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 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.

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

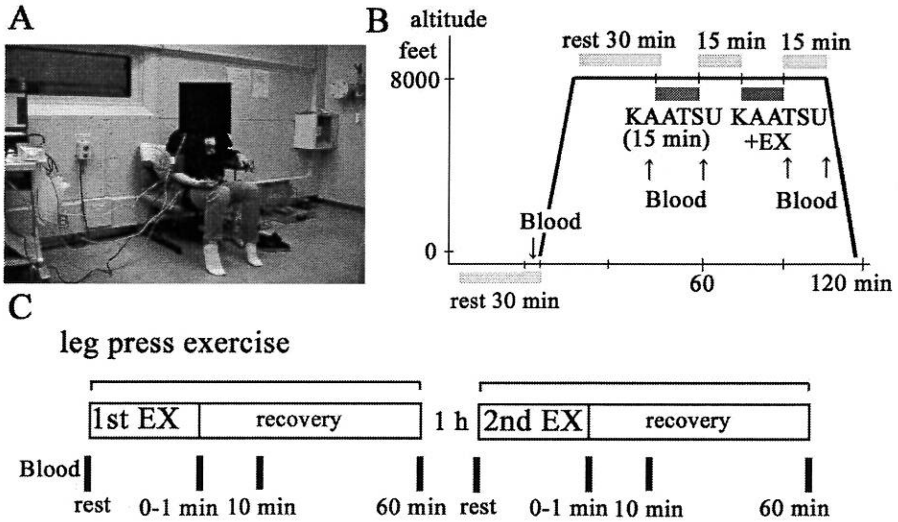

Figure 1. Experimental design. (A) The model of hypobaric chamber in JAXA (B) Protocol 1 (C) Protocol 2

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. O_2 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 heparin-lock 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 pre-heparinised 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 ± 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 (SpO_2) markedly decreased from 99 ± 0.3 % to 91 ± 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 SpO_2, but a series of short-term and low-intensity aerobic exercises combined with KAATSU increased SpO_2. 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 ± 78 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 ± 1.9 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 SpO_2 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.

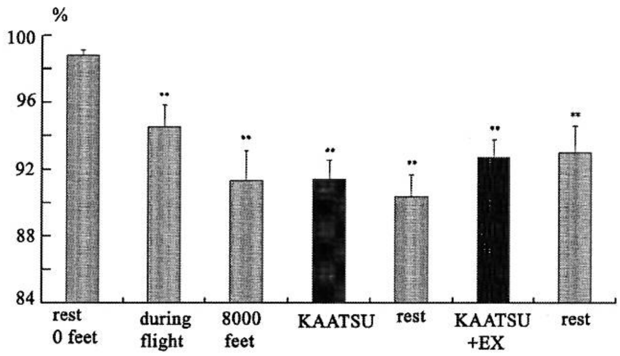

Figure 2. Time courses of SpO_2 under the hypobaric conditions (8000 feet) during KAATSU and a series of short-term and low-intensity aerobic exercises combined with KAATSU. All values are means ± S.E.M. obtained from 6 subjects. ** p<0.01 vs. rest (0 feet)

Table 1. Effects of KAATSU and exercises combined with KAATSU on various parameters under the conditions of 8000 feet in airflight

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 ± 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

* p<0.05 vs. 0 feet ** p<0.01 vs. 0 feet

Table 2. Effects of KAATSU and exercises combined with KAATSU on haemostatic parameters under 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

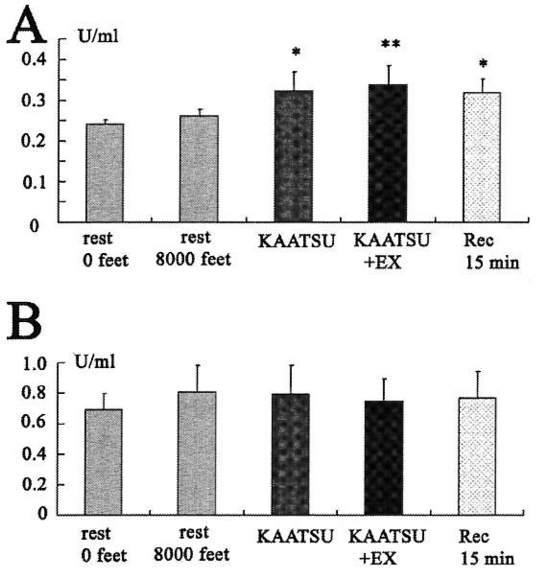

Figure 3. Effects of KAATSU only and low-intensity aerobic exercises combined with KAATSU on tPA activity (A) and PAI-1 activity (B) under the hypobaric condition (8000 feet). All values are means ± S.E.M. obtained from 6 subjects. * p<0.05, ** p<0.01 vs. rest (0 feet)

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 ± 4.9 bpm at rest to 107.1 ± 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 ± 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 ± 0.1 g/ml at rest and 2.3 ± 0.1 ng/ml immediately after the exercise). On the other hand, tPA antigen increased from 2.1 ± 0.1 ng/ml to 2.7 ± 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.

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

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 ± 0.7**		0.2 ± 0.9	
PV	(–)	–	–5.6 ± 1.0**	**	–0.5± 0.8	**	2.7 ± 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	

* 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

Parameter		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

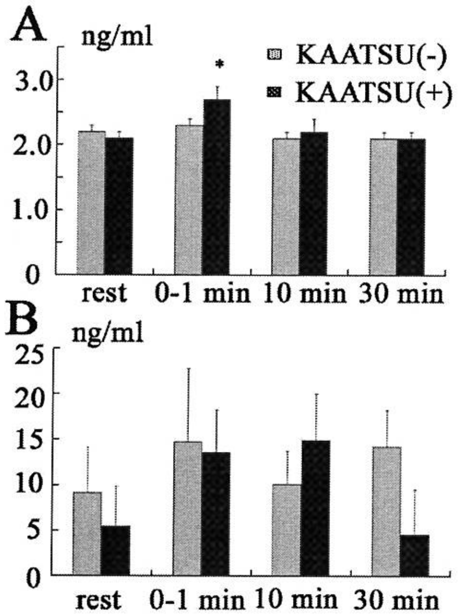

Figure 4. Effects of leg press exercises combined with and without KAATSU on tPA antigen (A) and PAI-1 antigen (B) after 24 h at -6° bed rest. The serum concentration of tPA and PAI-1 antigen in control rest (24 h bed rest), immediately after exercises (0-1 minute), 10 and 30 minutes after exercises are shown in leg press exercises with KAATSU and without. All values are means ± 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 exertion-related 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 SpO_2 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 with KAATSU on markers of coagulation and fibrinolysis

Parameters	Types of exercises	
	low-intensity aerobic exercises (8000 feet)	leg press exercises (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 SpO_2 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, SpO_2 markedly decreased to approximately 90%. However, tPA activity at rest (8000 feet) was not statistically different from rest (0 feet) (0.24 ± 0.01 vs. 0.26 ± 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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