). All experiments were conducted under the guidelines established by the Physiological Society of Japan and were approved by University of Electro-Communications Institutional Animal Care and Use Committee.

Repetitive restriction of muscle blood flow (RRMBF)

Under isoflurane anesthesia, rats were secured in a supine position, and the hip and knee joint of the right leg were bent in 90° flexion. The right leg was fixed mechanically and positioned on a firm and stable pedal as shown in Fig. 1a. Blood flow restriction to the right lower extremity was induced by compressing the proximal end of the right thigh with a Durable Digit Cuff (latex cuff; overall cuff size 16 mm width, 90 mm length; D.E. Hokanson, Inc.). The RRMBF stimulus, which consisted of an external compressive force of 100 mmHg for 5 min, followed by 3 min of rest, was repeated six times during a 48-min interval (Fig. 1b) as described in the previous clinical studies [11, 12].

Experimental protocol

Microvascular PO2 (PmvO2) measurement

Phosphorescence quenching as described previously [14] was utilized to determine PmvO₂ during a blood flow



Fig. 1 Experimental protocols. a Blood flow restriction (BFR) using a durable digit cuff. Under isoflurane anesthesia, rats were kept at supine position and the right leg was fixed on a firm and stable pedal at 2-3 cm above the chest level with both hip and ankle joint of 90° $(0^{\circ} \text{ at full extension})$. **b** Protocols of repetitive restriction of muscle blood flow (RRMBF). The RRMBF stimuli, consisting of external compressive force of 100 mmHg for 5 min followed by 3 min of rest, were repeated six times for 48 min. c Experimental protocol for microvascular PO₂ (PmvO₂) measurement during RRMBF (Experiment 1 EX1) and muscle tissue isolation (Experiment 2 EX2). Phosphorescence quenching method was utilized for the determination of Pmv_{O2} during RRMBF protocol within the medial region of the tibialis anterior muscle. In EX2, rats in each group (n = 6) were sacrificed in control (pre), immediately after (0 h), 1, 3, and 6 h after RRMBF, and the total tibialis anterior muscles were dissected from both right leg (BFR side) and left leg (control side)

restriction protocol applied to the medial region of the tibialis anterior muscle. Rats were anesthetized with isofurane anesthesia. To monitor arterial blood pressure and heart rate, the left carotid artery was cannulated (PE50). This catheter was also used to infuse a phosphorescent probe [palladium meso-tetra (4-carboxyphenyl) porphyrin dendrimer (R2)] at 15 mg/kg body wt. A lateral incision of the skin and overlying fascia was made to expose the tibialis anterior muscle of the right leg (Fig. 1a). The muscle surface was superfused with Krebs-Henseleit solution, equilibrated with 5 % CO₂-95 % N₂ at 38 °C, and adjusted to pH 7.4. The phosphor R2 was applied via the arterial cannula 15 min before the beginning of experiments, which were performed in a darkened room to prevent contamination by ambient light. The total duration of the experiments did not exceed 1 h.

 $PmvO_2$ was determined at 1-s intervals before and during RRMBF, and for 5 min during recovery [Fig. 1c, Experiment (EX) 1]. A PMOD 2000 frequency domain

phosphorometer (Oxygen Enterprises, Philadelphia, PA) was used to determine PmvO₂. The common end of the bifurcated light guide was placed approximately 2-3 mm above the medial region of the tibialis anterior muscle. The theoretical basis for phosphorescence quenching has been reported previously [15, 16]. Briefly, the Stern–Volmer relationship describes the quantitative O_2 dependence of the phosphorescent probe. R2 is a nontoxic dendrimer that binds to albumin at 38 °C and pH 7.4, with a quenching constant of 409 mmHg⁻¹ s⁻¹ and a 601 μ s lifetime of decay of in the absence of O_2 , under the physiological conditions extant herein [17]. The net negative charge of R2 also facilitates restriction of the compound to the vascular space. Thus, $PmvO_2$ reflects the pO₂ within capillary blood, which constitutes the principal intramuscular vascular space [15, 18].

Isolation of muscle tissue

Male Wistar rats (n = 30) underwent RRMBF under isoflurane anesthesia (50 mg/kg body wt) in 100 % O₂. Rats in each group (n = 6) were sacrificed immediately (~approximately 5 min after RRMBF) (0 h), 1 h, 3 h, and 6 h after RRMBF, and the entire tibialis anterior muscle was collected from both the right leg (blood flow restriction side) and left leg (control side). The harvested tissue was immediately flash frozen in liquid nitrogen and stored at -80 °C until use in experiments [Fig. 1c, Experiment (EX) 2].

RNA extraction and real-time quantitative reverse transcriptase/polymerase chain reaction (RT-PCR)

All cellular RNA was extracted from each tibialis anterior muscle using an RNeasy mini kit (Qiagen, Cambridge, MA). For RT-PCR, complementary DNA (cDNA) was synthesized from 1 µg of total RNA using a reverse transcriptase with random primers (Toyobo, Osaka). Realtime quantitative RT-PCR was performed using realtime Taq-Man technology and a sequence detector (ABI PRISM[®]7000, Applied Biosystems, Foster City, CA). Gene-specific primers and Taq-Man probes were used to analyze transcript quantity. The 18S ribosomal RNA level was analyzed as an internal control and used to normalize the values for transcript quantity of mRNA. The probes used in this study were purchased as part of the Assay-on-Demand service from Applied Biosystems (Foster City, CA): Assay ID Rn01441087_m1 for forkhead box O3A (FOXO3a), Rn00590197_m1 for muscle ring finger-1 (MuRF-1), Rn00569683_m1 for myostatin, Rn00577560_ m1 for hypoxia-inducible factor-1 α (HIF-1 α), and 4310893E for 18S rRNA endogenous control.

Proteins were separated on 10 % polyacrylamide gels for 60 min at 200 V and then transferred to Amersham Hypond-P membranes (GE Healthcare UK Ltd., Buckinghamshire, England) for 60 min at 72 mA using the semidry method. After the transfer, the membrane was blocked with 2-3 % skim milk in PBS (0.01 M phosphate buffer, 0.138 M NaCl, 0.027 M KCl, pH 7.4) with 0.1 % Tween 30 (PBS-T) at room temperature for 1 h. The membrane was then exposed to antibodies at the appropriate dilutions in blocking buffer overnight at 4 °C. The probed membrane was then washed three times in PBS-T for 15 min each time and was subsequently incubated with peroxidaselinked anti-rabbit IgG (Santa Cruz Biotechnology, Inc., CA; diluted 1:5000 with blocking buffer) for 1 h at room temperature. After three additional washes, bound antibodies were detected with a Chemi-Lumi One Super kit (Nacalai Tesque, Kyoto, Japan) and analyzed with an LAS-3000 mini-image analyzer (Fuji-Film, Tokyo, Japan).

Phosphorylation of Akt (Protein kinase B, PKB) on Ser473 was assessed using anti-phospho-Akt antibody (#4060, Cell Signaling Technology, Beverly, MA, USA) and anti-total Akt antibody (#4691, Cell Signaling Technology). Phosphorylation of mTOR on Ser2448 was measured using anti-phospho-mTOR antibody (#2971, Cell Signaling Technology) and anti-total mTOR antibody (#2972, Cell Signaling Technology). Phosphorylation of p70 S6 kinase (p70S6 k) on Thr389 was determined using antiphospho-p70 S6 kinase antibody (#9234, Cell Signaling Technology) and anti-total p70 S6 kinase antibody (#2708, Cell Signaling Technology). Ribosomal protein S6 phosphorylation (rpS6) on Ser240/244 was evaluated using anti-phospho-S6 antibody (#5364, Cell Signaling Technology) and anti-total S6 antibody (#2317, Cell Signaling Technology). Antibody dilutions were 1:1000 in each case. Phosphorylation of eEF2 on Thr56 was determined using anti-phospho-eEF2 antibody (#2331, Cell Signaling Technology) and anti-total eEF2 antibody (#2332, Cell Signaling Technology). 4E-BP1 phosphorylation on Thr37/46 was assessed using anti-phospho-4E-BP1 antibody at a dilution of 1:4000 (#2855, Cell Signaling Technology) and anti-total 4E-BP1 antibody (#9644, Cell Signaling Technology). AMPK phosphorylation on Thr172 was assessed using anti-phospho-AMPKa antibody (#2535, Cell Signaling Technology) and anti-total AMPKα antibody (#2532, Cell Signaling Technology). Phosphorylation of extracellular signal-regulated kinase 1/2 (ERK1/2) at Thr202/ Tyr204, p38 mitogen-activated protein kinase (p38MAPK) at Thr180/Tyr182, and c-Jun N-terminal kinase 1 (JNK1) at Thr183/Tyr185 was measured using anti-phospho-ERK1/2 antibody (#4370, Cell Signaling Technology) and anti-total ERK1/2 antibody (#4695, Cell Signaling Technology),

anti-phospho-p38MAPK antibody (#4511, Cell Signaling Technology) and anti-total p38MAPK antibody (#2307, Cell Signaling Technology), and anti-phospho-JNK1/2 antibody (# 9255, Cell Signaling Technology) and antitotal JNK1/2 antibody (#3708, Cell Signaling Technology), respectively. No change in total protein content was observed for any of the variables during these experiments.

Anti-REDD1 (regulated in development and DNA damage responses) rabbit polyclonal antibodies (NOVUS, NBP1-95,188) were obtained from Novus Biological. Anti-HIF-1 α antibody and anti-vascular endothelial growth factor (VEGF) rabbit polyclonal antibody were purchased from Abcam. Blots were loaded according to protein concentration (approx. 100–150 µg).

Data analysis

All values are expressed as mean \pm SEM. Differences between groups were compared by one-way ANOVA and Bonferroni's post hoc test. The level of significance was set at *P* < 0.05.

Results

PmvO₂ response during RRMBF

Figures 2a, b show dynamic $PmvO_2$ profiles in response to RRMBF. The typical data obtained from a rat are illustrated in Fig. 2a. During the application of RRMBF, dynamic $PmvO_2$ decreased rapidly and reached a level of approximately 5–8 torr. After release of the cuff pressure, dynamic P $PmvO_2$ returned to a control level. During RRMBF, similar dynamic $PmvO_2$ profiles were observed. Figure 2b shows mean $PmvO_2$ profiles during RRMBF obtained from four different rats. Baseline values of $PmvO_2$ were 36.1 ± 5.7 torr (n = 4). The minimum $PmvO_2$ level during RRMBF changed in the range of 4.8-9.0 torr (n = 4). The mean value was 5.9 ± 1.7 torr (n = 4), and gradually recovered during a rest period.

Animal data

Figures 2c, d show the effects of RRMBF on total weight of the tibialis anterior muscle and total muscle weight/ body weight. Total weight of the tibialis anterior muscle (Fig. 2c) and total muscle weight/body weight (Fig. 2d) increased significantly in rats sacrificed immediately after RRMBF, compared with control rats. It is therefore likely that the application of RRMBF using 100 mmHg in the present model induced venous pooling of blood in the lower leg and swelled the tibialis anterior muscle, a known phenomenon of KAATSU training [5, 19]. The increase in



Fig. 2 a, **b** Effects of RRMBF on microvascular pO_2 (PmvO₂) measurement. **a** Typical data recording of the dynamic Pmv_{O2} profiles in response to RRMBF. Note that during the application of RRMBF, the dynamic PmvO₂ rapidly decreased, and reached to a level of approximately 4.8–9.0 torr. After the release of BFR, the dynamic PmvO₂ returned almost to a control level. **b** The mean PmvO₂ profiles during RRMBF obtained from four different rats. The values of PmvO₂ at rest were 36.1 ± 5.7 torr (n = 4), and significantly decreased to 5.9 ± 1.7 torr significantly (n = 4) during RRMBF. **c**, **d** Effects of

total weight of the tibialis anterior muscle and total muscle weight/body weight had returned to control levels at 1 h after RRMBF.

Effects of RRMBF on mTOR signaling pathways

Figure 3 shows the effects of RRMBF on phosphorylation of p70^{S6k}, a downstream target of mTOR and a factor in synthesis regulation of some ribosomal proteins. As shown in Fig. 3a, p70S6 k phosphorylation at Thr389, a phosphorylation site associated with maximal activation of the kinase [20], in the right tibialis anterior muscle (BFR leg) was increased significantly 1 h after RRMBF, compared with control rats (p < 0.05). However, p70^{S6k} phosphorylation in the left tibialis anterior muscle (non-BFR leg) was not changed significantly at any time point in response to RRMBF (Fig. 3b). The enhanced phosphorylation of p70^{S6k} had returned to control levels 3 h after the stimuli (Fig. 3c). S6 phosphorylation at Ser240/244, a downstream target of p70^{S6k}, was also increased significantly 1 h after RRMBF (P < 0.05, Fig. 4a), which returned to the control level 3 h after the stimuli.

Figures 4b, c illustrate the effects of RRMBF on phosphorylation of 4E-BP1 and eEF2. Phosphorylation of

RRMBF on total muscle weight (c) and total muscle weight/body weight of the tibialis anterior muscle (d). The total muscle weight and total muscle weight/body weight of the tibialis anterior muscle were measured in control rats, and rats immediately (0 h), 1, 3, and 6 h after RRMBF. Each data were obtained from both sides of the muscles (n = 6). Note that the total muscle weight and total muscle weight/body weight of the tibialis anterior muscle increased significantly in rats sacrificed immediately after RRMBF. *P < 0.05 vs. control (pre)

4E-BP1 at Thr37/46 (Fig. 4b) and phosphorylation of eEF2 at Thr56 (Fig. 4c) did not change significantly at any time point in response to RRMBF, compared with control rats.

As shown in Fig. 5a, Akt phosphorylation at Ser473, a marker for Akt activation (Alessi et al. 1996), did not change significantly at any time point in response to RRMBF. Phosphorylation of mTOR on Ser2448, a site directly phosphorylated by Akt [21], tended to increase at 1 h after RRMBF (P = 0.06, Fig. 5b), but not statistically significantly.

Effects of RRMBF on AMPK phosphorylation and REDD1

AMPK phosphorylation at Thr172 did not change significantly at any time point in response to RRMBF (Fig. 5c). There was no difference in REDD1 total protein content in controls and after RRMBR (Fig. 5d).

Effects of RRMBF on myostatin, MuRF-1, and FOXO3a mRNA expression

We also investigated the effects of RRMBF on MuRF-1 and FOXO3a mRNA expression, factors associated with



Fig. 3 Phosphorylation of p70 S6-kinase (p70S6k) on Thr389 in rat tibialis anterior skeletal muscle before (control, **c**), immediately after (0 h), 1, 3 and 6 h after RRMBF. In **a**, **b** the representative immunoblots (phospho- p70S6k and total p70S6k) are shown above each group. The data in **a** were obtained from the right tibialis skeletal muscle (BFR side). The data in **b** were obtained from the right (BFR side) and left tibialis anterior muscle (non-BFR side). In this and following figures, proteins with a phosphorylation site are shown as a phospho to total ratio. Proteins without a regulatory phosphorylation site are indicated as total protein content. The ratio of p- p70S6k/total p70S6k is shown in **c**. The data for each time were obtained from six different rats. Note that RRMBF enhanced the phosphorylation of p70S6k in right tibialis anterior muscle (BFR leg), but not left muscle (non-BFR leg) 1 h after the stimuli. **P* < 0.05 vs. control (pre)

the regulation of muscle protein breakdown. As shown in Fig. 6, there were no significant differences in MuRF-1 (Fig. 6a, p > 0.05) and FOXO3a mRNA (Fig. 6b, P > 0.05) levels in controls and after RRMBF. Figure 6c shows the effects of RRMBF on myostatin mRNA level. There were no difference in myostatin mRNA in controls and after RRMBF.

Effects of RRMBF on muscle HIF-1 α mRNA, HIF-1 α and VEGF protein

To determine whether hypoxia induced by RRMBF stimulates hypoxia-related genes and proteins, we investigated the effects of RRMBF on HIF-1 α protein and gene expression. As shown in Fig. 6d, HIF-1 α mRNA expression was not significantly changed after RRMBF (P > 0.05, Fig. 6d). HIF-1 α protein expression was increased 1 h after RRMBF (P < 0.05), compared with controls (Figs. 6e, f), while it was not significantly changed at 0 h (~ approximately 5 min) after RRMBF. VEGF protein expression was not altered at any time point in response to RRMBF (Fig. 6g).



Fig. 4 Phosphorylation of S6 at Ser240/244 (**a**), 4E-BP1 at Thr37/46 (**b**), eEF2 at Thr56 (**c**) in rat tibialis anterior skeletal muscle before (control), immediately after (0 h), 1, 3, and 6 h after RRMBF. Two different representative immunoblots (phosphorylation and total) at times indicated are shown. The data were obtained from the right tibialis anterior muscle (BFR side) of six different rats. The phospho to total ratio is shown on the right. **P* < 0.05 vs. control (pre)

Effects of RRMBF on MAP kinase phosphorylation

Figure 7 shows the effects of RRMBF on phosphorylation of ERK1/2, p38MAPK, and JNK1/2. As shown in Fig. 7, phosphorylation of ERK1/2 (Fig. 7a) at Thr202/Tyr204, p38 MAPK (Fig. 7b) at Thr180/Tyr182, and JNK1/2 (Fig. 7c) at Thr183/Tyr185 did not change significantly at any time point in response to RRMBF.

Discussion

The major findings of our study are as follows. (1) During RRMBF, $PmvO_2$ decreased from 36.1 ± 5.7 to 5.9 ± 1.7 torr, and recovered gradually during a rest period. (2) RRMBF significantly increased p70S6k phosphorylation, a downstream target of mTOR, and ribosomal protein S6 phosphorylation at 1 h after stimulation. (3) Protein levels of REDD1, AMPK and MAPKs phosphorylation did not change significantly. (4) The mRNA expression level of FOXO3a, MuRF-1 and myostatin was not significantly altered during RRMBF. These results showed for the first time that RRMBF significantly decreased PmvO₂, and enhanced mTOR signaling pathways in skeletal muscle using a rat model, which may play a role in diminishing muscle atrophy under various conditions in human studies. Fig. 5 Phosphorylation of Akt at Ser473 (a), mTOR at Ser2448 (b), AMPK (Thr172) (c) and REDD1 protein (d) expression in rat tibialis anterior skeletal muscle before (control), immediately after (0 h), 1, 3, and 6 h after RRMBF. Two different representative immunoblots (phosphorylation and total) are shown above each group in (**a**–**c**). The data were obtained from right tibialis anterior muscle (BFR side) at times indicated. The phospho to total ratio is shown in lower part. In d, two different representative immunoblots (REDD1) obtained from right muscle are shown. The protein density of REDD1 is shown in D (right part). Each datum was obtained from six different rats



Our study showed the first model of RMBF using rats. In this model, we applied an RRMBF stimulus without exercise comprising an external compressive force of 100 mmHg, which is lower than systolic blood pressure of rats to avoid hemostasis. With this restriction, the total weight of tibialis anterior muscle and total muscle weight/ body weight ratio increased significantly in rats sacrificed immediately after RRMBF, suggesting that the application of RRMBF at 100 mmHg pressure does not completely block blood flow, and induces venous pooling of blood in the lower legs and swelling of the tibialis anterior muscle. It is a characteristic phenomenon of KAATSU training [5, 19, 22]. During RRMBF, PmvO₂ decreased significantly from 36.1 \pm 5.7 to 5.9 \pm 1.7 torr, and gradually recovered during a rest period. NMR techniques for myoglobin saturation have shown that resting intracellular PO_2 in human skeletal muscle is estimated to approximately 34 mmHg, and drops to approximately 23 mmHg, when subjects breathe ambient 10 % O₂ [23]. However, during near-maximal exercise, intracellular PO2 drops to values as low as 2–5 mmHg [24]. In addition, mean tissue PO₂ in lower limb muscle made ischemic by complete obstruction of blood flow drops to less than 1.9 mmHg [25]. Thus, the level of PmvO₂ reached in our study is higher than that induced by complete obstruction of blood flow, and that reached during the near-maximal exercise as described previously [24, 25].

Oxygen is an essential regulator of cellular metabolism. Under hypoxic conditions, cells rapidly activate a variety of adaptive responses that limit energy expenditure by inhibiting energy-intensive processes including protein translation [26, 27]. One related mechanism involves inhibition of mTOR activity, an anabolic pathway for skeletal muscle, observed following exposure to hypoxia $(1 \% O_2)$ [28]. HIF-1 α activity is known to increase under continuous hypoxia due to decreased rates of oxygen-dependent proline hydroxylation, ubiquitination, and proteasomal degradation of the HIF-1 α subunit. And, hypoxic regulation of mTOR activity occurs through a pathway involving the REDD1 gene [29], where REDD1 expression is highly induced in response to hypoxia via HIF-1 α [29, 30]. In our study employing RRMBF protocols, expression level of REDD1 protein did not change significantly. In general, the increased HIF-1 α protein elicited by hypoxia quickly returns to control levels immediately after the reoxygenation [31]. In the present study, expression level of HIF-1a protein did not significantly change immediately after RRMBF, while it increased 1 h after RRMBF. Hypoxia is also known to increase the expression of VEGF via HIF-1a [32], and VEGF protein expression did not significantly differ at any time point in response to RRMBF. Thus, it is likely that RRMBF used in our protocol did not activate HIF-1a activity enough to inhibit mTOR pathways and enhance VEGF expression. Alternatively, AMPK is an energy-sensing serine/threonine kinase activated by metabolic stressor that depletes ATP and increases AMP during exercise, hypoxia and glucose deprivation. Once activated, AMPK inhibits ATP consuming anabolic processes such as protein translation largely through inhibition of mTOR signaling [33]. However, AMPK phosphorylation also did not change significantly at any time point in response to RRMBF.



Fig. 6 a-c Skeletal muscle MuRF-1 (a), FOXO3a (b), and myostatin (c) mRNA in responses to RRMBF. The levels of MuRF-1 (a), FOXO3a (b) and myostatin (c) mRNAs were measured by the quantitative real-time RT-PCR, and normalized to those of the 18S ribosomal RNA levels. The relative abundance of mRNA is shown before (control), immediately after (0 h), 1, and 3 h after RRMBF. *P < 0.05vs. control (pre). d-f Skeletal muscle HIF-1a mRNA (d), HIF-1a protein (e, f) and VEGF protein (g) in responses to RRMBF. The levels of HIF-1a mRNA are normalized to those of the 18S ribosomal RNA levels, and the relative abundance of mRNA is shown before (control), immediately after (0 h), 1, and 3 h after RRMBF. *P < 0.05vs. control (pre). In e, two different representative immunoblots obtained from right muscle are shown above each group. The protein density of HIF-1 α and VEGF is illustrated in **f** and **g**, respectively. The data were obtained from six different rats. *P < 0.05 vs. control (pre)

Several studies have shown that activation of mTOR and p70S6 k phosphorylation in the recovery phase after highintensity resistance exercise is associated with increased protein synthesis [3, 34-36]. And, phosphorylation of p70S6k leads to S6 protein phosphorylation, which is thought to promote translation of the mRNAs that encodes ribosomal proteins and other translation factors. S6 protein activation leads to increased cellular capacity for protein synthesis. Recently, low-intensity resistance exercise under RMBF in healthy subjects has been reported to increase muscle strength and size even with low-intensity loads [6, 7]. Sessions of RMBF exercise have also been reported to increase protein synthesis through post-translation regulation in the Akt/mTOR pathway [8, 37]. They showed that the RMBF exercise increased p70S6k phosphorylation. Previous human studies have also reported that RRMBF



Fig. 7 Phosphorylation of extracellular signal-regulated kinase 1/2 (ERK1/2, **a**), p38 MAP kinase (p38MAPK, **b**) and c-Jun N-terminal kinase 1/2 (JNK1/2, **c**) before (control), immediately after (0 h), 1, 3, and 6 h after RRMBF. A representative immunoblot (phosphorylation and total) at time indicated is shown. The data were obtained from right tibialis anterior muscle (n = 6, BFR side) at times indicated. The phospho to total ratio is shown in *lower part*

even without a combination of exercise effectively diminishes post-operation disuse atrophy of knee extensors [11], and muscle weakness induced by immobilization without weight-bearing [12], but the underlying mechanisms have not been investigated. Here, we showed for the first time that RRMBF had significantly increased p70S6k phosphorylation, a downstream target of mTOR, and ribosomal protein S6 phosphorylation in a rat model. The degree of p70S6k phosphorylation in the first few hours after a session of high-intensity resistance exercise has been reported to be correlated with percentage change in muscle mass after several weeks of exercise in both rodents and humans [35, 38]. S6 phosphorylation is also regarded as a stimulation of protein synthesis [35, 36, 38]. Thus, RRMBF appears to increase protein synthesis due to enhanced p70S6k and S6 phosphorylation, possibly resulting in diminishing muscle atrophy as described previously in human studies [11, 12].

Several mechanisms for the effects of RRMBF on p70S6k and S6 phosphorylation in rat skeletal muscle may be proposed. Resistance exercise is an established and potent stimulus for enhancing muscle protein synthesis and subsequent muscle hypertrophy. The activation of mTOR signaling pathways is a primary mechanism by which resistance exercise stimulates translation initiation, elongation, and the rate of muscle protein synthesis [39]. Akt is an

upstream positive regulator of mTOR. However, RRMBF did not increase phosphorylation of Akt. Phosphorylation of mTOR on Ser2448 tended to increase at 1 h after RMBF, but not statistically significant. Thus, our study was unable to determine the involvement of Akt on increased p70S6k and S6 phosphorylation after RRMBF. However, we showed for the first time that RRMBF enhanced p70S6k phosphorylation, a downstream target of mTOR, in a rat model. RRMBF did not enhance the phosphorylation in control legs, suggesting that the local factors induced by RRMBF, but not systemic factors, are involved in enhanced p70S6k phosphorylation.

MAPK cascades are potential physiological mechanisms involved in the exercise-induced regulation of gene expression and protein synthesis in skeletal muscle [40]. The MAPK family members are separated into distinct parallel pathways, including ERK1/2, stress-activated protein kinase cascades (SAPK1/JNK) and SAPK2/p38 [41]. Downstream cytosolic targets for ERK1/2 include p90 ribosomal S6 kinase [42], and ERK1/2 is capable of phosphorylating S6 on its Ser235/236 regulatory site [43]. The kinases JNK and p38 MAPK are known as stressactivated protein kinases because of their activation by environmental stressors including osmotic shock and heat shock [44, 45]. RMBF induces an acute fluid shift from the vascular space into the muscles, and then muscle volume changes [5, 19, 22], which may be involved in the BFR exercise-induced anabolic response of skeletal muscle as proposed recently [22]. In our study, the total weight of the tibialis anterior muscle increased significantly in rats immediately after RRMBF, suggesting that application of RRMBF in the present model induced venous pooling of blood into the lower legs, and swelling the muscle. However, RRMBF did not increase phosphorylation of MAPK including ERK1/2. Thus, it is most likely that RRMBF enhances mTOR signaling pathways in rat skeletal muscle independently of MAPK signaling pathways. The formation of reactive oxygen species (ROS) in skeletal muscle has been also described in response to muscle stimulation, heat stress, and ischemia-reperfusion injury [46]. ROS have been proposed to play physiological roles on cell signaling in skeletal muscle [47]. The activity of ROS within muscle may increase in a hypoxic environment [48]. Also, because ischemia and reperfusion of skeletal muscle are associated with an increase of ROS release [49], ROS could be produced when muscle is kept hypoxic and subsequently exposed to reperfusion. Therefore, both lowered and elevated muscle oxygenation levels during RRMBF may enhance ROS production in skeletal muscle. ROS has been reported to activate the downstream target of phosphoinositide 3-kinase (PI3K)-Akt signaling [50]. However, RRMBF in the present study did not increase phosphorylation of Akt. Therefore, the basic mechanisms underlying

the enhancement of mTOR signaling pathways following RRMBF remain to be clarified.

Proteolytic events involve the ubiquitination and subsequent degradation of proteins induced by E3 ligases [51, 52]. Exercise-responsive genes involved in the muscle cell ubiquitin/proteolysis pathway include MuRF-1 and atrogin-1 (muscle atrophy F-box), both of which share a common transcription factor, FOXO3a. Atrogin-1 and MuRF-1 negatively regulate muscle mass. Recently, reduced expression of the proteolysis-related genes FOXO3a, atrogin-1, MuRF-1, and myostatin has been reported after acute BFR exercise [9, 10]. Suppression of myostatin has been found to induce muscle hypertrophy in rodents [53]. Also, immobilization increases the expression of myostatin in mice, while re-loading muscle decreases myostatin expression [54]. From these observations, it is likely that BFR combined with exercise inhibits skeletal muscle protein degradation and promotes myogenesis via ubiquitin/proteolysis pathways, resulting in increasing muscle mass. However, Drummond et al. [55] showed that mRNA expression level for myostatin and MuRF-1 3 h after performing BFR knee extension at 20 % 1RM was not different from that after control 20 % 1RM knee extension. In our study using animal model, RRMBF without exercise did not significantly change the mRNA expression level of FOXO3a, MuRF-1, and myostatin. Thus, the muscle cell ubiquitin/proteolysis pathways do not appear to be involved in the RRMBF-induced decrease of muscle atrophy as reported previously in human studies [11, 12].

Kaatsu training is an exercise training under the conditions with RMBF [5, 6]. The training is distinctive for producing increases in muscle size and strength through short-duration, and low load-intensity training. This training has been reported to enhance protein synthesis through post-translation regulation in the Akt/mTOR pathway [8, 37]. But, several papers have shown that RRMBF even without exercises appears to be a novel stimulus of skeletal muscle to induce a net positive protein balance and prevent atrophy. In the present paper, we showed the first evidence that RRMBF without exercises enhanced mTOR signaling pathways in skeletal muscle using a rat model. The hypertrophic effects of KAATSU training cannot be obtained by the continuous application of RMBF alone in clinical studies [56], suggesting that hypoxia only appears to be not enough to activate the mTOR pathway. In contrast, RRMBF appears to be a novel stimulus of skeletal muscle to induce a net positive protein balance and prevent atrophy. However, the further studies are needed to investigate the potencies of the hypertrophic effects of the RMBF exercises and RRMBF. In addition, we applied an RRMBF stimulus comprising an external compressive force of 100 mmHg, and used five repetitions for 5 min, and release of pressure for 3 min as described in the previous clinical studies [11, 12]. Therefore, the further detailed studies using different protocols of RRMBF including numbers and duration of RRMBF are needed to clarify the effects of RRMBF of the mTOR signaling pathways.

Exercise such as aerobic exercise is a well-established method for improving quality of life, and decreasing cardiovascular risk [57, 58]. However, it is difficult to apply to bedridden or postoperative patients. On the other hand, RRMBF can be used in such patients, and may prevent muscle atrophy in such patients as shown in the previous papers [11, 12]. But, further clinical studies are needed to clarify this possibility.

Conclusions

RRMBF significantly decreased $PmvO_2$, and enhanced mTOR signaling pathways in skeletal muscles using a rat model, which may play a role in preventing muscle atrophy in skeletal muscle under various conditions in human studies.

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Compliance with ethical standards

Conflict of interest There is no conflict of interests to disclose.

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RESEARCH ARTICLE

Muscle hypertrophy following blood flow-restricted, low-force isometric electrical stimulation in rat tibialis anterior: role for muscle hypoxia

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Nakajima T, Koide S, Yasuda T, Hasegawa T, Yamasoba T, Obi S, Toyoda S, Nakamura F, Inoue T, Poole DC, Kano Y. Muscle hypertrophy following blood flow-restricted, low-force isometric electrical stimulation in rat tibialis anterior: role for muscle hypoxia. J Appl Physiol 125: 134-145, 2018. First published March 22, 2018; doi:10.1152/japplphysiol.00972.2017.—Low-force exercise training with blood flow restriction (BFR) elicits muscle hypertrophy as seen typically after higher-force exercise. We investigated the effects of microvascular hypoxia [i.e., low microvascular O2 partial pressures (PmvO₂)] during contractions on muscle hypertrophic signaling, growth response, and key muscle adaptations for increasing exercise capacity. Wistar rats were fitted with a cuff placed around the upper thigh and inflated to restrict limb blood flow. Low-force isometric contractions (30 Hz) were evoked via electrical stimulation of the tibialis anterior (TA) muscle. The PmvO2 was determined by phosphorescence quenching. Rats underwent acute and chronic stimulation protocols. Whereas PmvO2 decreased transiently with 30 Hz contractions, simultaneous BFR induced severe hypoxia, reducing PmvO₂ lower than present for maximal (100 Hz) contractions. Lowforce electrical stimulation (EXER) induced muscle hypertrophy (6.2%, P < 0.01), whereas control group conditions or BFR alone did not. EXER+BFR also induced an increase in muscle mass (11.0%, P < 0.01) and, unique among conditions studied, significantly increased fiber cross-sectional area in the superficial TA (P < 0.05). Phosphorylation of ribosomal protein S6 was enhanced by EXER+ BFR, as were peroxisome proliferator-activated receptor gamma coactivator-1 α and glucose transporter 4 protein levels. Fibronectin type III domain-containing protein 5, cytochrome c oxidase subunit 4, monocarboxylate transporter 1 (MCT1), and cluster of differentiation 147 increased with EXER alone. EXER+BFR significantly increased MCT1 expression more than EXER alone. These data demonstrate that microvascular hypoxia during contractions is not essential for hypertrophy. However, hypoxia induced via BFR may potentiate the muscle hypertrophic response (as evidenced by the increased superficial fiber crosssectional area) with increased glucose transporter and mitochondrial biogenesis, which contributes to the pleiotropic effects of exercise training with BFR that culminate in an improved capacity for sustained exercise.

NEW & NOTEWORTHY We investigated the effects of low microvascular O_2 partial pressures ($PmvO_2$) during contractions on muscle hypertrophic signaling and key elements in the muscle adaptation for increasing exercise capacity. Although demonstrating that muscle hypoxia is not obligatory for the hypertrophic response to low-force, electrically induced muscle contractions, the reduced $PmvO_2$ enhanced ribosomal protein S6 phosphorylation and potentiated the hypertrophic response. Furthermore, contractions with blood flow restriction increased oxidative capacity, glucose transporter, and mitochondrial biogenesis, which are key determinants of the pleiotropic effects of exercise training.

blood flow restriction; COX4; GLUT4, hypoxia; mammalian target of rapamycin (mTOR); MCT1; microvascular Po₂; muscle hypertrophy; PGC-1α; rat skeletal muscle; rpS6 phosphorylation

INTRODUCTION

Skeletal muscle is a supremely plastic organ that responds to elevated chronic demands, such as exercise training, by evoking a spectrum of structural and functional adaptations that increases performance. Structural changes in muscle that underlie the hypertrophic response to isometric exercise training are facilitated by tipping the net balance between protein synthesis and degradation (8). An underlying premise herein is that the hypertrophic response is beneficial, especially in patients and/or elderly individuals whose morbidity/mortality is increased by muscle atrophy. In general, relatively high muscle force [e.g., \geq 70% of the 1-repetition maximum weight (1RM)] is used to elicit the hypertrophic response (3, 58). However, in patients with orthopedic or cardiovascular diseases, high-load exercise may be contraindicated because of various limitations, such as pain or hypertension. On the other hand, several studies have shown that low loads to failure (42, 43) or low-force (20%-30% 1RM) resistance exercise under blood flow restriction (BFR) can induce muscle hypertrophy and strength increases similar to those resulting from high-force (70% 1RM) training (30, 62). These studies suggest that the degree of muscle hypertrophy is not uniquely determined by mechanical stress and raise the question as to what other factors might control or potentiate the hypertrophic response.

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There is experimental support for muscle protein synthesis and hypertrophy being elicited by exercise training under hypoxic conditions (46, 63). Specifically, Nishimura et al. (46) recruited college-age men to undergo 6 wk of resistance training at 70% 1RM in a hypoxic chamber [inspired O₂ fraction (F_{IO_2}) 0.16] and demonstrated greater muscle hypertrophy of the elbow flexors and extensors compared with normoxic training. Moreover, D'Hulst et al. (16) found that 4 h of normobaric hypoxia (FIO, 0.11) elevated protein kinase B (Akt) and p70 kDa ribosomal protein S6 (rpS6) kinase (p70S6K, also known as S6K1) phosphorylation in the vastus lateralis, whereas in normoxia, (FIQ, 0.21) this response was not observed. However, these studies did not show the relationships between molecular changes and muscle hypertrophy. BFR induces skeletal muscle hypoxia and exacerbates any exercise-induced reduction of muscle oxygenation (35). Furthermore, Fujita et al. (21) and Fry et al. (19) showed that the phosphorylation of the mammalian target of rapamycin (mTOR) signaling pathway including mTOR, S6K1, and rpS6 and muscle protein synthesis were significantly increased 3 h after low-force (20% 1RM) knee extension

exercise with BFR. Collectively, these observations support the notion that skeletal muscle hypoxia in combination with mechanical stress may be important mechanistically for stimulating muscle hypertrophy via enhancement of protein synthesis. However, we are unaware that the relationships among muscle hypertrophy, muscle oxygenation, and key molecular control mechanisms have been investigated for exercise under BFR conditions.

The potential for BFR-induced muscle hypoxia in combination with low-force exercise to impact the hypertrophic processes is highlighted by multiple intramyocyte effects that are commonly found with far-higher-force exercise. For example, hypoxia challenges ATP production, which elevates the AMP/ ATP ratio and stimulates AMP-activated protein kinase α - α (AMPK α), serving as an intracellular energy sensor (20). In turn, AMPK α moves the glucose transporter 4 (GLUT4) to the cell membrane (25), promoting glucose uptake and sparing muscle glycogen (23). BFR also increases blood lactate immediately after low-force exercise akin to non-BFR, high-force exercise (54), and this low-force BFR training for 8 wk potentiates the extant metabolic changes, perhaps by inducing an ischemic stimulus that enhances muscle glucose transport and adenine nucleotide catabolism (12). Furthermore, in addition to muscle strength and hypertrophy, improvement in skeletal muscle endurance and maximum oxygen consumption has been reported following low-force exercise training with BFR (1). Thus, it is plausible that low-force exercise training with BFR may elicit the pleiotropic effects that resemble those normally associated with acute or chronic high-force exercise and in so doing reveal important clues as to the underlying control mechanisms.

The present investigation created a rat model in which to study how BFR in combination with low-force muscle contractions stimulates muscle hypertrophy and upregulates key metabolic pathways that are central to improving muscle functional capacity. Specifically, this model facilitated mimicking resistance exercise training (22, 33) under control and BFR conditions combined with measurements of microvascular O₂ partial pressures (PmvO₂), muscle tension level, and comprehensive muscle analyses. We tested the hypothesis that muscle hypoxia is essential for driving muscle hypertrophy. Moreover, we sought to determine how the ischemic-hypoxic condition induced by BFR influences key signaling mechanisms that determine metabolic function during contraction and which may be intrinsic to the reduced fatigability and improved exercise capacity of muscle after training.

MATERIALS AND METHODS

Animals. Male Wistar rats (n = 74, age 10–17 wk) were used in this study (Japan SLC, Shizuoka Laboratory Animal Center). All rats were housed in a temperature-controlled room at $23 \pm 1^{\circ}$ C at $55 \pm 10\%$ humidity with a light-dark cycle of 12 h and were maintained on rat feed (5L37, PMI Nutrition International, St. Louis, MO) and water ad libitum. All experiments were conducted under the guidelines established by the Physiological Society of Japan and were approved by the University of Electro-Communications Institutional Animal Care and Use Committee.

Experiment 1-1: acute experimental protocol for muscle contraction. Under isoflurane anesthesia, rats were secured in a supine position on a hot plate warmed to 37°C (BWT-100, Bio Research Center, Tokyo, Japan). The right (experimental) leg was attached to the hind limb movement device (Model RU-72, Motomura Systems, Tokyo, Japan) at a 90° knee joint angle (Fig. 1A) as previously described (26, 45). After the incision of the skin, a bipolar electrode TF210-104 (Unique Medical, Tokyo, Japan) was connected to the peroneal nerve, and isometric tibialis anterior (TA) contractions were produced by nerve stimulation (4-6 V; 30 Hz or 100 Hz square wave stimuli; 4-ms pulse duration, 0.7-s stimulus duration). Moreover, to ensure that the exposed surface of the muscle remained moist, the surface was continuously perfused with the Krebs-Henseleit solution (at pH 7.35 and equilibrated with 95% N_2 -5% CO_2 gas). First, we investigated the relationship between muscle tension and stimulus frequency (Hz), in which the tension elicited by 100 Hz was considered to be maximal (i.e., 100%, Fig. 2A, n = 3). Judging from this relationship, we defined the high-force condition as notionally equivalent to 1RM (maximum repetition) and the low-force condition as 50% (i.e., ~50% 1RM) of that which corresponded to the lowest frequency (i.e., 30 Hz) that yielded $48.0 \pm 1.2\%$ of the 100-Hz high-force contraction. To measure acute changes of PmvO₂ (Fig. 1B), 30 Hz and 100 Hz were also used as the low- and high-force contraction, respectively. In total, four sets of contractions (20, 15, 15, and 15 times every 3 s) at 1-min interset intervals were performed. Torque was monitored by computer using Power Lab/ 16s (AD Instruments, Colorado Springs, CO) via a strain gaugelinked motor device (Model RU-72, Motomura Systems) during all contraction bouts.

BFR protocol. BFR to the right lower extremity was induced by compressing the proximal end of the right thigh with a latex cuff (overall cuff size: 16-mm width, 90-mm length; Hokanson, Bellevue, WA) as previously described (45, 60). The pressure was set by using blood pressure meter (mercury blood pressure monitor, Kenz Medico, Saitama, Japan). This study (n = 5) determined the relationship between cuff pressure and $PmvO_2$ and resolved that 80 Torr provided a suitable BFR level at which $PmvO_2$ was lowered considerably but blood flow was not completely occluded. BFR was started 1 min before the start of electrical stimulation for each contractions (training) bout.

*PmvO*₂ measurements. Rats were divided into 3 groups (n = 6 each): low (30 Hz) or high (100 Hz) force isometric electrical stimulation exercise (EXER) and EXER under 80 mmHg cuff pressure (EXER+BFR). Phosphorescence quenching as described previously (28) was utilized during the BFR protocol to determine the *PmvO*₂ within the medial TA muscle region. A PE-50 catheter filled with heparinized saline was inserted in the right carotid artery and connected to a pressure transducer (DX-100, Nihon Kohden, Tokyo, Japan). This catheter was also used to infuse the phosphorescent probe

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B Acute experiment model (Exp.1)

Exp 1-1. PmvO₂ measurement

Exp 1-2. Muscle sampling (3 h after EXER or EXER+BFR) for biochemistry analysis



C Chronic experiment model (Exp.2)

Muscle sampling (24 h after 9th EXER or EXER+BFR) for biochemistry and histochemistry analysis



Fig. 1. Acute (Exp.1) and chronic (Exp.2) experimental model. *A*: blood flow restriction (BFR) treatment model using latex cuff. *B*: acute experimental protocol in Po₂ measurement (Exp. 1–1) and muscle sampling (Exp. 1–2). Exercise (EXER) groups (30-Hz or 100-Hz stimulation) and EXER with BFR groups (EXER+BFR). *C*: chronic experimental protocol (training model, Exp.2). Each set applied acute experimental protocol condition in B. PmvO2, microvascular oxygen partial pressure.

[palladium meso-tetra (4-carboxyphenyl) porphyrin dendrimer (R2)] at 15 mg/kg. A lateral incision of the skin and overlying fascia was made to expose the right TA muscle, and the light source probe/ collector of the PMOD 2000 Frequency Domain Phosphorometer (Oxygen Enterprises, Philadelphia, PA) was positioned 2–4 mm above the medial TA surface. Excitation light was applied at 524-nm wavelength; its phosphorescence decay was monitored to measure the lifetime and hence resolve $PmvO_2$. $PmvO_2$ was averaged for 5 s immediately before the end of contractions when the signal was stable.

The theoretical basis for phosphorescence quenching has been reported previously (53, 56). Briefly, the Stern-Volmer relationship describes the quantitative O_2 dependence of the phosphorescent probe. R2 is a nontoxic dendrimer that binds to albumin at 38°C and pH 7.4 (i.e., under the physiological conditions extant herein) with a quenching constant of 409 mmHg⁻¹·s⁻¹ and a 601 µs decay lifetime in the absence of O_2 (31, 50). The net negative charge of R2 also facilitates restriction of the compound to the vascular space. Thus, $PmvO_2$ reflects the Po₂ within capillary blood, which constitutes the principal intramuscular vascular space (50, 52).

Experiment 1-2: acute experimental model for biochemistry analysis. Electrical stimulation (4-7 V) was applied to the TA muscle by affixing a surface electrode to the abraded skin over the right TA (Vitrod A, Nihon Kohden, Tokyo). We used low force (30 Hz) and 80 mmHg applied pressure (i.e., a moderate degree of BFR) to produce the desired PmvO₂ decrease (Fig. 1B, Exp 1-1). In experiments to determine the phosphorylation response of muscle proteins to a single bout of contractions (Fig. 1B), the TA muscle was excised 3 h after exercise and immediately frozen in liquid nitrogen, then stored at -80° C until protein quantification as reported previously in rat electrical stimulation models (5). The phosphorylation responses of muscle hypertrophy proteins [mTOR, S6K1, rpS6, extracellular signal-regulated kinase (ERK), and AMPK] were examined in 11-wk-old rats (n = 24). Animals were separated into four groups (n = 6 each) depending on the presence or absence of EXER and BFR.

Experiment 2: chronic training model. Rats (n = 24, 11 wk old) were divided randomly into 4 groups (n = 6 each): control (CONT), low-force electrical stimulation exercise (EXER), exercise under BFR (EXER+BFR), and BFR only. For the latter groups, the EXER, BFR, or EXER+BFR conditions were performed three times a week for 3 wk (Fig. 1*C*). As above, BFR consisted of an external compressive force of 80 mmHg applied to the proximal portion of the right thigh. The TA isometric contractions were elicited by electrical stimulation (30 Hz). Rats in each group were killed 24 h after the last training bout to discriminate the "training" effects from those caused by the acute exercise per se. The entire TA muscle was collected from both the right leg (treated side) and left leg (nontreated side). The isolated tissue was immediately flash frozen in liquid nitrogen and stored at -80° C until analysis.

In chronic training experiments, we measured the force produced before exercise on the first day and the last day of training and compared them among groups. The maximal force was measured by applying 100 Hz electrical stimulation to produce a sustained isometric contraction.

Western blotting analysis. The frozen protein was dissolved in Lysis buffer (50 mM Tris, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Nonidet P-40, 10% glycerol, 200 mM NaF, 20 mM sodium phosphate, and 1 mM NaVO₄) and homogenized. Proteins were separated at 200 V using a 7.5%–10% polyacrylamide gel for

A



Fig. 2. Effects of blood flow restriction (BFR) on muscle oxygen partial pressure. *A*: relationships between muscle tension and the stimulus frequency (Hz). Tension elicited by 100 Hz was considered as 100% (i.e., equivalent to 1 maximal repetition; n = 3). *B*: relationship between cuff pressure (mmHg) and muscle oxygen partial pressure ($PmvO_2$, Torr). Significantly different between Rest and each condition, *P < 0.05, **P < 0.01; n = 5. *C*: time courses of alterations in $PmvO_2$ during muscle contraction induced by 30-Hz or 100-Hz electrical stimulation (EXER) and EXER under 80-mmHg cuff pressure (EXER+BFR). Open circle (30 Hz) and triangle (100 Hz) show EXER, and filled circle shows EXER+BFR. Significantly different between groups, *P < 0.05 vs. 30 Hz EXER, †P < 0.05 vs. 100 Hz EXER. Means \pm SE; n = 6/group.

~50 min. Transfer was performed at 72 mA for 60 min using the semidry method to Amersham High Bond P membrane (GE Healthcare UK, Buckinghamshire, UK). After the transfer, the membrane was blocked with 2%-3% skim milk in PBS (0.01 M phosphate buffer, 0.138 M NaCl, 0.027 M KCl, pH 7.4) with 0.1% Tween 30 at room temperature for 1 h. The membrane was then exposed to antibodies at the appropriate dilutions in blocking buffer overnight at 4°C. The probed membrane was then washed 3 times in PBS with 0.1% Tween for 15 min each time and was subsequently incubated with peroxidase-linked anti-rabbit IgG (Santa Cruz Biotechnology, Santa Cruz, CA; diluted 1:5,000 with blocking buffer) for 1 h at room temperature. After three additional washes, bound antibodies were detected with a Chemi-Lumi One Super kit (Nacalai Tesque, Kyoto, Japan) and analyzed with an LAS-3000 mini-image analyzer (Fuji-Film, Tokyo, Japan). Phosphorylation of Akt on Ser473 was assessed using anti-phospho-Akt antibody (#4060, Cell Signaling Technology, Beverly, MA) and anti-total Akt antibody (#4691, Cell Signaling Technology). Phosphorylation of mTOR on Ser2448 was measured using anti-phospho-mTOR antibody (#2971, Cell Signaling Technology) and anti-total mTOR antibody (#2972, Cell Signaling Technology). Phosphorylation of p70S6K (S6K1) on Thr389 was determined using anti-phospho-p70S6K antibody (#9234, Cell Signaling Technology) and anti-total S6K1 antibody (#2708, Cell Signaling Technology). Phosphorylation of ERK1/2 at Thr202/Tyr204 was measured using anti-phospho-ERK1/2 antibody (#4370, Cell Signaling Technology) and anti-total ERK1/2 antibody (#4695, Cell Signaling Technology). rpS6 phosphorylation on Ser240/244 was evaluated using anti-phospho-rpS6 antibody (#5364, Cell Signaling Technology) and anti-total S6 antibody (#2317, Cell Signaling Technology). Antibody dilutions were 1:1,000 in each case. AMPK phosphorylation on Thr172 was assessed using anti-phospho-AMPKα antibody (#2535, Cell Signaling Technology) and anti-total AMPKa antibody (#2532, Cell Signaling Technology). Cytochrome c oxidase subunit 4 (COX4, #4850, Cell Signaling Technology), peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1a, ab54481, Abcam, Cambridge, UK), GLUT4 (BAM1261, R&D Systems, Minneapolis,

MN), fibronectin type III domain-containing protein 5 (FNDC5, ab174833, Abcam), and anti-hypoxia-inducible factor(HIF)-1 α antibody (ab1) were purchased from Abcam. Blots were loaded according to protein concentration (approximately 100–150 μ g). To quantify the change by training, the levels were corrected with GAPDH levels measured using a GAPDH antibody (GT 239, Gene Tex, CA).

Histological staining. The frozen sample was adhered to a siliconcoated slide glass (s-9441, Matsunami Glass Ind., Osaka, Japan) as a transverse thin section of 10 μ m at -20° C by cryostat (CM 1510, LEICA). The staining was performed in hematoxylin-eosin, succinate dehydrogenase, and slow- and fast-type myosin heavy chain (MHC I, IIa, and IIb). For succinate dehydrogenase staining, sections were immersed in 0.2 M sodium phosphate buffer (pH 7.5) containing 0.2 M sodium succinate and 1.2 mM nitroblue tetrazolium at 37°C for 45 min. Mouse monoclonal slow MHC antibody (diluted 1:40 in PBS) supplied by Novocastrate Laboratories (Leica Microsystems, Wetzlar, Germany) was used to identify slow-twitch fibers. Mouse fast MHC monoclonal antibody isoforms were used, which specifically react with type IIa (1:1000; SC-71) and IIb (1:100; BF-F3). These were supplied by Developmental Studies Hybridoma Bank (University of Iowa, Iowa City, IA). Sections were immersed in PBS solution (pH 7.4) at room temperature for 20 min before immersion in primary antibody at 4°C overnight. Vectastain ABC kit (PK-6102, Vector Laboratories, Burlingame, CA) was used to elicit an antibody response against MHC antigen. Thereafter, pigment formation was performed using diaminobenzidine peroxidase substrate kit (SK-4100, Vector Laboratories) to develop color, and the muscle fiber type was quantified.

Statistical analysis. All values are expressed as means \pm SE. All statistical analyses were performed in Prism version 6.01 (GraphPad Software, San Diego, CA). Group differences in PmvO₂ during stimulation, muscle force, and muscle weight data were determined by a two-way analysis of variance and Tukey's post hoc test. One-way analysis of variance and Tukey's post hoc test were used for comparison of Western blotting analysis and histological data. The significance level was set at 0.05.

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RESULTS

 $PmvO_2$ measurements. When BFR pressure was increased from 20 mmHg to 140 mmHg at 1-min intervals, $PmvO_2$ decreased in a pressure-dependent manner up until 80 mmHg (Fig. 2*B*). This was selected as the applied BFR condition.

The average $PmvO_2$ value of TA muscle at rest (baseline) for all measured samples was 27.5 ± 2.0 Torr (Fig. 2*C*). $PmvO_2$ was decreased to 23.2 ± 4.3 Torr during low-force electrical stimulation (EXER 30 Hz, Fig. 2*C*) at 1 set, and it gradually increased to or even above the resting baseline value during the exercise. Application of BFR in combination with 30 Hz (low force, EXER+BFR) reduced $PmvO_2$ significantly to 7.6 ± 1.9 Torr. This $PmvO_2$ value was lower than that observed during high-force electrical stimulation (i.e., 13.5 ± 3.1 Torr, EXER 100 Hz, Fig. 2*C*).

Effects of acute isometric electrical stimulation with/without BFR on mTOR signaling pathways. Phosphorylation of mTOR on Ser2448, a site directly phosphorylated by Akt, did not increase in either of these groups (Fig. 3A). The S6K1 phosphorylation at Thr389, a phosphorylation site associated with maximal activation of the kinase (13), also did not increase in

either of these groups (Fig. 3*B*). On the other hand, rpS6 phosphorylation at Ser240/244, a downstream target of S6K1, was increased 3 h after EXER+BFR compared with CONT, EXER, and BFR alone (all comparisons P < 0.05, Fig. 3*C*). Given the hypotheses tested herein, of these comparisons, the significant difference between EXER and EXER+BFR was notable.

Mitogen-activated protein kinase cascades are also potential physiological mechanisms involved in the exercise-induced regulation of protein synthesis in skeletal muscle (68), and ERK1/2 is capable of phosphorylating S6 on its regulatory site (55). However, ERK1/2 phosphorylation at Thr202/Tyr204 (Fig. 3*D*) did not change in any condition. AMPK phosphorylation on Thr172 (Fig. 3*E*) was also not impacted.

Effects of exercise training with/without BFR on maximal force, fiber cross-sectional area, and fiber types. The time course of alterations in force generated during muscle contractions elicited by 30-Hz electrical stimulation (EXER) and EXER with 80 mmHg BFR (EXER+BFR) during the first and ninth training bout are shown in Fig. 4A. Note that the force generated in the first training bout gradually decreased during



Fig. 3. Effects of EXER or EXER+BFR on mTOR signaling pathways. Phosphorylation of mTOR (*A*) on Ser2448, S6K1 (*B*) on Thr389, S6 (*C*) on Ser240/244, ERK (*D*) on Thr202/Tyr204, and AMPK on Thr172 (*E*) in rat tibialis anterior (TA) muscles 3 h after each treatment. Representative immunoblots (phosphorylation and total protein) are shown each group. Proteins with a phosphorylation site are shown as a phospho-to-total ratio. Proteins without a regulatory phosphorylation site are indicated as total protein content. Means \pm SE; n = 6/group. Significantly different between groups, **P < 0.01. AMPK, AMP-activated protein kinase; BFR, blood flow restriction; CONT, control group; EXER, electrical stimulation exercise; mTOR, mammalian target of rapamycin; S6, ribosomal protein S6 (rpS6); S6K1, p70 kDa ribosomal protein S6 kinase.

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Fig. 4. Effects of EXER and EXER+BFR training on the exerting tension and muscle weight. A: time course of alterations in torque generated during muscle contraction by 30-Hz electrical stimulation (EXER) and EXER with 80 mmHg cuff pressure (EXER+BFR) at the first and ninth training. Open circle shows the first training, and filled circle shows the ninth training. B: muscle force (100 Hz) at the ninth training session in each group. Open circle shows the control leg (left side), and filled circle shows the treated side (right side). C: muscle wet weight at 24 h after the ninth training session. Inset: surgically removed tibialis anterior (TA) muscles from nontreated (left) and treated (right) hindlimbs. Means \pm SE; n = 6/group. Significantly different between nontreated (left) and treated (right) hindlimbs, **P < 0.01. There was no significant difference between the groups on the treated (right) hindlimbs in the peak tension (EXER vs. EXER+BFR, P = 0.795) and muscle weight (EXER vs. EXER+BFR, P = 0.340). BFR, blood flow restriction; CONT, control group.

the repetitive stimulation with and without BFR. However, the force rapidly decreased and attained a much lower level, especially during the third and fourth sets in EXER+BFR, compared with EXER alone. The force generated by 30-Hz electrical stimulation during the ninth training bout was increased in both EXER and EXER+BFR, compared with that during the first training bout. In Fig. 4*B*, the absolute maximal tension (100-Hz stimulation) at the left leg (nontreated side) was not significantly different among each group. However, the value after the ninth training bout was increased for both EXER and EXER+BFR but not for CONT and BFR groups. However, there were no statistical differences in maximal force between EXER and EXER+BFR groups after the ninth training bout (P = 0.340, Fig. 4*B*).

The effects of EXER with and without BFR on muscle mass after the ninth training session are shown in Fig. 4*C*. The muscle wet weight (left leg, nontreated side) was not significantly different among groups. However, EXER induced muscle hypertrophy (6.2%, P < 0.01), whereas CONT or BFR alone did not (Fig. 4*C*). EXER+BFR induced an increase in muscle mass (11.0%, P < 0.01), but although numerically greater, it was not significantly different between EXER and EXER+BFR. However, it was notable that there was substantial variability in the response, with the two most hypertrophied muscles belonging to the EXER+BFR group (Fig. 4*C*).

In EXER without BFR, fiber cross-sectional area (CSA) was not increased significantly in the superficial TA (CONT vs. EXER: P = 0.094, Fig. 5A). On the other hand, the condition of EXER+BFR led to a statistically significant increase in fiber CSA in the superficial portion (CONT vs. EXER+BFR: P =0.046, Fig. 5A). As expected, type II fibers were dominant in the superficial portion of TA muscle, but both type I and II fibers were present in the deep portion. No change in fiber type composition was detected under any condition (Fig. 5B).

Effects of exercise training with BFR on lactate transporters (*monocarboxylate transporter*). EXER and EXER+BFR both enhanced monocarboxylate transporter 1 (MCT1; Fig. 6A) and

cluster of differentiation 147 (CD147; Fig. 6*C*, P < 0.05) but not MCT4 (Fig. 6*B*) expression compared with CONT and BFR. EXER+BFR induced a much larger increase in MCT1 compared with EXER (P < 0.05).

Effects of exercise training with/without BFR on PGC-1 α and metabolite transport proteins. EXER+BFR, but none of the other conditions, significantly increased the critical regulator for mitochondrial biogenesis, PGC-1 α (Fig. 7A), and also GLUT4 (Fig. 7B). However, COX4 protein expression, indicative of muscle oxidative capacity, was increased by both EXER and EXER+BFR groups (Fig. 6C). EXER, EXER+BFR, and BFR conditions all enhanced FNDC5 (thought to be the origin of irisin) protein expression (Fig. 6D).

We also documented the effects of these experimental conditions on a broad spectrum of muscle proteins (Table 1), none of which was impacted significantly.

DISCUSSION

The foremost original findings of the present investigation are as follows: 1) Contrary to our hypothesis, muscle hypoxia (i.e., reduced $PmvO_2$) during low-force contractions is not a requisite condition for hypertrophy but, when induced via BFR, potentiates such hypertrophy. 2) Low-force contractions with BFR acutely enhance phosphorylation of rpS6. 3) EXER induced muscle hypertrophy (6.2%, P < 0.01), but EXER+ BFR induced an increase in muscle mass (11.0%, P < 0.01) and significantly increased CSA preferentially in the superficial muscle portion (P < 0.05). 4) Low-force contractions with BFR enhanced PGC-1 α protein expression, a critical regulator for mitochondrial biogenesis, and GLUT4. 5) EXER both with and without BFR elevated muscle oxidative capacity, as reflected by the protein content of COX4, FNDC5, MCT1, and CD147. However, EXER+BFR induced a much larger increase in MCT1 compared with EXER alone. These results provide the first data demonstrating that the reduced $PmvO_2$ accompanying low-force muscle contractions with BFR en-

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Fig. 5. Effects of EXER and EXER+BFR training on muscle fiber cross sectional area (CSA) and fiber types. A: muscle fiber CSA in TA muscles into deep and superficial portion. Significantly different between groups, *P < 0.05vs. CONT. Mean \pm SE; n = 6/group. B: muscle fiber composition ratio (%) in each group. Note that type II fibers were dominant in the superficial portion of TA muscle, but both type I and II fibers coexisted in the deep portion. There was no significant difference between groups for each composition. Mean \pm SE; n = 6/group. BFR, blood flow restriction; CONT, control group; EXER, electrical stimulation exercise; TA, tibialis anterior.



hanced the phosphorylation response of rpS6 in the mTOR signaling pathways, possibly resulting in the attendant muscle hypertrophy. Interestingly, these conditions also increased glucose transporter and mitochondrial biogenesis, which may underly the pleiotropic effects of exercise training with BFR, in which the activation of PGC-1 α is intrinsic to the improved capacity for sustained exercise.

There is experimental support for muscle protein synthesis and hypertrophy being elicited by exercise training under hypoxic conditions (8, 9, 11). However, until the present investigation, the O_2 pressures within the intact contracting muscles and their relationship to candidate mechanisms controlling the hypertrophic response to training (with BFR) had not been determined. $PmvO_2$ was significantly lowered from 27.5 mmHg at rest and 23.2 mmHg during blood flow-intact, low-force electrical stimulation to 7.5 Torr by application of the 80-mmHg pressure cuff concomitant with electrical stimulation. Indeed, this $PmvO_2$ was significantly lower than that present during flow-intact, high-force (100 Hz) contractions (13.5 Torr). It is important to note that the level of BFR utilized herein is not completely occlusive, which would be expected to reduce the $PmvO_2$ to values close to 0 Torr as demonstrated by Matusmoto et al. (36) using electron paramagnetic resonance oximetry in mouse quadriceps muscle. Thus, the level of $PmvO_2$ under the moderate BFR level used in the present investigation is intermediate between that induced by 100-Hz



Fig. 6. Effects of EXER or EXER+BFR training on monocarboxylate (lactate) transporter (MCT) proteins. Effects of EXER or EXER+BFR on the expression of MCT1 (*A*), MCT4 (*B*), and its chaperon, CD147, (*C*) in rat tibialis anterior (TA) muscles 24 h after the ninth training. Representative immunoblots are shown in each group. Expression level was obtained by adjusting each value by the internal control level of GAPDH. Means \pm SE; *n* = 6/group. Significantly different between groups, **P* < 0.05, ***P* < 0.01. BFR, blood flow restriction; CD147, cluster of differentiation 147; CONT, control group; EXER, electrical stimulation exercise.

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Fig. 7. Effects of EXER and EXER+BFR training on metabolite transport proteins and mitochondria related proteins. A: PGC1a. B: GLUT4. C: COX4. D: FNDC5. Data were obtained from right TA muscle (BFR side). Protein densities of PGC1 α (A), GLUT4 (B), COX4 (C), and FNDC5 (D) are shown. Expression level was obtained by adjusting each value by the internal control level of GAPDH. Representative immunoblots are shown above each group. Means \pm SE; n = 6/group. Significantly different between groups, *P < 0.05, *P < 0.01. Significance levels between EXER and CONT were P = 0.559(PGC1 α) and P = 0.094 (GLUT 4), respectively. BFR, blood flow restriction; CONT, control group; COX4, cytochrome c oxidase subunit 4; EXER, electrical stimulation exercise; FNDC5, fibronectin type III domaincontaining protein 5; GLUT4, glucose transporter 4; PGC-1a, peroxisome proliferatoractivated receptor gamma coactivator 1-alpha; TA, tibialis anterior.

intense muscle contractions and complete blood flow disruption.

By definition, aerobic organisms require O_2 to produce energy, and reduced O_2 availability or transient deprivation causes significant stress in all living cells, including skeletal muscles. Specifically, hypoxic stress to muscle tissues either

Table 1. Relative expression levels of various proteins ineach group

	CONT		EXER +	
	(n = 6)	EXER $(n = 6)$	BFR $(n = 6)$	BFR $(n = 6)$
SREBP-1(precursor)	0.262 (0.02)	0.304 (0.03)	0.328 (0.04)	0.317 (0.04)
SREBP-1 (mature)	0.238 (0.04)	0.237 (0.04)	0.346 (0.04)	0.187 (0.05)
REDD1	0.756 (0.07)	0.689 (0.03)	0.622 (0.05)	0.653 (0.04)
CD36	0.682 (0.05)	0.623 (0.06)	0.597 (0.08)	0.625 (0.03)
IL-6	0.258 (0.09)	0.471 (0.09)	0.273 (0.03)	0.492 (0.06)
Myostatin	1.468 (0.15)	1.262 (0.15)	1.034 (0.11)	1.222 (0.13)
BDNF (precursor)	1.070 (0.12)	0.800 (0.06)	0.730 (0.07)	0.740 (0.06)
HIF-1α	0.608 (0.17)	0.533 (0.09)	0.480 (0.07)	0.652 (0.14)
VEGF	0.634 (0.07)	0.675 (0.08)	0.563 (0.04)	0.802 (0.08)
Aquaporin-4	0.452 (0.05)	0.514 (0.05)	0.433 (0.06)	0.644 (0.07)

Data are given as mean (SE). Expression level was obtained by adjusting the internal control level of GAPDH. Each datum was obtained from six different rats. BDNF, brain derived neurotrophic factor; BFR, blood flow restriction group; CD36, cluster of differentiation 36; CONT, control group; EXER, exercise group; EXER+BFR, exercise with blood flow restriction group; HIF-1 α , hypoxia-inducible factor-1 α ; IL-6, interleukin-6; REDD1, regulated in development and DNA damage responses 1; SREPB-1, sterol regulatory element-binding protein 1; VEGF, vascular endothelial growth factor.

suppresses intracellular ATP production (4) or alters metabolic control [e.g., elevated perturbations of NADH, ADP, phosphocreatine, inorganic phosphate (Pi), or H⁺] as well as increasing reactive oxygen species (61, 71). An increase of reactive oxygen species reduces sarcoplasmic reticulum Ca2+ release/ reuptake and compromises contractile function (11). Pi accumulation is also considered to be an important contributor to muscle fatigue (61), as Pi binds to sarcoplasmic reticulum Ca^{2+} and inhibits muscle contraction (66). When low-force exercise is performed under BFR (27) or ischemia (65), muscle fatigability is enhanced and a decrease in the maximum voluntary muscle force and number of repetitions possible occurs in a similar manner as during high-force exercise. In fact, in exercise under BFR, the tension production was reduced substantially during the exercise bout because of muscle fatigue. Note in Fig. 4A that for EXER+BFR, tension decreases rapidly to its nadir compared with that seen for EXER alone. Takarada et al. (62) determined that this enhanced fatigability induced by exercise with BFR is mechanistically linked to a recruitment of type II (i.e., higher order) muscle fibers in addition to the type I fibers normally recruited in the free-flowing condition (24). This enforced recruitment of both fiber types at contraction intensities at which they would not normally both be active provides a putative explanation for the enhanced hypertrophic response induced by BFR. Such an explanation is also compatible with the present results showing that EXER+BFR increased fiber CSA preferentially in the superficial TA, where

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type II fibers predominate. Interestingly, a significant increase in maximum tension was observed in both EXER and EXER+BFR groups (Fig. 4*B*). Human voluntary contraction training programs provide evidence that low-force training with BFR promotes less adaptation of the nervous system compared with high-force resistance training (2, 62, 70). One of the reasons that a substantial increase of muscle strength was observed even at low force herein may be related to the electrical stimulation condition and enhanced recruitment of type II fibers. However, the involvement of other mechanisms, such as exercise-induced metabolites activating motor unit recruitment (17), cannot be ruled out in the present investigation. Hence, further studies are needed to resolve the precise mechanisms by which BFR promotes muscle strength and hypertrophy.

The mTOR signaling pathways involving phosphorylation of mTOR and sequential phosphorylation of S6K1/rpS6 play an essential role in increased muscle strength and hypertrophy (6, 29, 57, 67). Phosphorylation of the mTOR pathways increases within hours of exercise with BFR (19, 21, 44). For instance, Fujita et al. (21) found S6K1 phosphorylation 3 h following low-force (20% 1RM) knee-extension exercise with BFR in humans. Likewise, Fry et al. (19) showed that mTOR, S6K1, and rpS6 phosphorylation and muscle protein synthesis were increased 1 and 3 h after exercise with BFR. In particular, rpS6 phosphorylation increased ~17-fold compared with the untreated CONT group and ~3-fold compared with blood flow-intact exercise (19). Similarly, in our rat model, rpS6 phosphorylation increased ~13-fold compared with the CONT group and ~2.6-fold compared with EXER alone. We also investigated the relationship between PmvO₂ and phosphorylation of rpS6 as an index of the muscle hypertrophic signal. Interestingly, herein no significant change in the muscle hypertrophy signal (rpS6) was induced by BFR alone for 3 wk, which is inconsistent with our previous findings that BFR alone induced muscle hypertrophy (59). This disparity may potentially be explained by the longer experimental period (i.e., 6 vs. 3 wk herein) evaluated by Sudo et al. (58). But we did find that the low-force exercise condition with BFR herein markedly enhanced rpS6 phosphorylation. Thus, lowering PmvO₂ via BFR acts to potentiate the protein phosphorylation response driven by muscle contractions. However, in contrast to human studies (19, 21), the present investigation showed that lowforce electrical stimulation with BFR markedly enhanced the phosphorylation of rpS6, which occurred in the absence of altered mTOR and S6K1. This finding may have resulted from evaluating the response just 3 h after the exercise.

Reduced muscle oxygen pressures potentiate the hypertrophic signal within muscle as seen by the Akt, S6K1, and Eukaryotic translation initiation factor 4E (eIF4E)-binding protein 1 (4E-BP1) responses to 4 h to 4 days of hypoxic exposure (FI_{O2} 0.08 or 0.12) (16, 18). HIF-1 α represents one candidate signaling mechanism for this response because of its rapid upregulation during hypoxic stress (34). Surprisingly, however, HIF-1 α expression was not changed, at least 3 h after the exercise with BFR (Table 1), suggesting that HIF-1 α upregulation does not appear to drive the muscle hypertrophic signal (rpS6 phosphorylation). One limitation was that we only obtained tissue samples 3 h after acute exercise and could not therefore have detected subsequent changes. However, both rpS6 phosphorylation and hypertrophy occurred in the absence

of a hypoxic signal, suggesting that hypoxia itself, at least at the muscle microvascular level, is not an obligatory feature of these responses. Accordingly, the precise mechanisms controlling the hypertrophic response to low-level contractions with (and without) BFR remain to be resolved.

In marked contrast to the evidence herein, it is considered that to induce muscle hypertrophy, resistance exercise training is generally conducted at or above 70% 1RM (3, 39). The present investigation utilized neuromuscular electrical stimulation to mimic resistance exercise (22, 33), which has been successful for inducing hypertrophy and strengthening human muscles (at 75 Hz, isometric contractions) (22). Herein, lowforce (30 Hz) isometric contractions alone induced a significant hypertrophic effect (6.2% increase in muscle mass), disputing the notion that microvascular hypoxia during contractions is essential for muscle hypertrophy and increased maximal tension (100-Hz force production, Fig. 4A). However, in lowforce (30 Hz) isometric contractions alone, the torque generated gradually decreased during the repetitive stimulation (Fig. 4A). Thus, the force production in the present investigation did induce muscle fatigue, possibly stimulating muscle hypertrophy. On the other hand, a large increase in muscle mass (11.0%), though it was not statistically greater than found with EXER alone, and a significant increase in fiber CSA in the superficial portion were observed after the markedly hypoxic conditions induced by low-force contractions and BFR over 3 wk of training. This evidence supports the conclusion that hypoxia acts to potentiate the hypertrophic signaling as observed in the elevated rpS6 phosphorylation.

For the chronic exercise training response, as opposed to that present 3 h after a single bout of contractions (i.e., Fig. 3), no significant increase in phosphorylation of mTOR, S6K1, and rpS6 was observed in this study (data not shown). The most plausible explanation for this response is that their phosphorylation response usually reaches peak levels at 3 h after exercise and subsequently returns to baseline within 24 h. Second, the training effect itself may cause the muscle hypertrophy signal to gradually reduce (51). In general, the muscle hypertrophic response in repetitive resistance training increases the CSA and potentially the number of myofibers, such that the load on the individual muscle fibers suppresses further signaling and hypertrophy. Ogasawara et al. (48) reported that the phosphorylation response in S6K1 and rpS6, evoked by electrical stimulation (~30 V, 60 Hz) of the rat gastrocnemius muscle, decreases progressively with subsequent bouts of training. This notion is also supported by the observation that 9 wk of resistance training in older rats decreased the phosphorylation responses of Akt and mTOR, despite significant muscle hypertrophy (48). Interestingly, however, the phosphorylation response of AMPK α was increased (32).

In general, high-frequency isometric electrical stimulation (60-100 Hz) can induce skeletal muscle hypertrophy (48, 64), whereas lower-frequency electrical stimulation more resembles endurance-like training (5). However, the imposition of BFR during low-force isometric electrical stimulation as a training method herein does not permit discrimination between the effects of different stimulation frequencies. The low-force exercise with BFR used herein increases blood lactate immediately after exercise (54), and there is an upregulation of both glycolytic and aerobic systems after 8 wk of training (12). In

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addition to muscle strength and hypertrophy, there is evidence for improvement in endurance and maximum oxygen consumption resulting from low-force exercise with BFR training paradigms (1, 49). We showed that low-force isometric contraction with/without BFR increased COX4, a marker of muscle oxidative capacity (Fig. 7*C*), and provided the first evidence that exercise with BFR increases PGC-1 α protein expression. PGC-1 α constitutes a critical master regulator for mitochondrial biogenesis and energy metabolism (69), and PGC-1 α upregulation may allow greater capacity for modulating metabolic fluxes in skeletal muscle under the low-O₂ conditions imposed by simultaneous low-level contractions and BFR. Despite the fact that hypoxia is reported to enhance PGC-1 α protein expression (47), BFR alone did not increase PGC-1 α protein expression herein.

PGC-1 α induces the expression of multiple genes, including the substrate transporters GLUT4, FAT/CD36, MCT1, and FNDC5 (7, 10, 41). The concentrations of these substrate transporters, GLUT4 and fatty acid translocase/Cluster of Differentiation 36 (FAT/CD36) as well as MCT1 (9, 10, 37, 38, 40), are highly correlated with the oxidative capacities of skeletal muscle. In the present study, EXER+BFR enhanced GLUT4 protein and PGC-1a protein. EXER+BFR induced a much larger increase in MCT1 compared with EXER alone. FNDC5 and CD147, but not FAT/CD36, all increased with EXER alone. Benton et al. (7) reported that MCT1 and CD147, its chaperon, but not MCT4 are regulated by PGC-1a. This finding supports that BFR via its effect on PGC-1 α may help regulate MCT1 protein expression and, in so doing, potentially enhance lactate flux across the myocyte sarcolemma. Similarly, by elevating GLUT4, BFR may increase myocyte glucose uptake. Indeed, the hypoxic exposure itself raises the AMP/ ATP ratio, thereby activating AMPK α , which serves as an intracellular energy sensor moving GLUT4 to the cell membrane (25). In so doing, glucose uptake is promoted by the GLUT4, and ATP production is sustained/accelerated while sparing muscle glycogen (23). Thus, exercise under BFR increased oxidative capacity, glucose transporter, and mitochondrial biogenesis, which may be involved in the pleiotropic effects of the exercise training under BFR, in which the activation of PGC-1a provides a locus of control. Recently, Cannavino et al. (14) reported that mitochondrial dysfunction plays a major role in disuse atrophy, and inducing PGC-1a expression could be useful to treat/prevent muscle atrophy. Thus, low-force, electrically induced exercise with BFR appears to improve muscle mass and prevent muscle atrophy via the activation of PGC-1 α . In contrast, recent evidence in humans showed an attenuated expression of all four PGC-1 α isoforms when endurance exercise is performed with BFR (15), suggesting an exercise paradigm specificity to the BFR effects that is worthy of further attention.

In conclusion, although demonstrating that muscle hypoxia is not obligatory for the hypertrophic response to low-force, electrically induced muscle contractions, the reduced $PmvO_2$ induced by BFR during contractions enhanced rpS6 phosphorylation, possibly resulting in the attendant muscle hypertrophy. Furthermore, in addition to muscle hypertrophy, contractions with BFR also increased oxidative capacity, glucose transporter, and mitochondrial biogenesis, which are key determinants of the pleiotropic effects of exercise training in which PGC-1 α is upregulated.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

T.N. and T. Yamasoba conceived and designed research; T.N., S.K., T. Yasuda, T.H., S.O., and S.T. performed experiments; T.N., S.K., and Y.K. analyzed data; T.N., T. Yamasoba, T.I., and Y.K. interpreted results of experiments; T.N. and Y.K. prepared figures; T.N., F.N., T.I., D.C.P., and Y.K. drafted manuscript; T.N. edited and revised manuscript; T.N. approved final version of manuscript.

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ORIGINAL ARTICLE

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Hemodynamic and hormonal responses to a short-term low-intensity resistance exercise with the reduction of muscle blood flow

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Abstract We investigated the hemodynamic and hormonal responses to a short-term low-intensity resistance exercise (STLIRE) with the reduction of muscle blood flow. Eleven untrained men performed bilateral leg extension exercise under the reduction of muscle blood flow of the proximal end of both legs pressure-applied by a specially designed belt (a banding pressure of 1.3 times higher than resting systolic blood pressure, 160-180 mmHg), named as Kaatsu. The intensity of STL-IRE was 20% of one repetition maximum. The subjects performed 30 repetitions, and after a 20-seconds rest, they performed three sets again until exhaustion. The superficial femoral arterial blood flow and hemodynamic parameters were measured by using the ultrasound and impedance cardiography. Serum concentrations of growth hormone (GH), vascular endothelial growth factor (VEGF), noradrenaline (NE), insulin-like growth factor (IGF)-1, ghrelin, and lactate were also measured. Under the conditions with Kaatsu, the arterial flow was reduced to about 30% of the control. STLIRE with Kaatsu significantly increased GH $(0.11 \pm 0.03 \text{ to } 8.6 \pm 1.1 \text{ ng/ml}, P < 0.01), \text{ IGF-1}$ $(210 \pm 40 \text{ to } 236 \pm 56 \text{ ng/ml}, P < 0.01)$, and VEGF $(41 \pm 13 \text{ to } 103 \pm 38 \text{ pg/ml}, P < 0.05)$. The increase in

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Y. Sato · T. Nakajima (⊠) Department of Ischemic Circulatory Physiology, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan E-mail: masamasa@pb4.so-net.ne.jp Tel.: +81-3-38155411 Fax: +81-3-38140021 GH was related to neither NE nor lactate, but the increase in VEGF was related to that in lactate (r = 0.57, P < 0.05). Ghrelin did not change during the exercise. The maximal heart rate (HR) and blood pressure (BP) in STLIRE with Kaatsu were higher than that without Kaatsu. Stroke volume (SV) was lower due to the decrease of the venous return by Kaatsu, but, total peripheral resistance (TPR) did not change significantly. These results suggest that STLIRE with Kaatsu significantly stimulates the exercise-induced GH, IGF, and VEGF responses with the reduction of cardiac preload during exercise, which may become a unique method for rehabilitation in patients with cardiovascular diseases.

 $\begin{array}{l} \textbf{Keywords} \quad Resistance \; exercise \cdot \; Ischemia \; \cdot \\ Growth \; hormone \; \cdot \; Vascular \; endothelial \; growth \; factor \; \cdot \\ Hemodynamics \; \cdot \; Cardiac \; output \; \cdot \; Rehabilitation \; \cdot \\ Blood \; flow \; \cdot \; Preload \end{array}$

Introduction

Heavy resistance training has been known to be a potent stimulus for muscle cell growth and hypertrophy (MacDougall et al. 1997; Staron et al. 1984; Abe et al. 2000), then resulting in improvement of muscle strength and increased peak exercise capacity. This is due, in part, to the exercise-induced increase of endogenous anabolic hormones and growth factors such as growth hormone (GH) and insulin-like growth factor-1 (IGF-1) (Kraemer et al. 1990; Godfrey et al. 2003). The GH and IGF-1 have been also established as a regulator of cardiac growth, structure and function (Lombardi et al. 1997; Khan et al. 2002), and GH has been recently applied for the treatment of congestive heart failure (Fazio et al. 1996; Genth-Zots et al. 1999). However, the exerciseinduced GH elevation depends on specific exercise characteristics, and only certain heavy resistance exercise protocols such as a weight-lifting exercise can induce

significant elevation in serum GH (Lukaszewska et al. 1976; VanHelder et al. 1984; Godfrey et al. 2003). Therefore, it is difficult to apply the heavy exercise for patients, such as those with cardiac diseases. Alternatively, a variety of factors have influences on the exercise-induced GH responses, i.e. metabolic demands and hypoxia. Low-intensity resistance exercise with tourniquet ischemia or vascular occlusion has been shown to be a useful method for strength training (Shinohara et al. 1998; Takarada et al. 2000a; Moore et al. 2004), where the potent secretion of GH may play a part (Takarada et al. 2000b). High-intensity exercises [$\sim 80\%$ of one repetition maximum (RM)] produce a 100-fold increase in plasma concentration of GH (Kraemer et al. 1990), while a short-term low-intensity resistance exercise (STLIRE) when combined with the reduction of muscle blood flow (20% of 1 RM) produces a 290-fold increase (Takarada et al. 2000b). These results suggest that STLIRE with the reduction of muscle blood flow may be a novel method for training patients. However, the influence of such kinds of the exercise on the hemodynamics has not been investigated.

High endurance exercise training also stimulates both arteriogenesis and angiogenesis (Yang et al. 1990; Gute et al. 1996). The underlying mechanisms have not been known exactly. However, reduced oxygen tension and/ or related metabolic consequences have been suggested as possible stimuli, where growth factors such as vascular endothelial growth factor (VEGF) may play an essential role. During exercise, local muscle oxygen tension falls considerably (Richardson et al. 1995), followed by the secretion of VEGF immediately after a single bout of exercise in both animals and humans (Breen et al. 1996; Gustafsson et al. 2002). In addition, exercise (Gustafsson et al. 1999; Amaral et al. 2001; Gavin and Wagner 2001; Gustafsson et al. 2002) and hypoxia (Shweiki et al. 1992; Minchenko et al. 1994; Stein et al. 1995) up-regulate the expression of VEGF messenger RNA. Thus, it is likely that the exercise with the restricted blood flow reduces local muscle oxygen tension, and may modulate the exercise-induced VEGF responses.

Thus, we hypothesized that the hemodynamic parameters as well as hormonal responses including VEGF may be altered during STLIRE when combined with the reduction of muscle blood flow. Therefore, we compared the hemodynamic and hormonal responses to STLIRE with and without the reduction of muscle blood flow in healthy untrained men.

Methods

Subjects

Eleven normal healthy adult men, 26–45 years $(34 \pm 6 \text{ years})$, participated in this study. All were untrained volunteers without any diseases such as cardio-vascular and pulmonary diseases, who did not take any

medicine. They also had not regularly taken any sports. The informed consent was obtained prior to the study. Mean height was 175.8 ± 6.9 cm, and mean weight was 68.1 ± 7.8 kg. The body mass index was 22.1 ± 2.5 . The study protocol was approved by the ethics committee of the University of Tokyo.

Exercise protocols

All studies were performed in the afternoon at least 4 h after the lunch. An indwelling heparin-lock catheter was inserted into the superficial vein of left arm. After 30 min of rest on supine position, blood samples in control were collected. Then, the subject was seated against a backrest, and all subjects were asked to be kept both arms on the table. After taking rest measurements of hemodynamic parameters in sitting position for 3 min by using an impedance method (see below), both legs were pressure-applied by a special-designed belt, named as Kaatsu in Japanese (see below). Immediately after Kaatsu, the subjects performed bilateral leg extension exercise with the lower extremity positioned at $\sim 90^{\circ}$ flexion. The intensity of STLIRE was 20% of 1 RM, which was measured at least 1 week before the experiment. The subjects performed 30 repetitions without rest, and after a 20-seconds rest, they performed three sets again until exhaustion. All subjects stopped the exercise due to the leg fatigue. Immediately after the exercise sessions, the banding pressure was released and then blood samples were obtained at $0 \sim 1$, 10, and 30 min after the exercise. All blood samples were processed to serum or plasma before storage at -20° C until analysis. Nine men again performed the exercise without Kaatsu at the same intensity and quality as those for the exercise with Kaatsu. The sessions of experimental (with Kaatsu) and control exercise (without Kaatsu) were separated by 2-4 weeks.

Reduction of femoral muscle blood flow by Kaatsu

A method for inducing the reduction of muscle blood flow was similar as previously reported (Takarada et al. 2000b). Local application of external pressure over both legs (a banding pressure of 1.3 times higher than resting systolic blood pressure, 160–180 mmHg) was used to reduce exercise blood flow. Briefly, both sides of their thighs were pressure-applied at the proximal ends by means of specially designed belts (33 mm in width and 880 mm in length) just before the start of the exercise, and the pressure was released immediately after the exercise.

Measurement of hemodynamic parameters

To evaluate hemodynamic parameters, we used the Task Force Monitor (CNSystems Medizintechnik,

Graz, Austria) (Gratze et al. 1998, 1999; Fortin et al. 1998), which includes surface electrocardiograms (ECG), real time beat-to-beat stroke volume measurements by impedance cardiography (ICG) and beat-tobeat blood pressure measurements by vascular unloading technique (Penaz 1973) so that beat-to-beat changes of total peripheral resistance can be calculated. Oscillometric blood pressure was also recorded on the upper arm. ICG was performed by standard methods (Kubicek et al. 1966). A constant sinusoidal alternating current I_0 of 400 μ A and 40 kHz is passed through the thorax between a circular electrode placed around the neck and another electrode placed around the lower thorax aperture. The voltage u(t) is acquired by two further electrodes placed between the admitting electrodes, each at a distance of at least 3 cm from the outer electrodes to produce an interelectrode homogeneous current. The four electrodes consisted of aluminum tape, which is mounted on adhesive tape. The detected voltage u(t) is proportional to the thorax impedance $Z(Z(t) = u(t) \times I_0)$. The first derivative (d Z/d t) of the impedance signal Z(t) is supplied analog by ICG. The ECG was derived from two separate adhesive monitoring electrodes that are placed on the thorax to give maximal amplitude of the R wave. The signal flow, the components of ICG, and calibration of the finger blood pressure signal to the oscillometric blood pressure measurement were described (Gratze et al. 1999). The ECG, impedance signal and beat-tobeat blood pressure were sampled with 1000 Hz each. These data were used to calculate online all hemodynamic parameters. The measurements of hemodynamic parameters were heart rate (HR), blood pressure [systolic (sBP), diastolic (dBP) and mean (mBP)], left ventricular ejection time (LVET), stroke volume (SV), stroke index (SI), cardiac output (CO), cardiac index (CI), total peripheral resistance (TPR) and total peripheral pressure index (TPRI). The calculation of SI, CO, CI, TPR and TPRI was as follows.

SI = SV/BSA $CO = SV \times HR$ CI = CO/BSA $TPR = mBP \times 80/CO$ $TPRI = mBP \times 80/CI,$ where BSA was body surface area.

Biochemical analyses

Plasma level of lactate was measured at S.R.L. Inc (Tokyo) by the use of an enzyme system employing lactate oxidase combined with N-ethyl-N-(3-methylphenyl)- N-acetyl ethylenediamine and an auto-analyzer, HITACHI Type 7170. Growth hormone (GH) and insulin-like growth factor (IGF)-1 were measured with radioimmunoassay (S.R.L Inc., Tokyo). The VEGF was determined in duplicate by high-sensitivity an enzyme-linked immunosorbent assay (ELISA) using specific anti-VEGF antibody according to the manufacturer's instructions. Plasma noradrenaline (NOR) levels were measured in a plasma extract by high-performance liquid chromatography with the use of a cation exchange column, an acetonitrile/phosphate buffer mobile phase, and electrochemical detection. Plasma ghrelin concentration was measured using ELISA kit using specific anti-ghrelin antibody.

Measurement of femoral blood flow

The blood flow of superficial femoral artery was calculated from the cross-sectional area (CSA) of the artery and velocity time integral (VTI) using Aplio80 (Toshiba, Tokyo, Japan). The site recorded was \sim 5 cm distal to the portion of the Kaatsu belt. First, superficial femoral

Fig. 1 Measurement of femoral artery blood flow. In the pulse-Doppler method (A), velocity time integral (VTI), calculated as the integral area under the velocity curve, was measured. In the 2-dimensional mode, cross sectional area (CSA) was measured (B). A representative data recording of blood flow velocity (A) and diameter (B) of superficial femoral artery at rest (Pre-Kaatsu and Post-Kaatsu)



artery was identified in the 2-dimensional mode, and CSA was measured at the end-systolic period (Fig. 1B). Then, in the pulse-Doppler method (Fig. 1A), VTI, calculated as the integral area under the velocity curve, was measured. Adjustment of the angle for the measurement was within 60°. Blood flow per minute (ml/min) was obtained by multiplying CSA by VTI and heart rate. The blood flow was obtained from five out of 11 subjects at rest in sitting position before and after the application of Kaatsu, and just before releasing the pressure in the post-exercise.

Data analysis

All values are expressed as means \pm SD. Student's paired *t*-test was used to compare two sets of data from the same subjects. Comparison of time courses of parameters was analyzed by one-way ANOVA for repeated measures. When differences were indicated, a Dunnett's comparison was used to determine significance. Spearman rank correlation coefficient (*r*) was used to examine the relationship between individual exercise-induced changes. Differences were considered significant if *P* value was less than 0.05.

Results

Hemodynamic measurements

Figure 2 shows the effects of Kaatsu on blood flow in the superficial femoral artery. Application of Kaatsu significantly reduced blood flow at rest from 370 ± 71 to 133 ± 38 ml/min (approximately 30% of the control, n = 5, P < 0.05). The blood flow remained markedly depressed just before releasing the pressure in the post-exercise (195 ± 70 ml/min, P < 0.05).

Table 1 show the effects of STLIRE on HR and BP. HR, sBP, dBP, and mBP at the peak exercise in STLIRE



Fig. 2 Changes of blood flow of femoral artery. The blood flow of right superficial femoral artery was obtained at rest before and after Kaatsu, and immediately after the exercise with Kaatsu. Values are means \pm SD obtained from five subjects. * P < 0.05 vs. rest (Pre-Kaatsu)

with Kaatsu were much larger than those in STLIRE without Kaatsu. The peak exercise HR reached to 109 ± 15 bpm, which equals to $55 \pm 12\%$ of target heart rate calculated from the sex and age. The SBP, dBP, and mBP reached to 182 ± 18 , 105 ± 18 , and 127 ± 12 mmHg, respectively.

Figure 3 and Table 1 show the effects of STLIRE on hemodynamic parameters. In STLIRE with Kaatsu, CO increased from 5.1 ± 1.5 l/min at rest in sitting position to 6.2 ± 1.5 l/min (P < 0.01) at the peak exercise. In STLIRE without Kaatsu, CO increased from 5.2 ± 0.9 to 6.9 ± 1.5 l/min (P < 0.01). The increase in CO was not statistically different between both STLIRE (Fig. 3A, Table 1). But, SV significantly decreased in STLIRE with Kaatsu, compared with STLIRE without Kaatsu (Fig. 3B and Table 1, P < 0.05). The TPR did not change significantly at the peak exercise of STLIRE with and without Kaatsu (Fig. 3C). The LVET was decreased in STLIRE with and without Kaatsu (Fig. 3D, P < 0.05).

Changes in serum concentrations of NOR and lactate

Table 2 shows the time courses of the changes in serum lactate (A) and NOR (B) concentration during

 Table 1 Effects of a short-term low-intensity resistance exercise
 (STLIRE) with or without Kaatsu on hemodynamic variables

Variables	Rest	Peak exercise
Heart rate (bpm)		
With Kaatsu	73 ± 9	$109 \pm 15^{**}]^{**}$
Without Kaatsu	66 ± 9	$96 \pm 7^{**}$
Systolic blood pressure (mmHg)		
With Kaatsu	127 ± 12	$182 \pm 18^{**}]^*$
Without Kaatsu	118 ± 9	$155 \pm 12 * *$
Mean blood pressure (mmHg)		
With Kaatsu	98 ± 18	127±12**]*
Without Kaatsu	88 ± 9	$113 \pm 27^{**}$
Diastolic blood pressure (mmHg)		
With Kaatsu	86 ± 15	$105 \pm 18^{**}]^*$
Without Kaatsu	73 ± 9	99 ± 21 **
Cardiac output (l/min)		
With Kaatsu	5.1 ± 1.5	$6.2 \pm 1.5^{**}$
Without Kaatsu	5.2 ± 0.9	$6.9 \pm 1.5 **$
Stroke volume (ml)		
With Kaatsu	71 ± 27	$62 \pm 21^*]^*$
Without Kaatsu	74 ± 20	74 ± 20
Total peripheral resistant (dyn*s/cm ⁵)	ce	
With Kaatsu	1650 ± 615	1554 ± 642
Without Kaatsu	1473 ± 327	1471 ± 627
Left ventricular ejection time (ms)		
With Kaatsu	315 ± 39	$271 \pm 33*$
Without Kaatsu	314 ± 18	$290 \pm 33^{*}$

Values are mean \pm SD (n = 9). * P < 0.05, ** P < 0.01 vs. rest Significant differences between STLIRE with and without Kaatsu are also shown. * P < 0.05, ** P < 0.01 Fig. 3 Effects of short-term low-intensity resistance exercise (STLIRE) with and without Kaatsu on hemodynamic parameters (CO, SV, TPR, and LVET). Hemodynamic parameter at rest in sitting position and at the peak exercise are shown in STLIRE with Kaatsu (blue lines) and without (red lines). Values are means \pm SD obtained from nine subjects. * P < 0.05, ** P < 0.01 vs. rest. Significant differences between STLIRE with and without Kaatsu are also shown. * P < 0.05



STLIRE with and without Kaatsu. In STLIRE with and without Kaatsu, lactate significantly increased after the exercise. The increase in lactate concentration after exercise with Kaatsu was much higher than that without Kaatsu. In STLIRE with Kaatsu, NOR increased from 0.2 ± 0.06 ng/ml at rest to 0.54 ± 0.14 ng/ml (P < 0.01) immediately after the exercise, and gradually decreased after the exercise. On the other hand, it increased from 0.15 ± 0.03 to 0.32 ± 0.03 ng/dl (P <0.01) in the control exercise. Thus, the increase in NOR concentration attained in STLIRE with Kaatsu was also significantly higher than that without Kaatsu (Table 2). Effects of STLIRE with Kaatsu on plasma concentrations of GH, IGF-1, VEGF and ghrelin

Figure 4 and Table 2 show the time courses of plasma concentrations of GH, IGF-1, and VEGF. After the exercise with Kaatsu, GH, IGF-1, and VEGF significantly increased. The GH increased gradually after the exercise, and reached to the peak at 30 min after the exercise (Fig. 4A, Table 2). It increased from 0.11 ± 0.03 to 8.6 ± 1.1 ng/ml (P < 0.01). On the other hand, in STLIRE without Kaatsu, GH only slightly increased from 0.16 ± 0.08 to 0.48 ± 0.26 ng/ml (P < 0.05). Thus, the increase in GH elicited by STLIRE with Kaatsu was

Table 2 Effects of a short-term low-intensity resistance exercise (STLIRE) (STLIRE) with or without Kaatsu on hormonal variables	Variables	Supine	Ex	10 min	30 min	
	Lactate (mg/dl)					
	With Kaatsu	11.2 ± 4.5	$24.1 \pm 7.8 **]**$	$30.9 \pm 12.9 **]**$	$16.8 \pm 7.2]$ *	
	Without Kaatsu	8.6 ± 2.1	$15.2 \pm 5.7 **$	12.6 ± 5.4	9.7 ± 4.8	
	Noradrenaline (ng/ml)					
	With Kaatsu	0.2 ± 0.06	$0.54 \pm 0.14^{**}$]*	0.31 ± 0.08 **	$0.31 \pm 0.06 **$	
	Without Kaatsu	0.15 ± 0.03	$0.32 \pm 0.03 **$	0.32 ± 0.11 **	$0.3 \pm 0.08 **$	
	Growth hormone (ng/ml)					
	With Kaatsu	0.11 ± 0.03	0.49 ± 0.29	$3.2 \pm 1.45^*]^*$	$8.6 \pm 1.1^{**}$]**	
	Without Kaatsu	0.16 ± 0.08	0.31 ± 0.08	$0.48 \pm 0.26^{*}$	0.4 ± 0.2	
	IGF-1 (ng/ml)					
	With Kaatsu	210 ± 40	$236 \pm 56^{**}$]*	$230 \pm 40^{**}$]*	$226 \pm 58*$	
	Without Kaatsu	184 ± 25	200 ± 32	196 ± 36	192 ± 32	
Values are mean \pm SD ($n = 9$).	VEGF (pg/ml)					
* $P < 0.05$, ** $P < 0.01$ vs. rest (supine) Significant differences between STLIRE with and without Kaatsu are also shown. * $P < 0.05$, ** $P < 0.01$ <i>N.D.</i> not done	With Kaatsu	41 ± 13	$86 \pm 49*1*$	$104 \pm 24^{**}$]**	$103 \pm 38^{**}]^{**}$	
	Without Kaatsu	33 ± 5	49 ± 33	48 ± 30	47 ± 18	
	Ghrelin (ng/ml)					
	With Kaatsu	7.1 ± 3.5	8.9 ± 2.7	3.8 ± 1.8	7.4 ± 2.9	
	Without Kaatsu	ND	ND	ND	ND	
	Without Kaatsa	1	11.21	1.12.	T.1.2.	



Fig. 4 Time courses of changes in plasma concentrations of GH, IGF-1, and VEGF in STLIRE with Kaatsu (*blue lines*) and without (*red lines*). * P < 0.05, ** P < 0.01 vs. rest (supine). Significant differences between STLIRE with and without Kaatsu are also shown. * P < 0.05, ** P < 0.01

much higher than that without Kaatsu. The IGF-1 and VEGF also increased after the exercise with Kaatsu (Fig. 4B, C), and remained high during the recovery of the exercise. Thus, the increase in IGF-1 and VEGF elicited in STLIRE with Kaatsu was significantly higher than that without Kaatsu.

Figure 5A and C shows the relationships between the changes of serum lactate concentrations and those of VEGF and GH. There were statistical differences between the changes of serum lactate concentration and those of VEGF (r = 0.57, P < 0.05, Fig. 5A), but not GH (Fig. 5C). Figure 5B and D shows the relationships between the changes of serum NOR concentrations and those of VEGF and GH. There were no statistical differences between the changes of serum NOR concentration and those of VEGF and GH.

To investigate the possible involvement of ghrelin in GH release during STLIRE with Kaatus, the serum concentration of ghrelin was measured. Plasma concentration of ghrelin was not significantly altered before and after STLIRE with Kaatsu (Table 2), indicating that ghrelin is not involved in the exercise-induced GH secretion.

Discussion

The major findings of the present study are as follows; (1) STLIRE when combined with Kaatsu significantly stimulates the exercise-induced GH, IGF, and VEGF responses much higher than that without Kaatsu. (2) The TPR did not significantly change, but SV decreased due to the inhibition of venous return by Kaatsu, resulting in the reduction of cardiac preload during the exercise. These results suggest that STLIRE with Kaatsu significantly stimulates the exercise-induced GH, IGF, and VEGF responses with the reduction of cardiac preload during exercise, which may become a unique method for rehabilitation in patients with cardiovascular diseases. The GH secretion induced by the exercise is dependent on specific exercise characteristics, and only heavy resistance exercise protocols such as a weightlifting exercise can induce significant GH secretion (Lukaszewska et al. 1976; VanHelder et al. 1984; Godfrey et al. 2003). Therefore, it is difficult to apply the heavy exercise for patients, such as aged people and patients/athletes in rehabilitation programs, who cannot put high mechanical stress on muscle, tendon and joints. On the other hand, a variety of factors have influences on the exercise-induced GH responses, i.e. metabolic demands and hypoxia. Low-resistance exercise with tourniquet ischemia or vascular occlusion has been reported to be a useful method for strength training (Shinohara et al. 1998; Takarada et al. 2000a; Moore et al. 2004), where the potent secretion of GH is partly involved (Takarada et al. 2000b). In our study, we used STLIRE (20% of 1 RM) with Kaatsu. The maximal heart rate attained during the exercise reached to 109 ± 15 bpm, which equals to only $55 \pm 12\%$ of the maximal heart rate adjusted by age and sex. Rate-pressure product, which is accepted as a non-invasive estimate of myocardial oxygen demand during physical stress, was 198×10^2 mmHg × bpm. Even in spite of such mild strength of exercise, the increased plasma GH concentrations after the exercise were approximately 100-times as high as that at rest. The level of the increased GH concentration reached to the level as previously described, where high-intensity exercise ($\sim 80\%$ 1 RM) was used (Kraemer et al. 1990). Takarada et al. (2000b) reported the similar results of STLIRE (20% of 1 RM and 14 repetitions \times 5 sets, total 70 repetitions), where by using young men athletes aged 20-22 years, plasma GH concentration increased ~ 290 times as high as that before exercise. The value was larger than that in our study. The increased concentrations of NOR and lactate were also much higher than those in our study, suggesting that the difference between the level of the increased GH depends on the strength of the exercise. No such effect was seen after the exercise without Kaatsu, indicating that STLIRE with Kaatsu is a useful method for stimulating GH secretion.

The several mechanisms underlying GH release during STLIRE with Kaatsu may be proposed. Ghrelin has Fig. 5 Relation between the changes of serum VEGF and GH and those of serum lactate and NOR induced by STRILE with Kaatsu. Relationship between individual exercise-induced changes (peak level of post-exercise—pre-exercise level) (Δ VEGF, Δ lactate, Δ GH, and Δ NOR). (**A**, **C**) Relation between Δ VEGF/ Δ GH and Δ lactate (**B**, **D**) Relation between Δ VEGF/ Δ GH and Δ NOR



been known to potently stimulate GH release (Kojima et al. 1999; Takaya et al. 2000), and may be involved in exercise-induced GH elevation (Foster-Schubert at al. 2005). However, plasma concentration of ghrelin was not significantly altered before and after STLIRE with Kaatsu (Table 2), indicating that ghrelin is not involved in the exercise-induced GH secretion. Alternatively, under the conditions with Kaatsu, both lactate and NOR increased after the exercise, compared to the control exercise without Kaatsu. Thus, it is likely that exercise that produces greater demands on anaerobic glycolysis may stimulate serum GH elevation. However, since no consistent systematic relationships between GH and lactate/NOR were observed, a combination of anaerobic factors such as local ischemia and/or local accumulation of lactate in legs induced by the restriction of muscle blood supply may stimulate peripheral afferent nerves, then resulting in enhancement of GHreleasing hormone secretion and/or inhibition of somatostatin release from the pituitary gland (Glustina and Veldhuis 1998; Godfrey et al. 2003). Kaatsu by itself, in the absence of exercise, failed to induce any significant GH secretion in healthy males (data not shown), suggesting that exercise is also required. The greater GH secretion during Kaatsu exercise may be from afferents originating in fast twitch skeletal muscle fibers (Gosselink et al. 1998) since fast twitch fibers have been reported to be recruited preferentially during Kaatsu resistance exercise (Yasuda et al. 2004) or under dynamic ischemic training (Nygren et al. 2000). These mechanisms are speculative and further studies are needed to clarify the basic mechanisms of GH release induced by the Kaatsu exercise.

The IGF-1 as well as GH is involved in various physiological roles such as cell growth and maintenance of skeletal muscle. Heavy resistance exercise increases serum concentration of IGF-1 as well as GH (Kraemer et al. 1990), and we also showed that STLIRE with Kaatsu increased plasma IGF-1 concentration. The circulating GH stimulates synthesis and secretion of IGF-1 within the muscle, but the time courses in the changes of serum concentrations of GH and IGF-1 are quite different. Thus, the possibility that the elevation of IGF-1 observed in the present study is induced by the increase in GH is unlikely.

We also provided new evidence that STLIRE with Kaatsu increases serum VEGF concentration significantly, compared to the control exercise. The increase in VEGF was related to that in lactate. The VEGF has been reported to increase immediately after a single bout of exercise in both animals and humans (Breen et al. 1996; Gustafsson et al. 2002). In addition, in healthy subjects, exercise up-regulates the expression of VEGF messenger RNA (mRNA) (Gustafsson et al. 1999; Amaral et al. 2001; Gavin and Wagner 2001; Gustafsson et al. 2002). The underlying mechanisms have not been known exactly, but reduced oxygen tension and/or related metabolic consequences have been suggested as possible stimuli. Hypoxia acts as a stimulus of VEGF secretion and production (Shweiki et al. 1992; Minchenko et al. 1994; Stein et al. 1995), and during exercise, local muscle oxygen tension falls considerably (Richardson et al. 1995), suggesting that local muscle ischemia may act as a stimulus of VEGF secretion in the exercise. Thus, it is likely that in STLIRE with Kaatsu, the reduced oxygen tension and/or related metabolic consequences such as lactate accumulation induced by the restriction of muscle blood flow may be involved in VEGF secretion. VEGF has been known to play essential roles in exercise-induced angiogenesis (Amaral et al. 2001), proposing that STLIRE with Kaatsu may be a method for rehabilitation exercise to promote angiogenesis. Further clinical studies are needed to clarify this possibility.

It has been known that moderate to heavy resistance exercise markedly increases BP (Kilbom and Brundin 1976: Bosisio et al. 1980; Bezucha et al. 1982; Lewis et al. 1983). Heavy leg extension exercise appears to be a predominately dynamic type of exercise, but it has a substantial static component (Miles et al. 1987). During a static exercise, there is a rise in BP caused by an increase in CO, which is due to an increase in HR (Helfant et al. 1971; Perez Gonzalez 1981). In STLIRE with Kaatsu, CO increased without significant changes in TPR. The SV rather decreased by about 12%, as compared to control exercise, which was thought to be due to the reduction of venous return pressure-applied by the Kaatsu belt. Thus, the increase in BP was largely dependent on the increase in CO due to a significant increase in HR, but not SV. Therefore, the increase in BP observed in our exercise was typical of an exercise with a static component. The inhibition of venous return during STLIRE with Kaatsu can reduce cardiac preload during the exercise, which may be useful in rehabilitation in patients with cardiac diseases.

The GH and IGF-1 have been also established as a regulator of cardiac growth, structure, and function (Lombardi et al. 1997; Khan et al. 2002), and GH has been recently applied for the treatment of congestive heart failure (Fazio et al. 1996; Genth-Zots et al. 1999). The STLIRE when combined with Kaatsu appears to be a useful method to induce significant exercise-induced GH responses (EIGHR), while exercise at the same intensity but without Kaatsu fails to induce it. It remains unclear whether EIGHR can improve cardiac function in a similar manner to GH therapy (Fazio et al. 1996; Genth-Zots et al. 1999). However, GH has been also well known to increase serum IGF-1 production, which may also improve cardiac function, but further clinical trials are needed to clarify this possibility.

In conclusion, STLIRE with Kaatsu significantly stimulates the exercise-induced GH, IGF, and VEGF responses with the reduction of cardiac preload during exercise, which may become a unique method for rehabilitation in patients with cardiovascular diseases.

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Effects of low-intensity, elastic band resistance exercise combined with blood flow restriction on muscle activation

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We examined the effects of blood flow-restricted, lowintensity resistance exercise (termed kaatsu) using an elastic band for resistance on muscle activation. Nine men performed triceps extension and biceps flexion exercises (four sets respectively) using an elastic band for resistance with blood flow restriction (BFR) or CON (unrestricted blood flow). During a BFR session, subjects wore pressure cuffs inflated to 170–260 mmHg on the proximal region of both arms. Surface electromyography (EMG) was recorded from the triceps brachii and biceps brachii muscles, and mean integrated EMG (iEMG) was analyzed. Blood lactate concentration was obtained before (Pre) and immediately after two exercises (Post). During

Age-related skeletal muscle loss (sarcopenia) inhibits mobility and increases the risk of developing several diseases such as diabetes, osteoporosis, and heart disease (Visser et al., 2002; Guillet & Boirie, 2005). Highintensity resistance training can induce muscle hypertrophy and improve insulin resistance and type-2 diabetes in the elderly (Frontera et al., 1988; Fiatarone et al., 1990; Dunstan et al., 2002), suggesting that highintensity resistance training leads to prevention and/or improvement of sarcopenia in the elderly. However, the use of heavy weights with weight machines/free weights required for muscle adaptation with traditional resistance exercise may not be practical and may even be dangerous when carried out without proper supervision. Thus, the effectiveness of alternative exercise methods should be investigated.

Elastic bands/tubing have been used widely in rehabilitative medicine and in health enhancement for resistance training (Zion et al., 2003; Ribeiro et al., 2009; Colado et al., 2010). A previous study reported that a home-based resistance training program for older adults using elastic bands could serve as a practical and effective means of improving muscle strength (Mikesky et al., 1994). Elastic bands are also portable and are less expentriceps extension and biceps flexion exercises, muscle activation increased progressively (P < 0.05) under BFR (46% and 69%, respectively) but not under CON (12% and 23%, respectively). Blood lactate concentration at Post was higher (P < 0.05) under BFR than under CON (3.6 and 2.1 mmol/L, respectively). Blood lactate concentration at Post was significantly correlated with increased iEMG in both triceps extension (r = 0.65, P < 0.01) and biceps flexion exercises (r = 0.52, P < 0.05). We conclude that kaatsu training using elastic bands for resistance enhances muscle activation and may be an effective method to promote muscle hypertrophy in older adults or patients with a low level of activity.

sive and easier to use than weight machines/free weights. Elastic resistance training has thus been shown to be a feasible alternative to high-intensity resistance training (Colado & Triplett, 2008; Ribeiro et al., 2009; Andersen et al., 2010). However, as elastic resistance training is commonly performed at a low-to-moderate intensity level, this training typically has little or no effect on muscle hypertrophy (Mikesky et al., 1994; Hostler et al., 2001; Colado & Triplett, 2008).

In the past decade, several studies have reported that muscle hypertrophy can be produced with low-intensity resistance training [20–30% one-repetition maximum (1RM)] combined with muscular blood flow restriction (BFR), termed "kaatsu training," regardless of age (Takarada et al., 2000, 2002; Abe et al., 2005; Fujita et al., 2008). Kaatsu training is also a potentially useful method for promoting muscle hypertrophy with a low risk of injury (Nakajima et al., 2006; Madarame et al., 2010; Loenneke et al., 2011; Ozaki et al., 2011). Elastic resistance training with BFR may thus be an effective home-based resistance training program for promoting both muscle strength and hypertrophy. The mechanism by which BFR potentiates the training effect of lowintensity resistance training remains obscure but appears



Fig. 1. Illustrations show the body position used for exercises. Triceps extension (a) and biceps flexion (b) were performed with an elastic band.

to be related, in part, to an increase in muscle activation (Moritani et al., 1992; Takarada et al., 2000; Yasuda et al., 2008, 2009). The purpose of this study was thus to examine the effect of combined BFR and elastic band resistance exercise on muscle activation.

Methods

Subjects

Nine healthy men aged 23–41 years with resistance training experience volunteered for the study. All subjects received a verbal and written description of the study and provided written, informed consent prior to participating in the study. The study was approved by the Ethics Committee of the University of Tokyo.

Protocol

One week prior to experiments, all subjects completed an orientation session, which included measurement of resting blood pressure, range of motion (ROM), and familiarization with the elastic band exercises and BFR. During the orientation session, subjects sat in a chair with the testing arm placed on a table at heart level, and blood pressure was measured after a 3-min rest. The constancy of elbow joint ROM was calculated from coefficients of variation (CVs) of the mean angle values obtained during each exercise. In both triceps extension and biceps flexion exercises, the ROM of five repetitions was averaged to represent a single datum in each case. The CVs (n = 9) for ROM were 2.2% for triceps extension and 2.6% for biceps flexion exercises.

In an experimental session, subjects performed bilateral triceps extension and biceps flexion exercises with or without BFR. Subjects participated in two experimental sessions (i.e., one with BFR and the other without) that were scheduled 1 week apart. The order of exercises was randomized. Subjects were instructed to refrain from ingesting alcohol and caffeine for 24 h before the experimental sessions and from any strenuous exercise for 48 h before the sessions.

Exercises

During triceps extension exercise, subjects were seated comfortably on a rowing chair with the body supported in the vertical

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position (Fig. 1a). Elbow joint ROM during the exercise was approximately $140-10^{\circ}$ (with 0° being full extension). During biceps flexion exercise, subjects were seated comfortably on a chair (Fig. 1b). Elbow joint ROM during the exercise was approximately $45-140^{\circ}$ (with 0° being full extension). Both exercises were performed using an elastic band. The exercise duration was 2.4 s and included a 1.2-s concentric and 1.2-s eccentric exercise cycle controlled by a metronome (50 beats/min). The exercise session (30 repetitions followed by 3 sets of 15 repetitions, with 30 s between sets and exercises) was determined by reference to the previous studies (Yasuda et al., 2008, 2009).

BFR

In an orientation session, all subjects were trained to wear pressure cuffs (30-mm width; Kaatsu-Master, Sato Sports Plaza, Tokyo, Japan) at the most proximal region of both arms. The restriction pressure intensity of the cuffs (170–260 mmHg) was determined by ratings of perceived exertion (RPE) in the final set of two exercises because previous EMG studies using free weights showed that the value of RPE reached 17–18 in the same exercise protocol (Yasuda et al., 2008, 2009).

Prior to exercise with BFR, subjects were seated on a chair and the kaatsu arm cuff was tightened around the arm to a belt pressure of 40 mmHg. The cuff was then inflated to a pressure of 100 mmHg for 30 s and then deflated for 10 s. This procedure was repeated, increasing the inflation pressure 20–40 mmHg each time until the final cuff restriction pressure of 170–260 mmHg was achieved. Once the cuffs were inflated, they remained so for the entire experimental session, including rest periods between sets and exercises.

Electromyography (EMG)

The skin was shaved, abraded with a skin preparation gel (Skinpure, Nihon Kohden, Tokyo, Japan), and cleaned with alcohol wipes. During the experiment, skin impedance was less than 2 k Ω . The ground electrode was positioned on the lateral epicondyle. Bipolar (1-cm center-to-center) surface EMG (sEMG) electrodes (Ag/AgCl; Vitrode F; Nihon Kohden) were placed along the longitudinal axis of the triceps brachii and biceps brachii of the left upper arm. The electrode placements on the triceps brachii and biceps brachii were both at 60% of the upper arm limb length,
respectively. EMG signals were recorded and collected on a personal computer (T7300 Macintosh, Apple, Tokyo, Japan) for subsequent analysis. All EMG signals were digitized at a sampling rate of 1024 Hz with a bandwidth of 0 Hz–500 kHz (AB 6216; Nihon Kohden). To determine integrated EMG (iEMG), signals were fully rectified and integrated (Power Lab Chart 5 software, ADInstruments, Nagoya, Japan). During the experimental session, sEMG was recorded continuously, and each repetition was analyzed individually for iEMG. Each iEMG value was divided into groups of five successive repetitions, and the average for each group of five repetitions was represented as a single data point for statistical analysis. To determine the iEMG ratio of agonist muscles, iEMGs during each exercise was normalized to Pre, which was iEMG without BFR before the first set of each exercise.

Relative exercise intensity

To determine the relative exercise intensity of performing the triceps extension and biceps flexion exercises using the "extraheavy" bands (i.e., Blue Thera-Bands; Hygenic Corporation; Akron, Ohio, USA), the iEMGs for the band exercises were compared to the iEMGs of the same exercises using free weights at predetermined relative exercise intensities (i.e., 10%, 20%, 30%, 40%, and 50% of 1RM). Measurements were also made with the same elastic band (three to five repetitions) on another experiment day. The order of exercises and loadings was randomized for each individual.

Blood lactate concentration

With subjects rested in a seated position, venous blood samples $(15-50 \,\mu\text{L})$ were taken from the antecubital vein at baseline (Pre). Immediately following two exercises, the pressure cuff was quickly removed, and blood samples were obtained at 0 (Post) and 15 min (Post-15) after the two exercises. All samples were analyzed with a rapid lactate analyzer (Lactate Pro, Arkray, Kyoto, Japan).

Heart rate

Heart rate was measured at baseline (Pre) and immediately after the last set of each exercise (Post) with a heart rate monitor (Marquette Dash 3000 Patient Monitor, GE, Milwaukee, Wisconsin, USA).

RPE

RPE was measured using the Borg scale immediately after the last set of each exercise (Post) (Borg, 1973).

Statistical analysis

Results are expressed as mean \pm standard deviation (SD). A two-way analysis of variance with repeated measures (condition \times time) was used to evaluate the training effects for all dependent variables. Post-hoc testing was performed using Tukey's technique when appropriate. All calculations were made with JMP statistical software package v.8.0 (SAS Institute Inc., Tokyo, Japan). Pearson's product correlation was performed to determine the relationship between change in iEMG and blood lactate concentration or RPE. Statistical significance was set at P < 0.05.

Results

Descriptive characteristics for the subjects are shown in Table 1. None of the subjects had high systolic

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Table 1. Descriptive characteristics of nine male subjects

Variable	Mean	SD	Range
Age (years)	29.6	7.1	23–41
Height (cm)	174.7	4.4	166–180
Weight (kg)	72.3	8.7	61–88
Resting BP (mmHg)			
Systolic BP	117.8	13.2	96–127
Diastolic BP	71.8	8.3	59–84
ROM (°)			
Triceps extension	119.0	11.9	105–144
Biceps flexion	93.4	10.1	81–109
Relative exercise intensity (% 1RM)			
Triceps extension	15.0	8.3	6.0-27.6
Biceps flexion	19.9	6.7	10.2-33.6

1RM, one-repetition maximum; BFR, blood flow restriction; BP, blood pressure; ROM, range of motion; SD, standard deviation.

 $(\geq 130 \text{ mmHg})$ or diastolic $(\geq 85 \text{ mmHg})$ blood pressure. During the first five repetitions of each exercise, mean exercise intensity according to normalized iEMG was approximately 15% of 1RM for triceps extension and approximately 20% of 1RM for biceps flexion (Table 1).

Figure 2 shows representative EMG traces during BFR exercises. During the two exercises, all subjects maintained and completed all the prescribed exercises. During triceps extension exercise, iEMG increased (P < 0.05) progressively during exercises under BFR (approximately 46%) and was greater (P < 0.05) than CON under BFR in the last set (Fig. 3a). In biceps flexion exercise, there was a progressive increase (P < 0.05) in iEMG with BFR (approximately 69%), such that iEMG was greater (P < 0.05) under BFR vs CON from the second to the last set (Fig. 3b). No significant changes in muscle activation were observed under CON during triceps extension (approximately 12%) and biceps flexion exercises (approximately 23%).

At baseline, CON and BFR sessions produced no differences (P > 0.05) in blood lactate concentration $(1.0 \pm 0.1 \text{ and } 1.0 \pm 0.2 \text{ mmol/L}, \text{ respectively})$ or heart rate (67.4 \pm 9.6 and 66.0 \pm 9.2, respectively). Blood lactate concentration increased (P < 0.05) in both exercises. Blood lactate concentration at Post was higher (P < 0.05) under BFR than CON (3.6 and 2.1 mmol/L, respectively). Blood lactate concentration remained elevated at Post-15 under BFR, but not under CON (1.8 and 1.2 mmol/L, respectively). Following the triceps extension and biceps flexion exercises, heart rate was greater (P < 0.05) under BFR (99 ± 20/min and $109 \pm 22/\text{min}$, respectively) than CON (87 ± 15/min and 92 \pm 15/min, respectively), and RPE was greater (P < 0.01) under BFR $(17.4 \pm 1.6 \text{ and } 18.7 \pm 0.9,$ respectively) than CON (12.9 \pm 1.6 and 14.6 \pm 1.2, respectively).

Blood lactate concentration at Post was significantly correlated with increased iEMG in both exercises (Fig. 4). Following each exercise, iEMG was correlated



Fig. 2. Representative electromyography tracings from an experimental session with blood flow restriction. The exercises performed were triceps extension and biceps flexion was performed against elastic band resistance. The experimental session consisted of four sets of each exercise. The time line indicates the time spent performing each exercise and the 30-s rests in between. The total length of the experimental session was 9.5 min.



Fig. 3. Integrated electromyography (iEMG) ratio of triceps brachii and biceps brachii muscles during triceps extension (a) and biceps flexion (b) for each set performed. Pre was iEMG without blood flow restriction (BFR) before the first set of each exercise. *Difference from Pre, P < 0.05. *Difference from CON, P < 0.05.

with RPE in triceps extension (r = 0.60, P < 0.01) and biceps flexion exercises (r = 0.68, P < 0.01).

Discussion

Muscle activation has been shown to increase during low-load exercise with BFR (Takarada et al., 2000;

Yasuda et al., 2008, 2009). However, it was unknown whether increased muscle activation could be achieved during BFR exercise using an elastic band. Our findings show that muscle activation increased progressively in a BFR session when exercises were performed at a lowintensity level (approximately 20% 1RM) using an



Fig. 4. Relationships between integrated electromyography (iEMG) ratio of agonist muscles for each exercise and blood lactate immediately after two exercises.

elastic band for resistance. This result was similar to those in the previously reported studies using free weights (Yasuda et al., 2008, 2009).

In our study, blood lactate concentration increased following BFR exercise and was correlated with increased muscle activation in both triceps extension and biceps flexion exercises. Previously, it was suggested that greater muscle activation during low-intensity resistance exercise with BFR may have taken place to compensate for a deficit in force development secondary to changes in energy supply (Bigland-Ritchie et al., 1986; Moritani et al., 1986). In addition, venous blood oxygen saturation, oxygen partial pressure, carbon dioxide, accumulation of blood lactate concentration, and hydrogen ions are significantly changed following BFR exercises (Takarada et al., 2000; Yasuda et al., 2010), indicating that these changes could potentially stimulate muscle activation (Leonard et al., 1994). Taken together, these results suggest that BFR combined with elastic band exercise changes blood flow, energy supply, and venous occlusion sufficiently to induce an increase in muscle activation. A BFR exercise-induced increase in muscle activation may be one important factor in the muscle hypertrophy seen in active muscle following low-intensity resistance training with BFR in previous studies (Takarada et al., 2000; Yasuda et al., 2008, 2009). Consequently, the results of our study, together with those of the previous studies, suggest that BFR training

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using an elastic band as well as weight machines/free weights leads to significant muscle hypertrophy.

In general, because elastic resistance training is performed at a low-to-moderate intensity level, the training program is designed to be a high repetition form of training. Consequently, most studies have demonstrated that muscle strength and endurance capacity can be improved following elastic resistance training (Mikesky et al., 1994; Rogers et al., 2002; Colado et al., 2010). At the same time, elastic bands produced less muscle hypertrophy than weight machines (Colado & Triplett, 2008). Elastic resistance training also induced minor changes in muscle fiber size and fiber-type composition compared with high-intensity resistance training (Hostler et al., 2001). Thus, low-intensity elastic resistance training combined with BFR may be an effective training program for promoting muscle hypertrophy in practical applications.

Previous studies have demonstrated that elastic resistance training is well tolerated, as indicated by nonexacerbation of chronic disease conditions and lack of training-induced injury (Mikesky et al., 1994; Zion et al., 2003). Home-based resistance training using elastic bands has therefore been used widely for older adults and for patients with a lower level of activity (Aniansson et al., 1984; Mikesky et al., 1994; Rogers et al., 2002; Zion et al., 2003; Colado & Triplett, 2008; Colado et al., 2010). In the present study, the cuff pressure (170-260 mmHg) produced near exhaustion in subjects, as reported previously (Yasuda et al., 2008, 2009) because increased iEMG during BFR exercise was related to a high value of RPE. Consequently, high values of RPE were observed in both triceps extension and biceps flexion (17.4 and 18.7, last set, respectively). However, because RPE was generally lower in BFR exercises (14.9-15.8, last set) vs high-intensity resistance exercise (17.6-18.7, last set), there were no incidents of any pain reported in kaatsu training (Yasuda et al., 2010, 2011). Although kaatsu training is performed with restricted venous blood flow and pooling of blood in the extremities, it has no impact on blood clotting function as assessed by changes in fibrin d-dimer and fibrin degradation products after exercise or training (Madarame et al., 2010; Clark et al., 2011). Furthermore, serious side effects of kaatsu training have not been reported in approximately 13 000 people, including older adults (>80 years old) and patients with various physical conditions (Nakajima et al., 2006). All these findings suggest that elastic resistance training with BFR is a relatively safe training method, but it should be noted that a potential for adverse effects exists, especially when subjects perform until near or complete exhaustion.

Two limitations of this study should be mentioned. First, the material properties of an elastic band are an important variable for determining exercise intensity. Previous studies reported that increase in muscle

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activation depends on the resistance level and stretch distance of the elastic band/tubing during exercise (Mikesky et al., 1994; Patterson et al., 2001; Andersen et al., 2010). However, the type of band used in our study (Blue, Thera-band) presented the same resistance level, and elbow joint ROM was defined arbitrarily. There were consequently large individual differences in the relative exercise intensity.

Second, cuff pressure intensity (170-260 mmHg) for arms was higher than that in the previous studies (Takarada et al., 2000; Yasuda et al., 2008, 2010, 2011), as discussed earlier. In this study, relative exercise intensities during triceps extension and biceps flexion (15.0% and 19.9%, respectively) were lower than those in previous EMG studies (20% 1RM; Yasuda et al., 2008, 2009). Furthermore, because the same elastic band was used for all subjects, there were large differences between individuals in the relative exercise intensity. In the biceps flexion exercise, cuff pressure intensity was negatively correlated with relative exercise intensity (r = -0.80, P < 0.05). Taken together, we speculate that a high level of cuff pressure intensity is required during BFR training at low intensity; as a result, the potential for adverse effects is increased. More work is needed to understand how BFR training using an elastic band would be advantageous for developing safe and effective methods of promoting muscle hypertrophy in older adults, and especially in patients capable of tolerating only low-load resistance exercise.

Perspective

Blood flow-restricted, low-intensity resistance exercise (kaatsu training) using machines/free weights leads to increased muscle activation, the response to which is an important factor in muscle hypertrophy. However, as an elastic band is inexpensive, compact, and easy to use compared with machines/free weights, elastic band resistance training could be used in home-based training programs for older subjects or for patients with a lower level of activity. As in previous BFR studies using machines/free weights, our results suggest that lowintensity, elastic band resistance exercise combined with BFR enhances muscle activation. Kaatsu training using elastic bands may therefore be an effective method to promote muscle hypertrophy in older adults or in patients capable of tolerating only low-load resistance exercise.

Key words: vascular occlusion, kaatsu, elastic band, EMG, upper limb.

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Muscle size and arterial stiffness after blood flow-restricted low-intensity resistance training in older adults

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Previous studies have shown that blood flow-restricted low-intensity resistance training (BFR-RT) causes muscle hypertrophy while maintaining arterial function in young adults. We examined the effects of BFR-RT on muscle size and arterial stiffness in older adults. Healthy subjects (ages 61–84 years) were divided into BFR-RT (n = 9) or non-training control (CON; n = 10) groups. The BFR-RT group performed 20% and 30%, respectively, of onerepetition maximal (1-RM) knee extension and leg press exercises, 2 days/wk for 12 weeks. The BFR-RT group wore elastic cuffs (120–270 mmHg) on both legs during training. Magnetic resonance imaging-measured muscle cross-sectional area (CSA), 1-RM strength, chair stand

Age-related skeletal muscle loss (sarcopenia) inhibits mobility and increases the risk of developing several diseases such as diabetes, osteoporosis, and heart disease (Visser et al., 2002; Guillet & Boirie, 2005). Highintensity resistance training (HI-RT) can induce muscle hypertrophy and improve insulin resistance and type-2 diabetes in the elderly (Frontera et al., 1988; Fiatarone et al., 1990), suggesting that HI-RT leads to the prevention and/or improvement of sarcopenia in the elderly (Aagaard et al., 2010). However, HI-RT induced about a 20% reduction in arterial compliance in young and older adults (Miyachi et al., 2003, 2004). In general, reductions in arterial compliance or increases in arterial stiffness reduce the arterial buffering function of the pulsation of blood pressure and blood flow, which contribute to elevations in systolic blood pressure, left ventricular hypertrophy, coronary ischemic disease, and reductions in arterial baroreflex sensitivity (O'Rourke, 1990; Tanaka et al., 1998; Monahan et al., 2001). This means that prevention and treatment of arterial compliance or stiffness are also important. Thus, even if traditional HI-RT is an effective tool in reversing sarcopenia and/or osteoporosis, this type of training may have deleterious effect on arterial compliance or stiffness in older adults.

(CS) test, and cardio-ankle vascular index testing (CAVI), an index of arterial stiffness, were measured before and 3–5 days after the final training session. Muscle CSA of the quadriceps (8.0%), adductors (6.5%), and gluteus maximus (4.4%), leg extension and leg press 1-RM strength (26.1% and 33.4%), and CS performance (18.3%) improved (P < 0.05) in the BFR-RT group, but not in the CON group. In CAVI testing, there were no changes in both two groups. In conclusion, BFR-RT improves muscle CSA as well as maximal muscle strength, but does not negatively affect arterial stiffness or humeral coagulation factors in older adults.

In the past decade, several studies have reported that low-intensity resistance training combined with blood flow restriction (BFR-RT), referred to as "KAATSU Training," elicits muscle hypertrophy and strength gains similar to those elicited during traditional HI-RT (Takarada et al., 2000; Wernbom et al., 2008; Karabulut et al., 2010). Additionally, previous studies have reported that BFR-RT could improve and/or maintain arterial compliance in young adults (Ozaki et al., 2011, 2013). Therefore, BFR-RT may be a useful method for promoting muscle hypertrophy with a low risk of increased arterial stiffness in older adults.

Recently, some studies have demonstrated that sarcopenia is muscle specific and greater quadriceps muscle loss was found in older adults (Miyatani et al., 2003; Abe et al., 2011). However, no previous studies have examined the effect of BFR-RT on lower body muscle size in older adults, and only a single study has investigated changes in skeletal muscle size with BFR-RT in older adults (Takarada et al., 2000). Thus, the purpose of the present study was to examine the effects of BFR-RT on thigh muscle size and arterial stiffness in older adults.

Methods

Participants

Twenty-one older men and women (aged 61-84 years) volunteered to participate in the study and were selected according to the exclusion criteria used to define "medically stable" older participants for exercise studies proposed by Greig et al. (1994). In addition, volunteers who suffered from a chronic disease such as severe hypertension, orthopedic disorders, deep venous thrombosis, peripheral vascular disease, or cognitive dysfunction were excluded from the study. None of the participants had participated in resistance-type training for a minimum of 3 years prior to the study. All participants were free of overt chronic disease as assessed by medical history, physical examination, and complete chemistry and hematologic evaluation. Subjects in the nontraining control (CON) group continued their daily physical activity, but no additional exercise routine was imposed. All participants were informed of the risks associated with the methods, procedures and risks, and signed an informed consent document before participation. The principles of the World Medical Association Declaration of Helsinki and the American College of Sports Medicine Guidelines for Use of Human Subjects were adopted in this study. Twenty-one individuals (5 men, 16 women) enrolled in the study, but two participants dropped out following randomization to the BFR-RT group because of reasons unrelated to the research study. Consequently, 19 participants were randomized to either the BFR-RT group {3 men, 6 women: n = 9; age [mean \pm standard deviation (SD)]: 71.3 \pm 7.1 years} or the CON group (2 men, 8 women: n = 10; age: 67.7 ± 6.0 years).

Training protocol

To develop the thigh muscles especially for quadriceps muscles, the participants in the BFR-RT group performed bilateral knee extension and leg press exercise training 2 days/week for 12 weeks. This training was performed under the close supervision of those with technical knowledge in BFR training. One week before the start of the training study, both groups performed practice sessions for the functional ability test and one-repetition maximum (1-RM) test. In addition, BFR-RT participants were familiarized with the BFR stimulus. Three or four days before training, the 1-RM of both exercises was determined. Training intensity and volume were set at 20% or 30% of 1-RM and 75 repetitions (30, 20, 15, and 10 reps, with around 30-s rests between sets, respectively) for knee extension and leg press exercises (90-s rests between exercises), respectively. This protocol is typical of submaximal BFR studies (Abe et al., 2005; Yasuda et al., 2010, 2012b). Once the cuffs were inflated, they remained so for the two exercises, including rest periods between sets and exercises. During knee extension and leg press exercises, participants were seated comfortably on isotonic knee extension (13260, VR1, Cybex International, Inc., Medway, Massachusetts, USA) and leg press machines (Pro 1 Seated Leg Press, Life Fitness, Schiller Park, Illinois, USA), with the body supported in a vertical position. Knee joint range of motion (ROM) was approximately 90-0° and hip joint ROM was maintained at 110° (with 180° being full extension) during knee extension exercises, while knee joint ROM and hip joint ROM were approximately 90-0° and 125-70°, respectively during leg press exercises. The exercise duration was 2.0 s (1.0-s concentric and 1.0-s eccentric exercise cycle) for knee extension exercises and 2.6 s (1.3-s concentric and 1.3-s eccentric exercise cycle) for leg press exercises. During all training sessions, heart rate was recorded at baseline (pre) and immediately after the last set of each exercise (post; Model 9560, Onyx II, Nonin Medical Inc., Plymouth, Minnesota, USA). Ratings of perceived exertion, calculated on a scale (6-20) to measure subjective feelings of exertion and fatigue, were recorded immediately after the last set of each exercise (Borg, 1982). The training loads of each exercise for the BFR-RT group were adjusted based on 1-RM testing performed every 3 weeks.

Blood flow restriction

During the training sessions, subjects wore a specially designed elastic pressure cuff (50 mm width, KAATSU Master, Sato Sports Plaza, Tokyo, Japan) around the most proximal portion of the both legs. On the first day of training, the cuff was set at 120 mmHg. The pressure was increased by 10–20 mmHg at each subsequent training session until a pressure of approximately 270 mmHg was reached. The restriction pressure was selected in accordance with previous studies (Yasuda et al., 2012a). Immediately after the two exercises, the pressure cuff was quickly removed. The amount of time under moderate blood flow restriction was approximately 11 min.

Measurements schedule

Subject testing took place before the start of the study (pre) and 3–7 days after (post) the 12-week training period. The orders of measurements were magnetic resonance imaging (MRI), venous blood samples, arterial function [flow-mediated dilatation (FMD), cardio-ankle vascular index testing (CAVI), and ankle brachial pressure index (ABI)] tests, functional ability test, and 1-RM strength measurements. The MRI measurement was obtained between 11:00 and 12:00 h. Venous blood samples and arterial function tests (after 6–7 h fast) or functional ability test and 1-RM strength were determined on separate days (few days interval). The subjects were instructed to refrain from ingesting alcohol and caffeine for 24 h prior to pre- and post-training measurements.

MRI-measured muscle CSA

Muscle CSA was obtained using a MRI scanner (1.5-T MRI, Hitachi, Tokyo, Japan). A T-1 weighted spin-echo axial plane sequence was performed with a 540-ms repetition time and a 20-ms echo time. Subjects rested quietly in the magnet bore in a supine position with their legs extended. The top edge of the great trochanter was used as the origin point, and continuous transverse images with 10-mm slice thickness (0-mm interslice gap) were obtained from the top edge of the great trochanter to the lateral condyle of femur at pre- and post-training measurements. All MRI data were transferred to a personal computer for analysis using specially designed image analysis software (sliceOmatic, Tomovision Inc., QC, Canada). Skeletal muscle tissue cross-sectional area (CSA) data for the quadriceps, adductors and hamstrings at 50% of thigh length (Fig. 1) and for the gluteus maximus at the top edge of the great trochanter were digitized. The coefficient of variation of this measurement was less than 1.0% (Yasuda et al., 2012b).

Estimation of 1-RM strength

One RM was estimated by the 10-RM method (Baechle & Earle, 2008) using a weight stack machine. Bilateral knee extension and leg press maximum dynamic strength (1-RM) were assessed using an isotonic knee extension (VR1, Cybex International, Inc.) and leg press machines (Seated Leg Press, Life Fitness). After warming up, the testing load was set (approximately 80% of predicted 1-RM). Each subject reached muscular failure for the load, and partial repetitions (where participants failed to lift through the entire ROM) did not count as RMs. If a subject had to perform a given repetition number for a given condition again, as a result of ease in obtaining the desired repetitions or failure to attain the repetition number, a 5-min rest period was given and the condition was attempted again at an altered load. No participant had to perform a given repetition number test condition more than three times. Each participant performed the knee extension exercise, rested for 5 min, and then performed the leg press exercise. During estimated 1-RM testing as well as training sessions, the parallel leg



Fig. 1. Typical magnetic resonance images showing transverse sections of the thigh taken before (pre) and after (post) 12 weeks of leg extensions and leg presses with blood flow restriction. The images show identical sections at the mid-thigh in the same subjects (YN).

stance width was set at 100% of the shoulder-width for leg press exercises.

Arterial function tests

FMD, CAVI and ABI measurements were conducted in the supine position. The participants were instructed to fast 6-7 h before testing and refrain from ingesting alcohol, caffeine for at least 12 h prior to testing. After the participants were asked to rest in the lying position in a quiet, dark, air-conditioned room (23-25°C) for 5 min, a standard cuff was positioned around the right arm, 2–3 cm below the antecubital fossa and their systolic and diastolic blood pressures were assessed using oscillometric methods (UA-767PC, A&D Co., Ltd, Tokyo, Japan). Then, after the participants had rested again for at least 15 min in a supine position in the same room, endothelium-dependent FMD of the brachial artery was measured using an established noninvasive method (Corretti et al., 2002). Using a 10-MHz linear array transducer probe, the longitudinal image of the right brachial artery was recorded at baseline and then continuously from 30 sec before to at least 2 min after the cuff deflation that followed syprasystolic compression (50 mmHg above systolic blood pressure) of the right forearm for 5 min. The diastolic diameter of the brachial artery was determined semiautomatically using an instrument equipped with software for monitoring the brachial artery diameter (UNEX EF, Unex Co. Ltd, Nagoya, Japan). %FMD was calculated as reported previously (Tomiyama et al., 2008; Yeboah et al., 2009). Then, after a 20-30min rest, CAVI and ABI were measured noninvasively using a VS-1500 system (Fukuda Denshi Co., Ltd, Tokyo, Japan). CAVI was obtained by substituting the stiffness parameter into an equation for determining vascular elasticity (Shirai et al., 2006).

Blood sampling and biochemical analyses

Venous blood samples were obtained from the antecubital vein and measured for fibrin/fibrinogen degradation products (FDP), D-dimer and creatine kinase (CK). The plasma concentrations of these samples were measured at a commercial laboratory (SRL Inc., Tokyo, Japan) by following latex immunoassay for FDP and D-dimer and spectrophotometry for nicotinamide adenine dinucleotide phosphate formed by a hexokinase and D-glucose-6phosphate-dehydrogenase-coupled enzymic system for CK.

Functional ability test

A chair-stand test required participants to stand up from a seated position, as many times as possible, within 30 s (Rikli & Jones, 1990).

Statistical analyses

Results are mean \pm SD. Statistical analysis was performed by a two-way analysis of variance (ANOVA) with repeated measures

[trials (BFR-RT vs CON) × time (pre vs post)]. Post-hoc testing was performed using Tukey's test when a significant *F*-value was detected. Percent changes from pre were also compared between groups using Tukey's test. Statistical significance was set at P < 0.05.

Results

Before training, there were no significant differences between the two groups for age and anthropometric variables except for systolic blood pressure (Table 2). After the training program, mid-thigh girth was increased (P < 0.01) in the BFR-RT group, but not in the CON group (Table 1). There were no changes in body mass, BMI and lower leg girth in both groups. During training sessions in the BFR-RT group, heart rates were slightly higher (P < 0.05) in the leg press exercise [118 ± 18 beats per minute (BPM)] than in the knee extension (112 ± 19 BPM) exercise. The ratings of perceived exertion tended to be higher (P = 0.09) in the knee extension exercise (15.3 ± 1.5) than in the leg press (14.3 ± 1.8) exercise. During both exercises, the participants did not perform contraction efforts until exhaustion.

After 12 weeks of BFR training, CSA was increased by 8.0% (pre, 44.0 \pm 9.5 cm²; post, 45.1 \pm 9.4 cm²) in the quadriceps, 6.5% in the adductors (pre, 22.2 \pm 8.4 cm^2 ; post, $22.6 \pm 8.2 \text{ cm}^2$) and 4.4% in the gluteus maximus (pre, $37.5 \pm 7.3 \text{ cm}^2$; post, $39.8 \pm 4.4 \text{ cm}^2$), but not in the hamstrings (pre, $21.1 \pm 4.6 \text{ cm}^2$; post, $21.1 \pm 4.1 \text{ cm}^2$) in the BFR-RT group (Fig. 2A–D). In the CON group, no change was observed in each muscle CSA (pre, $43.5 \pm 9.5 \text{ cm}^2$; post, $42.7 \pm 9.0 \text{ cm}^2$ for quadriceps, pre, $21.2 \pm 8.6 \text{ cm}^2$; post, $21.0 \pm 8.6 \text{ cm}^2$ for adductors, pre, $19.9 \pm 3.6 \text{ cm}^2$; post, $20.3 \pm 3.6 \text{ cm}^2$ for hamstrings and pre, $36.4 \pm 7.2 \text{ cm}^2$; post, $35.6 \pm$ 7.5 cm² for gluteus maximus; Fig. 2A–D). Knee extension and leg press 1-RM strength were also increased 26.1% (pre, 50 \pm 20 kg; post, 64 \pm 26 kg) and 33.4% (pre, $145 \pm 47 \text{ cm}^2$; post, $191 \pm 56 \text{ kg}$) in the BFR-RT group but not in the CON group (pre, 52 ± 26 kg; post, 55 ± 27 kg for knee extension and pre, 143 ± 56 kg; post, 142 ± 51 kg for leg press; Fig. 3A,B).

There were no changes (P > 0.05) between pre- and post-training in heart rate, systolic and diastolic blood

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Table 1. Changes in anthropometric variables and skeletal muscle size after 12	2 weeks o	i training p	eriod
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	BFR-RT			CON		
	Pre	Post	%	Pre	Post	%
Anthropometric variables						
Age, years	71 (7)			68 (6)		
Standing height, m	1.61 (0.08)			1.58 (0.06)		
Body mass, kg	53.9 (9.3)	54.0 (9.0)	0.4	53.4 (9.1)	53.4 (9.0)	0.1
BMI, kg/m ²	20.8 (2.6)	20.9 (2.5)	0.4	21.3 (2.9)	21.3 (2.8)	0.1
Mid-thigh girth, cm	44.0 (3.7)	44.7 (1.5)**	1.5##	46.5 (3.7)	46.3 (3.9)	-0.5
Lower leg girth, cm	33.7 (3.0)	33.5 (3.1)	-0.7	34.0 (2.9)	33.9 (2.9)	-0.4

Notes: Data are given as mean (\pm standard deviation). BFR-RT, blood flow-restricted resistance training; BMI, body mass index; CON, non-resistance training; mid-thigh girth, at 50% thigh length; lower leg girth, at 30% lower leg length. **P < 0.01, Pre vs Post; #P < 0.01, BFR-RT vs CON.



Fig. 2. Percent changes in quadriceps (a), adductors (b), hamstrings, (c) and gluteus maximus (d) muscle cross-sectional area (CSA). Data are given as mean (\pm standard deviation). ***P* < 0.01, **P* < 0.05. Blood flow-restricted resistance training (filled symbols) vs non-resistance training (unfilled symbols).

pressures, CAVI, ABI, FDP, D-dimer and CK for either group; however, the BFR-RT group tended to improve (P = 0.09) in FMD, whereas the CON group did not change (Table 2).

Chair-stand performance improved (P < 0.05) 18.3% in the BFR-RT group (pre, 14.8 ± 3.1 times; post, 17.4 ± 4.2 times), but not in the CON group (pre, 18.0 ± 3.3 times; post, 18.4 ± 3.9 times). The change in the chair-stand test results correlated with the change in muscle CSA for the quadriceps (r = 0.53, P < 0.05) and gluteus maximus (r = 0.50, P < 0.05), but not for the adductors (r = 0.27, P > 0.05), as well as changes in muscle strength for knee extension (r = 0.55, P < 0.05) and leg press (r = 0.47, P < 0.05).

Discussion

It has previously been observed that BFR-RT leads to increased muscle size and maintenance of arterial compliance in young adults (Ozaki et al., 2013). However, there are no published data investigating thigh muscle size and arterial stiffness following BFR-RT in older adults. Our findings show that low-intensity knee extension and leg press training with BFR can lead to a significant increases in thigh (quadriceps and adductors) and hip (gluteus maximus) muscle CSA as well as maximal contractile strength in older adults. In addition, we observed no changes in hemodynamic parameters (heart rate, systolic and diastolic blood pressures), arte-



Fig. 3. Percent changes in knee extension (a) and leg press (b) one-repetition maximal (1-RM) strength. Data are given as mean (\pm standard deviation). ***P* < 0.01, Blood flow-restricted resistance training (filled symbols) vs non-resistance training (unfilled symbols).

Table 2.	Changes in hemod	ynamic parameter	, arterial function,	coagulation system ar	nd muscle damage after	12 weeks of training period
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	BFR-RT		CON		
	Pre	Post	Pre	Post	
Heart rate, bpm	62 (11)	62 (8)	58 (7)	59 (7)	
Systolic BP. mmHa	151 (15)##	143 (20) [#]	126 (10)	121 (12)	
Diastolic BP. mmHg	90 (9)	91 (11)	79 (10)	80 (12)	
CAVI. m/sec	9.1 (1.4)	9.0 (1.5)	8.7 (0.8)	8.5 (1.1)	
ABI. unit	1.13 (0.07)	1.15 (0.06)	1.13 (0.10)	1.13 (0.09)	
FMD. %	2.8 (2.0)	4.4 (2.5) [§]	4.4 (1.8)	3.9 (2.5)	
FDP. 10 ⁻⁵ g/L	2.9 (1.1)	3.7 (3.0)	2.7 (0.7)	3.0 (2.0)	
D-dimer. 10 ⁻⁵ a/L	0.5 (0.4)	1.2 (1.6)	0.3 (0.3)	0.4 (0.2)	
CK, IU/I	165 (145)	137 (63)	108 (53)	115 (54)	

Notes: Data are given as mean (\pm standard deviation). BFR-RT, blood flow-restricted resistance training; CON, non-resistance training; BP, blood pressure; CAVI, cardio-ankle vascular index; ABI, ankle-brachial pressure index; FMD, flow-mediated dilation; FDP, fibrin/fibrinogen degradation products; CK, creatine kinase. ^{##}P < 0.01, [#]P < 0.05, BFR-RT vs CON; [§]P, 0.09, Pre vs Post.

rial stiffness (CAVI), coagulation factors (FDP and δ -dimer) and muscle damage (CK), suggesting that BFR-RT was an useful method for preventing and/or improving sarcopenia in old healthy adults.

In this study, BFR-RT (at 20-30% 1-RM) produced a hypertrophic potential of 0.33% per session (8.0% increase in quadriceps muscle CSA over 24 training sessions), which is similar to that observed following HI-RT at 80% 1-RM (0.26-0.45%) in elderly adults (Frontera et al., 1988; Fiatarone et al., 1990). Additionally, the observed gains in knee extension and leg press 1-RM strength (1.09% and 1.39% per session, respectively) were comparable with the previous BFR-RT study (Karabulut et al., 2010) at 20% 1-RM (1.06% and 1.07% per session, respectively), which thus appears equally effective as HI-RT at 80% 1-RM for improving thigh muscle strength in older adults. Therefore, our data suggested that BFR-RT (at 20-30% 1-RM) as well as HI-RT (at 80% 1-RM) can provide an effective hypertrophic stimulus on selected thigh muscles in older adults.

Few studies have attempted to elucidate the cellular and molecular mechanisms of adaptation in skeletal muscle as well as the cardiorespiratory system in response to low-intensity BFR exercise (Manini & Clark, 2009). Previous studies demonstrated that a single session of low-intensity (at 20% 1-RM) knee extension

exercise with BFR increased both vastus lateralis (VL) muscle protein synthesis (40-50% at 3 h post-exercise) and the Akt/mammalian target of rapamycin (mTOR) signaling pathway in young and older men (Fujita et al., 2007; Fry et al., 2010). These anabolic responses may contribute significantly to BFR training-induced muscle hypertrophy and strength gain. On the other hand, the same laboratory using the same technique reported that high-intensity (at 70% 1-RM) knee extension exercise increased VL muscle protein synthesis (48% at 2 h postexercise) through the mTOR pathway in young men (Drever et al., 2006). This means that increases in postexercise muscle protein synthesis are probably similar between high-intensity resistance exercise and lowintensity BFR resistance exercise. Recently, Nielsen et al. (2012) revealed that BFR-RT (23 training sessions) leads to marked proliferation of myogenic stem cells and resulting myonuclei addition in skeletal muscle, which is accompanied by substantial myofiber hypertrophy. Therefore, BFR-RT as well as HI-RT indicates that myogenic stem cell-derived myonuclei provides an improved capacity for myofibrillar gene transcription, which is likely to contribute to an enhanced activity of muscle protein synthesis.

Previous cross-sectional studies found that individuals who performed HI-RT on a regular basis demonstrated

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lower levels of arterial compliance than their sedentary peers (Bertovic et al., 1999; Miyachi et al., 2003). Consistent with the cross-sectional studies, 8-16 weeks of HI-RT induced approximately 20% reductions in arterial compliance (Miyachi et al., 2004). On the other hand, our results show that arterial stiffness was maintained following 12 weeks of BFR-RT in older adults. The finding is consistent with previous observations of an unaffected arterial stiffness following BFR-RT in young adults (Ozaki et al., 2013). It is not clear what physiological mechanisms explain the reduced arterial compliance following resistance training, but previous HI-RT and BFR-RT studies reported that resistance training-reduced arterial compliance was associated with elevations of systolic arterial pressure during training sessions (London & Guerin, 1999; Ozaki et al., 2013). The training load, the number of repetitions, and the rest time between sets during BFR training sessions were similar between this study and a previous BFR-RT study (Ozaki et al., 2013). Therefore, we speculate that the magnitude of change in blood pressure responses during BFR exercise is an influencing factor for resistance training-induced arterial compliance in older adults as well as young adults.

Recently Yoshizawa *et al.* (2009) demonstrated that moderate-intensity resistance training (at 60% 1-RM) did not increase arterial stiffness in middle-aged women, which may have great importance for health promotion with resistance training. However, they did not mention the changes for muscle size. In addition, the magnitude of increase in 1-RM leg press strength following training period was less than one-half that reported for current study (14.8 vs. 33.4%) although the training frequency and period (2 days/week for a 12-week period) were same between two studies. This means that moderate-intensity resistance training is not enough method for preventing and/or improving sarcopenia in old healthy adults.

Arterial compliance is influenced by vascular endothelial function (Wilkinson et al., 2004). In general, vascular endothelial cells play an important role in the regulation of vascular activity by producing vasoactive substances such as nitric oxide (NO), but the endothelial function is not improved by HI-RT (Okamoto et al., 2009). In this study, NO-dependent brachial artery FMD tended to improve following BFR-RT. Moreover, a previous study demonstrated that NO production in muscle is enhanced following BFR exercise (Larkin et al., 2012). Therefore, there is a high possibility that BFR-RT has a beneficial effect on endothelial function, unlike HI-RT. In this study, interestingly, brachial artery FMD was tended to improve following leg exercise training. This is in agreement with previous study showing that cycle exercise improves brachial artery FMD (Schmidt et al., 2002). Previously, Madarame et al. (2008) demonstrated that "cross-transfer" effect for the endogenous anabolic hormones of blood flow-restricted muscles was observed in non-restricted muscles. These findings indicate that a beneficial effect on endothelial function following BFR-RT could be expected to affect both blood flow-restricted and non-restricted sites.

Previous studies reported that resistance training increases muscle size and strength, and improves functional performance in daily tasks for older adults (Hunter et al., 2001; Suetta et al., 2004; Bottaro et al., 2007; Kryger & Andersen, 2007). Additionally, it is well known that knee extension muscle CSA and strength play important role in the chair-stand performance for older adults (Corrigan & Bohannon, 2001; Takai et al., 2009). In the present study, we showed that 12-week BFR-RT led to improved chair-stand performance where the magnitude of improvement was associated with the change in the muscle CSA and strength for both the quadriceps and gluteus muscles, respectively. Recently, Yoshioka et al. (2012) revealed that hip extensors (gluteus maximus, etc.) as well as knee extensors are fundamental muscles in the chair-stand task. Taken together, the present improvement in the chair-stand task likely was due to the observed increases in muscle size and strength for the quadriceps and gluteus maximus.

The present results showed that muscle hypertrophy occurring not only in the thigh muscles directly affected BFR, but also in the gluteus maximus muscle proximal to the area directly affected by BFR. This finding is consistent with that of previous investigations in young adults (Abe et al., 2005; Yasuda et al., 2010). The mechanisms behind trunk (non-restricted blood flow) muscle hypertrophy following BFR-RT are not completely known, but have been hypothesized to occur from an accumulation of metabolites leading to increased muscle fiber recruitment (Yasuda et al., 2009) and the "cross-transfer" effect for the growth of restricted-blood flow skeletal muscles (Madarame et al., 2008) in older adults as well as young adults.

Some potential limitations of this study may be mentioned. We measured CAVI, which reflects changes in both central and peripheral muscle arterial compliance, although previous studies measured central and/or peripheral arterial compliance. Hence, more work is needed to understand the relationship between BFR-RT and arterial function.

In conclusion, low-intensity knee extension and leg press training with BFR elicited marked gains in thigh muscle CSA and strength, and did not negatively affect arterial stiffness in older healthy adults. Also, chair-stand ability was improved by this training, which may be mainly due to an increase in quadriceps and gluteus maximus muscle size. Thus, our results demonstrated that low-intensity resistance training with BFR was an useful method for preventing and/or improving sarcopenia in old healthy adults.

Perspective

BFR-RT leads to improve lower body muscle size and strength and improve and/or maintain arterial

compliance in young healthy adults. However, the effect of BFR-RT on lower body muscle size and arterial function in old healthy adults was unclear. As previous BFR-RT studies in young healthy adults, our results suggested that BFR-RT (20–30% of 1-RM) could improve lower body muscle size and maintain arterial stiffness in old healthy adults. Thus, BFR-RT is an useful method for preventing and/or improving not only sarcopenia in old healthy adults, but also disuse muscle atrophy in elderly patients capable of tolerating only low-load resistance training (i.e., multiple sclerosis patients, hip/knee arthritis patients, etc).

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Key words: sarcopenia, vascular occlusion, muscle hypertrophy, arterial stiffness, strength.

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ORIGINAL ARTICLE

Effect of low-load resistance exercise with and without blood flow restriction to volitional fatigue on muscle swelling

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Abstract

Purpose The effects on muscle swelling were compared between low-load resistance exercise to exhaustion with (BFR) and without blood flow restriction (NBFR).

Methods Ten young men [aged 27 (SD 5) years, standing height 1.74 (SD 0.05) m, body mass 70.3 (SD 4.3) kg] performed 20 % of one repetition maximal dumbbell curl exercise to exhaustion (four sets, rest intervals were 30 s for BFR and/or 3 min for NBFR, respectively). One arm was randomly chosen for BFR exercise and the other arm performed NBFR exercise. During the BFR exercise session, an elastic cuff was worn proximally on the testing arm at 160 mmHg. Electromyography (EMG) signals were recorded from surface electrodes placed on the biceps brachii muscle and analyzed for integrated EMG (iEMG). Biceps brachii muscle thickness (MTH) was measured using B-mode ultrasound.

Results The total number of exercise repetitions was greater (p < 0.01) in NBFR (221 ± 67 reps) than in BFR (111 ± 36 reps). During the exercise session, iEMG for biceps brachii muscles increased (p < 0.01) during BFR and NBFR (3.94 and 4.45 times of baseline value). Immediately after the exercise, MTH sharply increased (p < 0.01) with BFR and NBFR (1.21 and 1.20 times of baseline value). These results demonstrate that both BFR and NBFR exercises lead to pronounced muscle activation and muscle swelling.

Conclusion Low-load resistance exercise to exhaustion is an effective method for promoting muscle swelling

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regardless of BFR. Furthermore, our data indicate that the increase in muscle swelling for both NBFR and BFR is maintained even 60 min after the exercise.

Keywords Muscle thickness · Electromyography · Vascular occlusion · Biceps brachii · Metabolite

Abbreviations

1-RM	One repetition maximum
BFR	Blood flow restriction
HL	High-load resistance training
iEMG	Integrated electromyography
MTH	Muscle thickness
NBFR	No blood flow restriction

Introduction

In the past decade, numerous studies have reported that low-intensity resistance training [20–30 % one-repetition maximum (1-RM)] combined with blood flow restriction (BFR) elicits muscle fatigue and hypertrophy similar to that induced by traditional high-load resistance training (HL, >70 % 1-RM), regardless of age (Abe et al. 2005; Karabulut et al. 2010; Loenneke et al. 2012d; Takarada et al. 2000; Yasuda et al. 2009, 2010a, 2014). BFR training was originally developed in Japan and it is better known as KAATSU training (Sato 2005). This technique may serve as an alternative training method to improve muscle size and strength in elderly patients who are capable of tolerating only low-load resistance training (i.e., multiple sclerosis patients, arthritis patients, etc.).

Recently, low-load (30 % 1-RM) resistance exercise to volitional fatigue was reported to stimulate muscle protein synthesis for a longer duration than HL (90 % 1-RM) or

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work-matched resistance exercise (Burd et al. 2010). In addition, Ogasawara et al. (2013) revealed that low-load (30 % 1-RM) bench press training to volitional fatigue results in muscle hypertrophy similar to HL (75 % 1-RM) bench press training. Therefore, there is a high possibility that low-load resistance training to volitional fatigue is an effective method for the promotion of muscle protein synthesis and hypertrophy, regardless of whether or not BFR is applied.

Acute cell swelling has been shown to stimulate protein synthesis and suppress proteolysis (Berneis et al. 1999; Häussinger et al. 1993). A previous study showed that increased leg circumference, an index of muscle swelling, was more pronounced in BFR than in non-BFR immediately after low-intensity knee extension exercise (Fry et al. 2010). Interestingly, following a single bout of low-intensity BFR bench press exercise, acute changes in muscle size were observed in both the blood flow restricted triceps muscle as well as the blood flow non-restricted chest muscle. The muscle cross-sectional area of both the triceps and chest muscles increased following BFR bench press training (Yasuda et al. 2010b). Therefore, it appears that BFR training-induced muscle cell swelling may contribute significantly to the anabolic benefits of BFR (Loenneke et al. 2012a; Yasuda et al. 2012). However, previous BFR or NBFR studies (Fahs et al. 2014; Martín-Hernández et al. 2013; Yasuda et al. 2012) have only evaluated muscle swelling at the beginning and end of the resistance exercise, giving no inference as to the time-course of change. We hypothesized that low-load resistance training to volitional fatigue is an effective method for the promotion of muscle swelling regardless of BFR, and the time-course of the increase in muscle swelling will be similar for both conditions. Thus, the purpose of the present study was to investigate the effect of low-load resistance exercise to volitional fatigue with and without BFR on muscle swelling.

Methods

Subjects

Ten healthy young men volunteered for the study. A priori sample size estimation indicated that eight subjects would be needed to detect a group by time interaction for measurements of muscle size with an effect size of 1.5-1.8, alpha level of 0.05, and a power of 0.80. Subjects were excluded if they were hypertensive (blood pressure > 140/90 mmHg) or obese (BMI > 30 kg/m²). The subjects were classified as "recreationally active"; three of ten participated in regular aerobic type exercises (jogging, or cycling; 2–3 times/week for approximately 30 min). Three other subjects had light to moderate resistance training

experience and performed upper body, but they were not professionally trained. Each subject was informed of the risks associated with the exercise, measurements to be taken and the purpose of the study, which conformed to the Declaration of Helsinki and was approved by the Ethics Committee for Human Experiments, University of Tokyo. Written informed consent was obtained from each subject prior to participation.

Protocol

One week prior to experiments, all subjects completed an orientation session which included measurement of resting blood pressure and familiarization with arm curl exercise and BFR. During the orientation session, subjects sat in a chair with the testing arm placed on a table at heart level, and blood pressure was measured after 3 min rest. Then, 1-RM for each arm was determined. Subjects performed 5-6 unilateral arm curls with a low load (approximately 30-40 % predicted 1-RM) as a warm-up and to familiarize subjects with the arm curl exercise. After the warm-up period, the intensity was set at about 80 % of predicted 1-RM. Following each successful lift, the intensity was increased by about 5 % until the subject could not complete the lift through the entire range of motion. A test was considered valid only when the subject used the proper form and completed the entire lift in a controlled manner without assistance. On average, five trials were necessary to complete a 1-RM test (2-3 min rest between each attempt) (Yasuda et al. 2008, 2009, 2010a, 2012).

In the experiments, the subjects randomly performed unilateral arm curl exercises with two different exercise conditions that were scheduled 1 week apart (Visit 1 and Visit 2). The order of exercise conditions was randomized. Subjects were instructed to refrain from drinking alcohol and caffeine for 24 h before the sessions and from performing any strenuous exercise for 48 h before the sessions.

Exercise

The subjects performed four sets of arm curl exercise without BFR (NBFR, with 3 min rest period between sets) and with BFR (with 30 s rest period between sets). In each set of exercise, the subjects repeated the movements until exhaustion, which was defined as failure to keep up with the metronome. This protocol is typical of previously reported BFR and NBFR studies (Kubo et al. 2006; Ogasawara et al. 2013). Exercise intensity was 20 % of the predetermined 1-RM. During each exercise condition, subjects sat on the arm curl bench, with their arm positioned in front of their body such that the shoulder was supported at 45° flexion. The range of elbow joint motion during exercise was completed from 0° to 150° (0° being full extension).

Fig. 1 Experimental timeline



Contraction duration was 2.4 s with a 1.2:1.2 s concentriceccentric contraction duty cycle controlled by a metronome (50 beats per min) (Yasuda et al. 2008, 2009). One arm was randomly chosen to perform BFR exercise, while the other arm performed NBFR exercise, but the use of dominant or non-dominant arm was randomized between subjects. The protocols are summarized in Fig. 1.

Blood flow restriction

Before the experiment, all subjects were familiarized with the specialized pressure cuff (30 mm width, Kaatsu-Master, KAATSU Japan Co., Ltd, Tokyo, Japan). The pressure was set to 160 mmHg, according to previous studies (Yasuda et al. 2008, 2009) for the upper arm which reported that 160 mmHg is the optimal level of BFR for altering blood flow, energy supply, and increasing muscle activation when the predetermined exercise protocol is the same among all BFR conditions. The restriction pressure intensity was applied to the upper arms as previously described (Yasuda et al. 2009, 2010a, 2011). During acclimatization, no sign of discomfort or pain was observed in the subjects. In the BFR session experiment, subjects were seated on a chair and the cuff was tightened around the arm to a "belt pressure" (i.e., the pressure the cuff is applying to the arm prior to inflation) of 30 mmHg on the most proximal region of the testing arm. The cuff was then inflated to 160 mmHg (Yasuda et al. 2009, 2010a, 2011). In the BFR session, the cuff was inflated for the entire exercise session including the rest periods between sets and contractions.

Ultrasound-measured muscle thickness

The muscle thickness of the elbow flexors was measured using B-mode ultrasound (Acuson Sequoia 512, Siemens, Tokyo, Japan) at the biceps brachii muscle (at 60 % distal between the lateral epicondyle of the humerus and the acromial process of the scapula). Briefly, the measurements were carried out while the subjects stood with their elbows extended and relaxed. A 10.0 MHz scanning head (5.5 cm length probe) was placed on the skin perpendicular to the tissue interface. The scanning head was coated with a water-soluble transmission gel to facilitate acoustic contact without depressing the dermal surface. The subcutaneous adipose tissue-muscle interface and the muscle-bone interface were identified from the ultrasonic image. The perpendicular distance from the adipose tissue-muscle interface to the muscle-bone interface was taken as muscle thickness (MTH). Ink markers on the elbow flexors were used to ensure similar positioning over repeated MTH measurements (Yasuda et al. 2012). Multiple images were recorded by the same investigator (TY) each time and printed for analysis. Then, each image analysis was performed by the same investigator who was blinded to the both condition and time assignments of the subjects. Test-retest reliability of MTH measurements using intraclass correlation coefficient (ICC_{3.1}) was 0.97. The MTH was recorded before the resistance exercise (pre), during rest periods between sets (1st, 2nd and 3rd set), immediately after the resistance exercise (post), and at 15, 30 and 60 min after the resistance exercise (Fig. 1).

Electromyography (EMG)

The skin was shaved, abraded with a skin preparation gel (Skinpure, Nihon Kohden, Japan), and cleaned with alcohol wipes. During the experiment, skin impedance was less than 2 k Ω . The ground electrode was positioned on the lateral epicondyle. Bipolar (2-cm center-to-center) surface EMG (sEMG) electrodes (Ag/AgCl; Vitrode F; Nihon Kohden; Tokyo, Japan) were placed over the muscle belly (mid-portion) along the longitudinal axis of the biceps brachii of the testing upper arm (Yasuda et al. 2008, 2009). The electrode placement on the biceps brachii was at 60 % of the upper arm limb length. EMG signals were recorded and collected on a personal computer (T7300 Macintosh, Apple, Japan) for subsequent analysis. All EMG signals were digitized at a sampling rate of 1,024 Hz with a bandwidth of 0 Hz to 500 kHz (AB 6216; Nihon Kohden; Tokyo, Japan). The load cell signal was low-pass filtered with a 10 Hz cutoff. Raw EMG signals were digitized and stored on hard disk in a computer using the Chart software program (LabChart 7 software, ADInstruments, Japan). To determine integrated EMG (iEMG), signals were fully rectified and automatically integrated ("Integral" function in LabChart 7 software) on the same computer. During the experimental session, sEMG was recorded continuously and each iEMG value was divided into groups of five successive repetitions (12 s). When the last iEMG group of each set was less than five repetitions, the iEMG value was divided according to the number of repetitions. The highest of iEMG group over each entire set was represented as a single data point for statistical analysis. iEMG during each set was normalized to pre, which was iEMG of the same exercise without BFR (three to five repetitions) before the first set of each visit day. The coefficient of variation (CV) for this measurement from test to retest was 5.7 %.

Blood sampling and biochemical analyses

Venous blood samples (2 mL) were obtained from the antecubital vein and measured for hematocrit, lactate concentration (n = 10), and creatine kinase (CK, n = 3). Hematocrit and lactate concentration were obtained before (pre), immediately after [post (without cuff)], and at 15, 30 and 60 min after the resistance exercise (Fig. 1). CK was obtained at pre, post, and daily for 4 days after the resistance exercise. Lactate concentration was analyzed with a rapid lactate analyzer (Lactate Pro, Arkray, Tokyo, Japan) and hematocrit and CK were measured at a commercial laboratory (SRL Inc., Tokyo, Japan).

Heart rate

During two exercise sessions, heart rate was measured during each set (Model 9560, Onyx II, Nonin Medical Inc., Plymouth, MN, USA).

Ratings of perceived exertion (RPE)

RPE was measured using the Borg scale (6–20) every 10 repetitions and immediately after each set (Borg 1973).

Visual analog scale (VAS)

VAS, drawn as a 100-mm line with 0 mm indicating "no pain" and 100 mm indicating "extremely sore", was used to quantify soreness levels. The subject was instructed to mark a point on the line describing their soreness after they palpated their upper arm with their fingers (palpation of ~5 mm skin indention), while the investigator passively extended and flexed the subject's forearm. The highest value of soreness from these three methods for each subject was used for muscle soreness analysis. VAS was measured before, immediately after (without cuff), and daily for 4 days after the exercise bout of each arm (Thiebaud et al. 2013). VAS of each subject was measured at the same time of day throughout the investigation.



Fig. 2 Exercise repetitions during each set. **p < 0.01 vs. 1 set; $^{\#p} > 0.01$, $^{\#}p < 0.05$ vs. BFR



Fig. 3 Change in biceps brachii muscle thickness during and immediately after exercise. **p < 0.01 vs. pre

Statistical analysis

Results are expressed as mean \pm standard deviation (SD). Two-way ANOVA with repeated measures (condition \times time) was used to evaluate the training effects for all dependent variables. When significant main effects and/or interaction were observed, post hoc testing was performed using the Tukey technique. Statistical significance was set at p < 0.05. Effect sizes (ESs, Cohen's d) in MTH and lactate concentration were calculated with the following formula: [(α mean - pre mean)/pre SD, $\alpha = 1$ st set, 2nd set, 3rd set, post, 15, 30, or 60 min; d = 0.2–0.5 is a small effect, d = 0.5–0.8 is a moderate effect, and d > 0.8 is a large effect] (Cohen 1988).

Results

The mean age, height, body mass and BMI for the subjects were 27 \pm 5 years, 173.6 \pm 4.9 cm, 70.3 \pm 4.3 kg and



Fig. 4 Change in integrated EMG (iEMG) during each set. **p < 0.01 vs. pre

 23.3 ± 1.6 , respectively. On each visit day, there were no differences (p > 0.05) between BFR and NBFR for 1-RM (13.6 ± 2.5 and 13.7 ± 2.1 kg), heart rate (67.4 ± 8.0 and 64.7 ± 11.0 BPM), systolic (124 ± 7 and 120 ± 8 mmHg) and diastolic blood pressures (75 ± 9 and 71 ± 13 mmHg).

Exercise repetitions decreased progressively (p < 0.01)in the two conditions for arm curl exercise from the 1st to 4th set and NBFR was greater (p < 0.01) than BFR at the 1st and 2nd sets. The total number of (4 sets) exercise repetitions was greater (p < 0.01) in NBFR (221 \pm 67 reps) than in BFR (111 \pm 36 reps) (Fig. 2). MTH for biceps brachii increased (p < 0.01) with BFR and NBFR (1.21 and 1.20fold the baseline value), which was similar from the 1st set to 60 min (Fig. 3). All ESs for the differences in MTH were large for BFR (0.88-1.51 from 2nd set to 60 min) and NBFR (0.84-1.81 from 1st set to 60 min) except for 1st set for BFR (0.71). During the exercise session, iEMG for biceps brachii muscles increased (p < 0.01) progressively in BFR and NBFR (3.94 and 4.45 times baseline value); the iEMG increases for BFR and NBFR were similar from the 1st to 4th sets (Fig. 4).

Immediately after the exercise session, the lactate concentration was similarly increased with both conditions (Fig. 5). Immediately after the exercise session, hematocrit was increased with both BFR (pre: $46.4 \pm 2.5 \%$, post: $48.4 \pm 2.7 \%$) and NBFR (pre: $46.6 \pm 1.7 \%$, post: $48.2 \pm 2.0 \%$) to similar levels. During the exercise session, the heart rate increased progressively similarly in both BFR and NBFR from the 1st (96 ± 10 and 95 ± 13 BPM), 2nd (96 ± 10 and 97 ± 15 BPM), 3rd (93 ± 8 and 99 ± 12 BPM), and 4th set (93 ± 7 and 99 ± 17 BPM). All ESs for the differences in lactate concentration were large for BFR (0.8-10.4 from post to 60 min) and NBFR (1.6-10.9 from 1st set to 60 min) except for 60 min for NBFR (0.64).



Fig. 5 Change in lactate concentration immediately after exercise. **p < 0.01, vs. pre

In summation of four sets, high RPE score (more than 15) showed a longer duration (p < 0.01) in NBFR (130 ± 56 reps) than in BFR (90 ± 39 reps). In both BFR and NBFR, VAS significantly increased: 39 ± 27 and 47 ± 23 mm at 24 h, 54 ± 26 and 69 ± 23 mm at 48 h, 50 ± 22 and 58 ± 17 mm at 72 h, and 28 ± 21 and 32 ± 17 mm at 96 h. There was no significant difference in VAS between the two conditions in any set.

In both BFR and NBFR, CK gradually increased: 165 \pm 73 and 160 \pm 74 U/L at pre, 169 \pm 72 and 162 \pm 78 U/L at post, 420 \pm 429 and 583 \pm 559 U/L at 24 h, 3,668 \pm 5,442 and 3,825 \pm 3,767 U/L at 48 h, 9,102 \pm 7,267 and 8,788 \pm 7,570 U/L at 72 h, and 13,415 \pm 7,267 and 11,305 \pm 8,712 U/L at 96 h.

Discussion

The main findings of the present study were: (1) increased muscle swelling following exercise and increased muscle activation during resistance exercise did not differ between BFR and NBFR, and, (2) time-course of the increase in muscle swelling was similar between two conditions, (3) low-load resistance exercise to volitional fatigue induced an increase in muscle swelling 15 min after exercise, which was mainly dependent on muscle damage or inflammation of the muscle tissues regardless of BFR.

It is known that acute cell swelling due to osmotic water shifting into the cell stimulates anabolic processes, both through an increase in protein synthesis and a decrease in proteolysis (Berneis et al. 1999; Häussinger et al. 1993). Recent BFR studies (Martín-Hernández et al. 2013; Loenneke et al. 2012a; Yasuda et al. 2012) suggest that BFR training leads to pronounced acute changes in muscle size,

an index of muscle swelling, the response to which may be an important factor for promoting muscle hypertrophy. However, recent studies (Mitchell et al. 2012; Ogasawara et al. 2013) have reported that NBFR training to volitional fatigue results in muscle hypertrophy similar to high-load training, but their studies were unclear regarding the relationship between NBFR and muscle swelling. In the present study, we revealed the effect size for muscle swelling was similar in both BFR and NBFR exercises [1.5 (large) and 1.8 (large) for post, respectively]. Therefore, it would appear that low-load resistance exercise to volitional fatigue could stimulate muscle protein metabolism induced by muscle swelling. On the other hand, Fahs et al. (2014) demonstrated that BFR training induced a greater increase in muscle size compared with non-BFR training although similar muscle swelling was observed following low-load resistance training to volitional fatigue with and without BFR. In addition, Loenneke et al. (2012b) observed that the induced increase in muscle size after a brief application of BFR (in the absence of exercise) may have been mediated through an acute fluid shift. Taken together, these findings suggest that acute muscle swelling may be a requisite factor for muscle hypertrophy but by itself is not enough.

In previous BFR studies, greater muscle activation during low-load BFR resistance exercise was hypothesized to occur as a compensation for a deficit in force development, secondary to changes in energy supply; these changes resulted from the decreased oxygen available to the muscle and an accumulation of metabolites (Bigland-Ritchie et al. 1986; Moritani et al. 1986; Yasuda et al. 2010a). In this study, muscle activation during the exercise session increased progressively in both BFR and NBFR conditions, although the number of exercise repetitions was approximately two-fold greater in NBFR than in BFR. Additionally, the magnitude of the increased blood lactate concentration and hematocrit following exercise was similar between the two conditions. Therefore, it can be speculated that increased muscle activation during NBFR was also caused by a mismatch in energy demand/energy supply, although the metabolic product per unit exercise repetition with NBFR was approximately one-half that with BFR. Taken together, muscle swelling and muscle activation during low-load resistance exercise to volitional fatigue could reach a plateau with NBFR as well as with BFR, even though the number of exercise repetitions was approximately two-fold greater in NBFR than in BFR.

Previous studies (Loenneke et al. 2012a, c; Yasuda et al. 2012) reported that an increase in muscle swelling with BFR was due to a fluid shift from the plasma into the muscle under BFR. In the present study, however, the increased muscle swelling with both NBFR and BFR remained at least 60 min post-exercise whereas plasma volume and blood lactate concentration returned to baseline at 15 min

post-exercise. On the other hand, increased muscle soreness scores (assessed by VAS) with NBFR and BFR (54 and 69 mm, respectively) were very high score compared with a previously reported BFR study (20 mm; Thiebaud et al. 2013). In addition, muscle damage (CK, n = 3) gradually increased to 13,415 U/L for BFR and 11,305 U/L for NBFR at 96 h post-exercise. These low-intensity exercise results suggest that muscle soreness and muscle damage with BFR and NBFR were comparable with that reported with a high-intensity exercise study (Nosaka and Newton 2002), which performed maximal eccentric exercise of unilateral elbow flexors (3 sets of 10 reps). In contrast, previous BFR studies of low-load BFR exercise not to volitional fatigue reported that there are no changes in markers of muscle damage between before and after an acute bout of exercise (Abe et al. 2006; Fujita et al. 2008). Taken together, it is speculated that low-load resistance exercise to volitional fatigue induced an increase in muscle swelling 15 min after exercise, which was mainly dependent on muscle damage or inflammation of the muscle tissues regardless of BFR.

Some limitations of this study should be discussed. First, the rest period between sets was different between NBFR (3 min rest period between sets) and BFR (30 s rest period between sets). Based on previous studies (Stull and Kearney 1978; Salles et al. 2009), it can be speculated that NBFR with 30 s rest periods between sets recovered maximal strength and exercise repetitions were approximately twice as much as 3 min rest periods between sets for the upper bodies. Therefore, it is likely that increased MTH in NBFR was a little suppressed after the 2nd set when NBFR with 30 s rest periods between sets was applied. In this study, the highest effect size in MTH was 1.5 for BFR and 1.8 for NBFR (at the 3rd set and post, respectively), but MTH at the 1st set of NBFR already reached a large effect size (1.3). Taken together, there is a high possibility that increased muscle swelling between the two conditions with 30 s rest periods between sets are also similar. Second, because the number of repetitions at the 1st set (67 reps) were higher compared with a previous BFR study (25 reps) (Kubo et al. 2006), it appears that the optimal level of BFR was higher than 160 mmHg in this study. Notably, this study used an arbitrary pressure. Previous studies (Loenneke et al. 2012b, 2013; Yasuda et al. 2008) reported that differences in limb size do result in differences in BFR, thus it is always necessary to pay attention to the relationship between the level of BFR and the exercise and/or training effect. Third, NBFR exercise was limited to highly motivated individuals who are capable of tolerating the perceptual response of the exercise session because the high RPE score (more than 15) was approximately a 1.5-fold longer period in NBFR than in BFR. Fourth, our results were acute and may not necessarily be translated to chronic adaptation. Hence, more studies are necessary to understand the relationship between NBFR exercise and safety and/or arterial function.

In conclusion, low-load resistance exercise to volitional fatigue is an effective method to promote muscle swelling and muscle activation regardless of BFR, and the time-course of the increase in muscle swelling was similar for both conditions. Furthermore, our data indicate that the increase in muscle swelling for both NBFR and BFR is maintained even 15–60 min after the exercise, which may be mainly dependent on muscle damage or inflammation of the muscle tissues.

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