

Effects of Resistance Training on Arterial Compliance and Plasma Endothelin-1 Levels in Healthy Men

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Summary

Arterial compliance (AC) is an index of the elasticity of large arteries. Endothelial dysfunction has been reported to result in reduced arterial compliance, which represents increased arterial stiffness. A reduction in AC is elicited by high-intensity resistance training, however the mechanisms are obscure. Because a single bout of resistance exercise causes a transient increase in circulating plasma endothelin-1 in humans, some vasoconstrictors may play a role in the mechanisms. The present study aimed to investigate whether resistance training-induced decrease in AC is associated with changes in circulating vasoconstrictors levels in young men. Young sedentary men were assigned to control (n=5) or training (n=9) groups. The training group performed four-week high-intensity resistance training (weight training exercise; three sessions/week). We measured AC and plasma levels of endothelin-1, angiotensin II, and norepinephrine before and after intervention. Resistance training significantly decreased AC, whereas the changes in plasma levels of neither endothelin-1, nor angiotensin II, nor norepinephrine were significantly different between the control and the training groups. Moreover, we found no significant correlations between changes in circulating plasma levels (endothelin-1, angiotensin II, and norepinephrine) and in the AC. Despite of no alteration of the resting circulating plasma levels (endothelin-1, etc.), we cannot exclude a possibility that the tissue/local concentrations of vasoconstrictors (endothelin-1, etc.) around the vessels might be increased and also involved in a reduction of AC in the training group. Taken together, the present results suggest that circulating vasoconstrictors (endothelin-1, etc.) in plasma are not involved in a reduction in AC by the resistance training.

Key words

Resistance training • Carotid arterial compliance • Vasoconstrictor
• Young men • Endothelin • EDCF

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Introduction

Arterial compliance (AC) is an index of the elasticity of large arteries. It is used as an indicator of arterial stiffness. Endothelial dysfunction has been reported to result in reduced AC, which represents increased arterial stiffness (Marti *et al.* 2012). Endothelin-1 (ET-1) is an endothelium-derived potent vasoconstrictive peptide with vasoproliferative activity (Barton and Yanagisawa 2008, Davenport *et al.* 2016, Houde *et al.* 2016, Miyauchi *et al.* 1990, Miyauchi and Goto 2013, Yanagisawa *et al.* 1988). Therefore, it is speculated that ET-1 released from vascular endothelium may be involved in alteration of AC in various physiological/pathophysiological conditions in humans. However, only few studies were reported concerning the alteration of AC and ET-1 in humans. Moreover, there is no report concerning the relations between alteration of AC and ET systems by resistance training in humans.

As for the alteration of AC and exercise/training, it has been reported that there is a differences between the

aerobic exercise and the resistance exercise training (Collier *et al.* 2008). It was reported that aerobic exercise training increases AC in young normotensive subjects (Seals *et al.* 2003). The aerobic exercise training has also been shown to slow or lessen the progression of arterial stiffness associated with aging (Tanaka *et al.* 2000). On the contrary to the aerobic exercise, Collier *et al.* (2008) reported that resistance training decreases AC in the pre-hypertensive humans. Thus, there is a difference between the aerobic and the resistance training in the alteration of AC. On the other hands, Collier *et al.* (2008) also reported that the resistance training decreased significantly blood pressure (BP) to similar extent to that by the aerobic exercise in the pre-hypertensive humans, suggesting that both the resistance training and the aerobic exercise training are beneficial in lowering BP in the pre-hypertensive humans (Collier *et al.* 2008). Thus, the resistance exercise is recommended as a complement kinesiotherapy to an aerobic training kinesiotherapy-program (Pescatello *et al.* 2004, Pollock *et al.* 2000) and offers many benefits for the aging population, including the prevention and treatment of hypertension, diabetes mellitus, metabolic syndrome, osteoporosis and sarcopenia (Eves and Plotnikoff 2006, Garber *et al.* 2011, Hurley and Roth 2000, Layne and Nelson 1999, Fielding 1995, Miller *et al.* 1994, Pollock *et al.* 2000, Williams *et al.* 2007). It has also been reported that the high-intensity resistance training decreases AC in healthy men (Miyachi *et al.* 2004, Miyachi 2013).

ET-1 is a potent vasoconstrictor peptide with vasoproliferative activity (Barton and Yanagisawa 2008, Davenport *et al.* 2016, Houde *et al.* 2016, Miyauchi *et al.* 1990, Miyauchi and Goto 2013, Yanagisawa *et al.* 1988). Generally, various effects of ET-1 are mediated via the ET type A receptor (ET_A) and the ET type B receptor (ET_B) which were the G protein-coupled receptors (Barton and Yanagisawa 2008, Davenport *et al.* 2016, Houde *et al.* 2016, Miyauchi and Goto 2013). Angiotensin II (Ang II) and norepinephrine (NE) also have a potent vasoconstrictive effect (Jiang *et al.* 2000, Miyauchi *et al.* 1990). We previously reported that the plasma ET-1 levels were increased in humans at various pathophysiological situations such as myocardial infarction (Miyauchi *et al.* 1989), renal failure (Miyauchi *et al.* 2012), and pulmonary hypertension (Ishikawa *et al.* 1995). We previously also demonstrated that an application of systemic ET_A/ET_B receptor antagonists or systemic α -adrenergic blockers increase AC in humans (Maeda *et al.* 2009, Sugawara *et al.* 2009). Moreover,

a single bout of resistance exercise (concentric exercise) causes a transient increase in circulating ET-1 in humans (Okamoto *et al.* 2008). Thus, it is likely that these vasoconstrictors (ET-1, etc.) may contribute to decreasing AC caused by the resistance training. However, there is no report concerning the relation between alteration of AC and ET systems caused by resistance training in humans. Therefore, to answer this question, the present study aimed to investigate whether resistance training-induced decrease in AC is associated with changes in circulating plasma vasoconstrictors levels (ET-1, Ang II, and NE) in young men.

Methods

Subjects

A total of 14 healthy men (age 21-35 years) were enrolled in a longitudinal study. They were assigned to the control (n=5) and training groups (n=9). All subjects were recruited from the local community through flyers, e-mails, and information sharing. None of the subjects had participated in any resistance or endurance training regularly. All subjects were non-smokers and cardiovascular disease-free and not taking cardiovascular medications. The subjects were instructed to maintain current eating behaviors for the duration of the intervention. The present study was conducted in accordance with the Declaration of Helsinki and was approved by the ethical committee of the University of Tsukuba (Tai 27-69: permitted on October 13, 2015). All subjects provided written informed consent.

Experimental design

We measured hemodynamics, muscle strength, AC, and plasma vasoconstrictors levels (ET-1, Ang II, and NE) before and after the intervention of the resistance training for four weeks in the exercise group. As the resistance training, the weight training exercise of the biceps curls were performed (75 % of one-repetition maximum [1RM], 10 repetitions/set, five sets/session, three sessions/week) for four weeks in the exercise group in the present study. In the control group, we also measured hemodynamics, muscle strength, AC, and plasma vasoconstrictors levels (ET-1, Ang II, and NE) before and at the end of the four-week study.

Measurements

Before each test, subjects abstained from caffeine and fasted for at least 12 h. All subjects were

studied at least 48 h after they last exercised, to avoid acute effects of exercise. All measurements were performed at constant room temperature (23–25 °C), in a quiet room, after the subjects had rested in a supine position for at least 15 min. The subjects were tested at the same time of the day throughout the study period to avoid potential diurnal variations.

Strength testing

The maximal muscular strength of all subjects was tested before and after intervention using biceps curls. All subjects performed warm up exercise, constituting 10 repetitions with 5 kg weights, and afterwards, 1RM was obtained based on the established guideline (Beachle *et al.* 2008). All repetitions of maximal strength test were performed in 3-s eccentric (lowering) and 3-s concentric (lifting) phases. Subjects repeated the actions at approximately constant velocities and frequencies with the aid of a metronome. The day-to-day coefficient of variation was 1.0 ± 1.8 % for 1RM.

AC (indicator of arterial distensibility)

Common carotid artery echography immediately after applanation of tonometrically obtained arterial pressure from the carotid artery permits noninvasive determination of dynamic AC. Common carotid artery diameter was measured using images derived from an ultrasound device (Logiq E; GE Healthcare, Tokyo, Japan) equipped with a high-resolution linear array transducer as previously described (Lage *et al.* 1993, Miyachi *et al.* 2004). A longitudinal image of the cephalic portion of the common carotid artery was acquired 1–2 cm distal to the carotid bulb. Computer images were analyzed using image analysis software (Image J, Maryland, USA). All image analyses were performed by the same investigator. The arterial lumen diameter at minimal diastolic relaxation and maximal systolic expansion was measured at three points per frame, with the points then being averaged. Carotid arterial pressure waveforms were obtained with arterial applanation tonometry using an array of 15 micro-piezoresistive transducers (form PWV/ABI; Colin Medical Technology, Komaki, Japan) (Cortez-Cooper *et al.* 2003). These waveforms were calibrated by equating the mean arterial pressure and diastolic BP of the carotid to those of the brachial artery with each parameter being averaged over 10–15 continuous beats. Brachial BP was measured with the oscillometric method using the automated polygraph apparatus (form PWV/ABI; Colin

Medical Technology, Komaki, Japan). Heart rate was computed from ECG. The AC was calculated using the equation $[(D1 - D0)/D0]/[2(P1 - P0)]\pi(D0)^2$, where D1 and D0 are the maximal and minimum diameters and P1 and P0 are the highest BP and lowest BP, respectively (Miyachi *et al.* 2004). The daily coefficient of variation of AC was 6.8 ± 2.7 % in our laboratory.

Measurement of plasma vasoconstrictors (ET-1, Ang II, and NE) levels

Each blood sample was placed in a chilled tube containing EDTA (2 mg/ml) and then centrifuged at $3,000 \times g$ for 15 min at 4 °C. The plasma samples were stored at -80 °C until the assay. Plasma concentrations of ET-1 were measured by an enzyme-linked immunoassay kit (R&D systems, Minnesota, USA). Plasma concentrations of Ang II were measured by an enzyme-linked immunoassay kit (Enzo Life Sciences, New York, USA). The intra-assay coefficients of variation of ET-1 and Ang II were 7.5 ± 7.1 % and 2.6 ± 2.4 % in our laboratory, respectively. Plasma concentrations of NE were assayed by high-performance liquid chromatography (Tosoh Co, Tokyo, Japan). The intra-assay coefficient of variation of NE was 6.0 ± 1.0 %, as previously described (NE, CA test TOSOH, Tosoh Co).

Body composition

Anthropometric measurements were taken with subjects' barefoot and wearing only light clothing. Height was measured to the nearest 0.1 cm using a stadiometer (AD-6227R, A&D Co., Ltd., Tokyo, Japan). Body mass and body fat percentage were measured to the nearest 0.1 kg on a calibrated digital scale (InBody 770, InBody Japan, Tokyo, Japan) and adjusted for the estimated clothing mass by subtracting 0.5 kg. Body mass index (BMI) was calculated as weight/height^2 (kg/m²). Daily coefficients of variation for the two trials were 0.2 ± 0.1 %, 0.5 ± 0.2 %, and 3.4 ± 2.7 % for body mass, BMI, and body fat percentage in our laboratory, respectively.

Resistance training intervention

Subjects in the training group performed the resistance exercise of the weight training exercise (biceps curls) three times per week throughout the 4-week study period. During training sessions, subjects performed biceps curls for five sets of 10 repetitions at 75 % of 1RM with an inter-set rest period of 2 min (Okamoto *et al.* 2009a, Okamoto *et al.* 2015). We selected biceps curls

because of their similarity to whole-body resistance training in terms of inducing an increase in arterial stiffness (i.e. decrease in AC) (Kawano *et al.* 2006, Miyachi *et al.* 2004, Okamoto *et al.* 2009a, Okamoto *et al.* 2015). The eccentric (lowering) and concentric (lifting) phases of all repetitions were each performed for 3 s (Okamoto *et al.* 2009a). The subjects repeated the actions at approximately constant velocities and frequencies with the aid of a metronome. Those who were able to complete 10 repetitions in the third set were given increased loads during subsequent exercise sessions. Each training session lasted for approximately 20 min. The resistance exercise was performed until concentric failure, afterward remaining sets were completed with the support of assistants. Except for routine activities during training, all other exercises (resistance training and anaerobic and aerobic exercises) were prohibited.

Statistical analysis

All data were expressed as means \pm standard deviations of the mean unless otherwise indicated. The Shapiro-Wilk tests were used to evaluate the normal distribution. The unpaired sample t-tests or Mann-Whitney U-tests were used to examine significant differences in subject characteristics between the two groups (the control and training groups). A two-way analysis of variance with repeated measures was used to analyze hemodynamics, muscle strength, AC, and plasma levels of ET-1, Ang II, and NE. When a significant interaction was observed, the simple main effects of group or time were determined. Correlations between changes in the AC and in the plasma levels of ET-1, Ang II, or NE were assessed using Spearman's rank correlation coefficient (r_s) across all subjects. In all analyses, a two-tailed P value of <0.05 was determined

to be statistically significant. Statistical analyses were conducted using SPSS software version 24.0 for Windows (IBM SPSS Japan Inc., Japan).

Results

In the training group, all subjects completed every training session (three sessions/week for four weeks; i.e. a total of 12 training sessions during the four weeks). At the baseline just before starting the intervention (training), no significant differences were found in any of the parameters between the control and training groups such as body mass index, body fat (%), plasma ET-1, plasma Ang II, plasma NE and the AC (Tables 1 and 2, Figs 1 and 2).

Before intervention, plasma levels of ET-1 in the training group tended to be higher than those in the control group. However, it was not statistically significant ($P=0.13$). Moreover, before the intervention, plasma levels of NE in the training group also tended to be higher than those in the control group, however it was not statistically significance ($P=0.26$). Thus, at the baseline levels just before starting the training, there were no significant differences in circulating plasma vasoconstrictor levels (ET-1, Ang II, and NE) between the control and training groups.

The interactions were significant on neither the BP nor the heart rate in between the control and training groups (Table 2); that is, neither the BP (systolic BP, mean BP, and diastolic BP) nor the heart rate changed at the end of the four-week study in the control group (Table 2), and neither the BP (systolic BP, mean BP, and diastolic BP) nor the heart rate changed at the end of the four-week study in the training group. On the other hands, we found significant interaction in the 1RM between the two groups ($F=42.61$, $P<0.05$); that is, the

Table 1. Baseline characteristics of subjects.

Variables	Control	Training	<i>P</i> -value
<i>Number of subjects</i>	5	9	-
<i>Age, years</i>	24 \pm 3	25 \pm 4	0.90
<i>Height, cm</i>	173 \pm 6	174 \pm 7	0.75
<i>Body mass, kg</i>	76.3 \pm 11.6	69.3 \pm 13.6	0.11
<i>Body mass index, kg \cdot m⁻²</i>	25.6 \pm 4.0	23.0 \pm 4.4	0.29
<i>Body fat, %</i>	19.9 \pm 6.9	20.3 \pm 9.4	0.94

Values are means \pm SD.

Table 2. Changes in hemodynamics and muscle strength.

Variables/Group	Time-point		Interaction
	Baseline	After intervention	
<i>Systolic BP, mm Hg</i>			
Control	116 ± 12	115 ± 8	$F = 0.05$
Training	122 ± 6	121 ± 8	$P = 0.83$
<i>Mean BP, mm Hg</i>			
Control	83 ± 9	85 ± 7	$F = 0.31$
Training	90 ± 6	91 ± 7	$P = 0.59$
<i>Diastolic BP, mm Hg</i>			
Control	65 ± 7	65 ± 4	$F = 0.04$
Training	73 ± 7	73 ± 4	$P = 0.86$
<i>Heart rate, bpm</i>			
Control	55 ± 3	52 ± 5	$F = 1.58$
Training	62 ± 8	62 ± 5	$P = 0.23$
<i>1RM biceps curls, kg</i>			
Control	23 ± 3	23 ± 4	$F = 42.61$
Training	20 ± 2	23 ± 2*	$P < 0.05$

Values are means ± SD. BP, blood pressure; 1RM, one repetition maximum. * $P < 0.05$ vs. baseline. Control group: $n=5$; Training group: $n=9$.

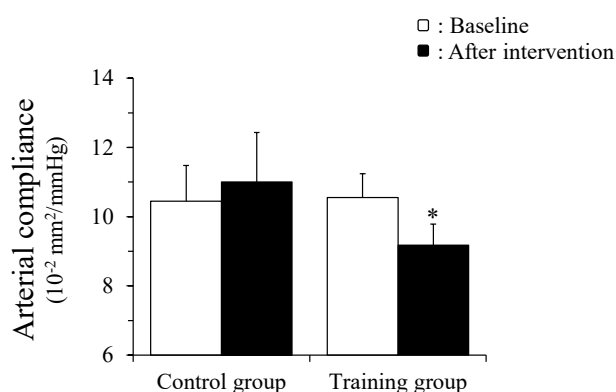


Fig. 1. The AC before and after the four-week study in the control group ($n=5$) and the training groups ($n=9$). Two-way ANOVA reveals a significant interaction on AC. * $P < 0.05$: significant difference compared with the baseline in the training group using *post-hoc* analysis. Data are presented as mean ± SE. AC, arterial compliance; ANOVA, analysis of variance.

1RM was significantly increased after the intervention of the four-week training in the training group ($P < 0.05$), whereas the 1RM did not change at the end of the four-week study in the control group (Table 2).

After the intervention, we found a significant interaction on changes in the AC between the groups using a two-way analysis of variance with repeated measures [$F_{(1, 12)} = 10.46$, $P < 0.05$] which was followed by *post-hoc* test. The *post-hoc* test showed that the AC of the

training group was significantly decreased at the end of the four-week study (Fig. 1, from 10.6 ± 2.1 to 9.2 ± 1.8 10^{-2} mm²/mm Hg, $P < 0.05$), whereas the AC of the control group did not change at the end of the four-week study (Fig. 1, from 10.5 ± 2.3 to 11.0 ± 3.2 10^{-2} mm²/mm Hg, $P = \text{N.S.}$).

Figure 2 shows the plasma vasoconstrictors (ET-1, Ang II, and NE) levels before and after the four-week study. The interactions of each value at the respective time-point (each value at Baseline, After intervention) and the groups (Control group, Training group) on the plasma vasoconstrictors (ET-1, Ang II, and NE) levels were not significant (ET-1: $F_{(1, 12)} = 0.83$, $P = \text{N.S.}$; Ang II: $F_{(1, 12)} = 0.30$, $P = \text{N.S.}$; NE: $F_{(1, 12)} = 1.03$, $P = \text{N.S.}$), that is, the plasma vasoconstrictors (ET-1, Ang II, and NE) levels did not change at the end of the four-week study in the training group and the plasma vasoconstrictors (ET-1, Ang II, and NE) levels did not change at the end of the four-week study in the control groups (Fig. 2).

The abscissa of the Figure 3 shows the changes of the plasma levels of ET-1 (upper), Ang II (middle), and NE (lower) before and after the four-week intervention in the control group and the training group. The ordinate of the Figure 3 shows the changes of the AC before and after the four-week intervention in the control

group and the training group. We found no significant correlations between the changes (before and after the intervention) in plasma vasoconstrictors (ET-1, Ang II, and NE) levels and the changes in the AC in the control group and the training group (ET-1: $r_s=0.29$, $P=0.32$; Ang II: $r_s=-0.002$, $P=0.99$; NE: $r_s=-0.25$, $P=0.39$) (Fig. 3).

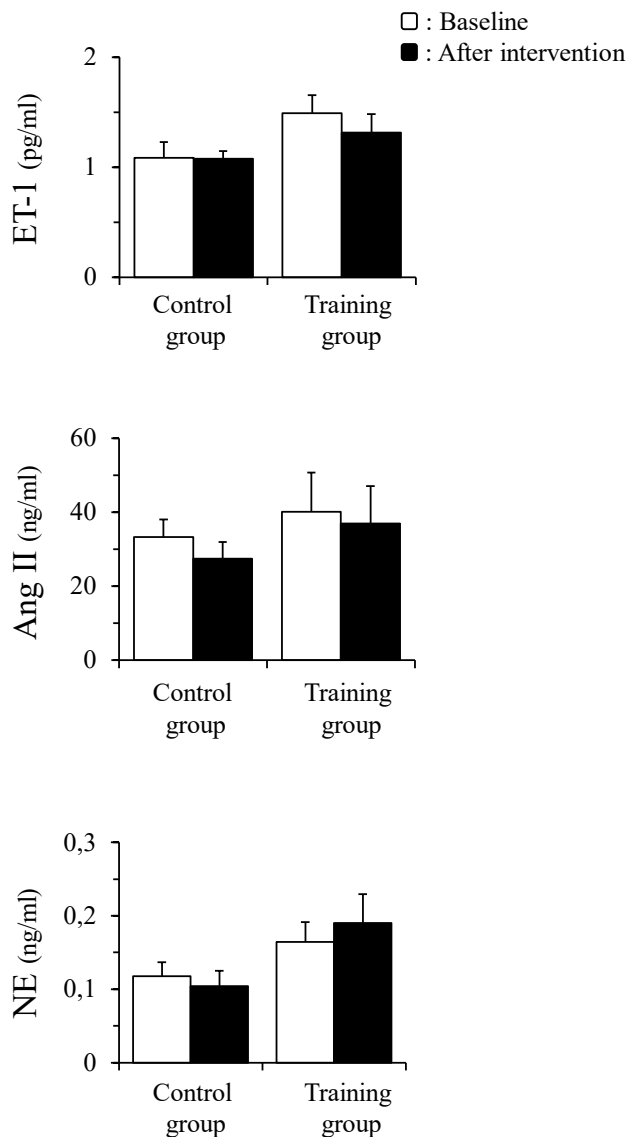


Fig. 2. The plasma vasoconstrictors (ET-1, Ang II, and NE) levels before and after the four-week study in the control group ($n=5$) and the training group ($n=9$). The interactions of each value at the respective time-point (each value at Baseline, After intervention) and the groups (Control group, Training group) on the plasma vasoconstrictors (ET-1, Ang II, and NE), determined using two-way ANOVA, were not significant; that is, the plasma vasoconstrictors (ET-1, Ang II, and NE) levels did not change at the end of the four-week study in the training group and also in the control groups. Data are presented as mean \pm SE. ET-1, endothelin-1; Ang II, angiotensin II; NE, norepinephrine; ANOVA, analysis of variance.

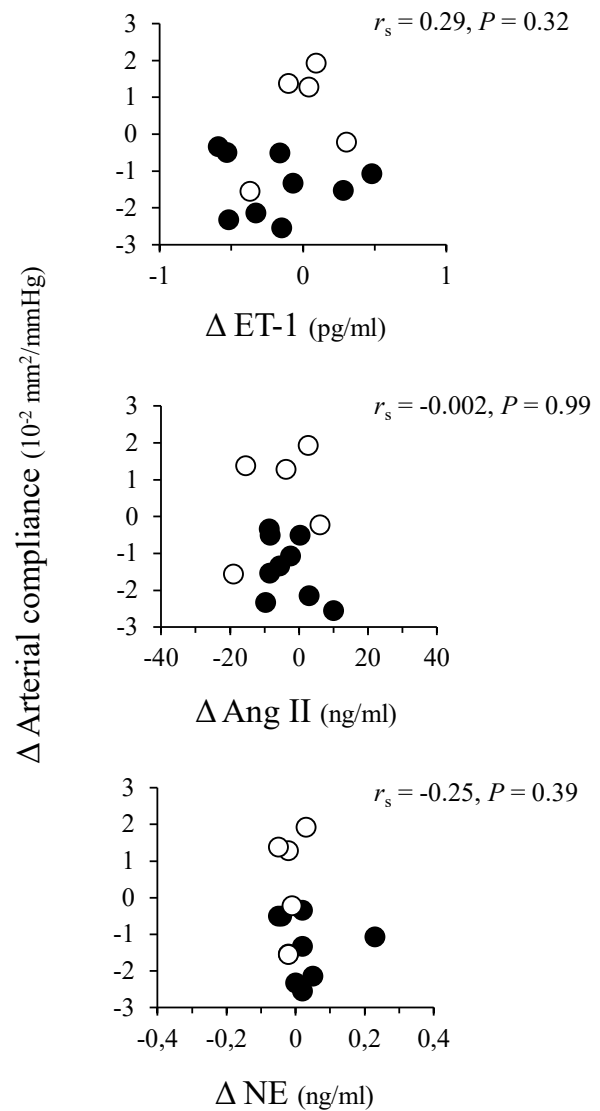


Fig. 3. The abscissa shows the changes of the plasma levels of ET-1 (upper), Ang II (middle), and NE (lower) before and after the four-week intervention in the control group ($n=5$) and the training group ($n=9$). The ordinate shows the changes of the AC before and after the four-week intervention in the control group and the training group. The correlations between the changes in the AC and the changes in three types of plasma vasoconstrictors (ET-1, Ang II, and NE) levels before and after the intervention are shown in this Figure. There were no significant correlations between the changes in plasma vasoconstrictors (ET-1, Ang II, and NE) levels and the changes in the AC before and after the intervention assessed by the Spearman rank correlation coefficient analysis. The control (○) and training (●) groups are shown. ET-1, endothelin-1; Ang II, angiotensin II; NE, norepinephrine; AC, arterial compliance.

Discussion

It has been reported that a single bout of resistance exercise (concentric exercise) caused a transient increase in circulating plasma ET-1 in humans (Okamoto *et al.* 2008). In the present study, the training group performed four-week resistance training (weight

training exercise of biceps curls, three sessions/week). Therefore, it is thought that the repeated bout of the resistance exercise might cause a repeated increase in plasma ET-1 as well as a repeated increase in the tissue ET-1 around the vessels during the four-week intervention of the resistance training. In the present study, the resistance training in young men significantly decreased the AC (indicator of arterial distensibility), which was in accordance with previous reports concerning the resistance exercise training and the reduction in the AC (Collier *et al.* 2008, Miyachi *et al.* 2004, Miyachi 2013). The decrease in the AC represents an increase in the arterial stiffness. Because ET-1 has potent vasoconstrictive activity and vasoproliferative activity, it was possible that a repeated increase in the tissue ET-1 around the vessels in the training group might cause a repeated over-stimulation of the ET receptors (ET_A and ET_B) and hence might affect a vascular structure and might result in contributing to a decrease in AC in the training group of the present study.

The present study showed that the four-week resistance training in young men significantly decreased the AC, whereas the resistance training did not change the basal circulating vasoconstrictors (ET-1, Ang II, and NE) levels. However, we cannot exclude a possibility that, at the basal condition after the four-week exercise training, the tissue/local ET-1 concentrations around the vessels may be increased and hence involved in a reduction of AC induced by the four-week resistance training. Indeed, there are several reports in which there is a difference between plasma and tissue ET-1 levels: the tissue ET-1 are elevated whereas the circulating plasma ET-1 levels are not changed in some conditions in the animal models of various diseases. In the deoxycorticosterone (DOCA)-salt hypertensive rats, the tissue ET-1 content of the thoracic aorta and mesenteric artery was significantly higher than that of the control normotensive rats, whereas the plasma ET-1 levels did not differ between the two groups (Wang *et al.* 2005). Therefore, similar to the case of the DOCA-salt hypertensive rats, it has a possibility that the tissue/local vascular-regulatory system by ET-1 may be enhanced in the training groups of our study, although the current resistance training did not change the resting circulating plasma levels of ET-1 in the young men. Taken together, the present results suggest that circulating plasma vasoconstrictors (ET-1, etc.) are not involved in a reduction in AC by the high-intensity resistance training.

As for the aerobic exercise and plasma ET-1, we previously reported that a single bout of aerobic exercise causes a transient increase of the plasma ET-1 levels in humans (Maeda *et al.* 1994, Maeda *et al.* 1997). It has been reported that a single bout of resistance exercise (concentric exercise) causes a transient increase in circulating plasma ET-1 in humans (Okamoto *et al.* 2008). Therefore, it is thought that, regardless of types of exercise (the aerobic exercise or the resistance exercise), a single bout of exercise causes a transient increase of the plasma ET-1 levels in humans. Moreover, using animal models of exercise, we previously reported that a single bout of exercise causes an organ-specific increase in ET-1 production in some organs such as the heart (Maeda *et al.* 1998a) and the kidney (Maeda *et al.* 1998b), but not the lung (Maeda *et al.* 1998a, Maeda *et al.* 1998b) in the rats. Therefore, it is considered that endogenous ET-1 may contribute to regulation of the cardiovascular systems under the exercise in both humans and animals.

A previous study has reported that circulating plasma ET-1 was increased about 1.4-fold immediately after a single bout of resistance exercise (Okamoto *et al.* 2008). We previously reported that high-intensity cycle ergometer exercise increased about 3-fold circulating plasma ET-1, and the increase remained until 60 min after exercise (Maeda *et al.* 1994, Maeda *et al.* 1997).

It has been reported that the stimulation of the ET receptors (ET_A and ET_B) causes an stimulation of the intracellular signal-transduction systems such as G protein, protein kinase C (PKC), Rho kinase, mitogen-activated protein kinase (MAP kinase) (Barton and Yanagisawa 2008, Davenport *et al.* 2016, Houde *et al.* 2016, Miyauchi and Goto 2013), therefore, it is possible that the ET receptors-intracellular signal-transduction systems may be changed after the four-week exercise training in the training groups; that is, it has a possibility that a repeated increase in the tissue ET-1 around the vessels by the resistance training during four weeks may cause a repeated over-stimulation of the vascular ET receptors (ET_A and ET_B) during the four weeks, therefore may result in an alteration of the ET receptors (ET_A and ET_B) – intracellular signal transduction systems (G protein, PKC, Rho kinase, MAP kinase, etc.) of the vascular smooth muscle cells in the young men with resistance training at the basal condition without changing the basal circulating plasma vasoconstrictors (ET-1, etc.) levels in the training group. Therefore, we cannot exclude a possibility that the four-week exercise might cause a change of the vascular ET receptors-

intracellular signal transduction systems and hence might cause a decrease in the AC in the exercise group.

As for Ang II, there are also several reports in which there is a difference between circulating and tissue Ang II levels: the tissue Ang II levels are elevated whereas the circulating Ang II levels are not changed in some conditions in the animal models of various diseases. In spontaneously hypertensive rats (SHR) at the age of 14-week (typical hypertensive stage), the tissue Ang II content of the kidney was significantly higher than that of the age-matched Wistar-Kyoto rats, whereas the plasma Ang II levels did not differ between the two groups (Kobori *et al.* 2005). Therefore, similar to the case of the SHR rats, it has a possibility that the tissue/local vascular-regulatory system by Ang II may be enhanced in the training groups of our study, although the resistance training did not change the resting circulating plasma levels of Ang II in the young men.

It is well known that NE-release from the sympathetic nerve endings to the vessels is greatly increased during resistance exercise (Kraemer *et al.* 1999). In the present study, the subjects of the training group performed four-week resistance training (biceps curls, three sessions/week). Therefore, it is thought that the repeated bout of the resistance exercise might cause a repeated increase in the tissue NE around the vessels during four weeks. Because NE has vasoproliferative activity and vasocontractile activity *via* the $\alpha 1$ adrenalin receptors (Piascik and Perez 2001), it was speculated that a repeated increase in the tissue NE around the vessels in the training group during the four-week intervention might affect a vascular structure and this might contribute to a decrease in AC in the training group of the present study. On the other hand, the present data showed that the resting circulating plasma NE levels were not changed by the intervention of the four-week resistance training in the young men.

In general, the exercise training has different effects on the BP in the hypertensive and normotensive subjects; the exercise training causes BP decrease in the hypertensive subjects, whereas the exercise training does not cause BP decrease in the normotensive subjects, suggesting that baseline BP values affect BP changes elicited by exercise training. One meta-analysis demonstrated that the resistance training decreases systolic BP of pre-hypertensive humans, whereas it does not change that of normotensive humans (Cornelissen and Smart 2013). Collier *et al.* (2008) also reported that the resistance training decreased significantly BP to similar

extent to that by the aerobic exercise in the pre-hypertensive humans. In the JNC-7 report (The Seventh Report of the Joint National Committee on Hypertension), the pre-hypertension has been defined as systolic BP of 120-139 mm Hg or diastolic BP of 80-89 mm Hg, and the stage-1 hypertension has been defined as systolic BP of 140-159 mm Hg or diastolic BP of 90-99 mm Hg (Chobanian *et al.* 2003). In the above report by Collier *et al.* (2008), systolic BP and diastolic BP of the subjects are 136.4 ± 3.4 and 78.2 ± 1.5 , respectively. On the other hand, the subjects of the present study were normotensive (systolic BP 122 ± 6 mm Hg, diastolic BP 73 ± 7 mm Hg), and the current exercise intervention did not change the level of the BP in the healthy men. Thus, it is possible that a difference in the baseline BP values by our study (normotensive subjects) and the Collier's study (pre-hypertensive subjects) may be a major reason for the difference in change in BP by resistance training. Our consideration is also consistent with the reports that high-intensity resistance training does not change systolic BP in the normotensive humans (Miyachi *et al.* 2004, Okamoto *et al.* 2009b).

It has been reported that the circulating ET-1 is cleared mainly *via* the lung and the kidneys (De Nucci *et al.* 1989, Burkhardt *et al.* 2000, Staniloae *et al.* 2004, Langleben *et al.* 2006, Miyauchi and Goto 2013). Moreover, it has been reported that endogenous ET-1 is cleared *via* the endothelial ET_B (Berthiaume *et al.* 2000, Burkhardt *et al.* 2000, Miyauchi and Goto 2013, Angus *et al.* 2017). When ET-1 is intravenously administered as a bolus, it disappears quite rapidly from the blood stream with a half-life of a few minutes (Miyauchi and Goto 2013). The lung appears to be one of the most important tissues for uptake, since approximately 60 % of ET-1 is removed after a single passage through the pulmonary circulation (De Nucci *et al.* 1989, Burkhardt *et al.* 2000, Staniloae *et al.* 2004, Langleben *et al.* 2006, Miyauchi and Goto 2013). In the lung tissues, ET_B is highly expressed and ET-1-ET_B complex may be taken up into cells through an internalization process and degraded by lysosomal enzymes, e.g. aspartic proteases (Berthiaume *et al.* 2000, Burkhardt *et al.* 2000, Miyauchi and Goto 2013, Angus *et al.* 2017). Neutral endopeptidase and lysosomal cathepsin G are also concerned with enzymatic degradation of ET-1 (Burkhardt *et al.* 2000, Miyauchi and Goto 2013). Thus, the circulating ET-1 level is considered to be determined by the balance between the synthesis of ET-1 by endothelium and the clearance by

the organs (such as the lungs and the kidneys). We should point out that the ET-1 clearance studies in men have been limited, and that no clearance studies were performed in the present study. It has a possibility that the exercise intervention of the current study could alter the clearance of the circulating ET-1 in the healthy men. However, we did not measure this parameter and this might be a limitation of our study.

In the present study, we also have the following limitations. In the first, the sample power in the present study was 0.84. Because it is considered that a power should be more than 0.80 and an α level should be less than 0.05 in the statistical evaluation for the differences (Cumming 2014), the present sample power (0.84) would meet this criteria and therefore it was considered that the present study would be in adequate sample size. Nevertheless, because the sample size of the present study was not large, larger sample size study will be needed to confirm our findings. Thus, it has a possibility that larger sample size study will result in the different conclusion from the current manuscript, and therefore, we write this possibility as a study limitation of current study. In the second, muscle strength may have improved with exercise intervention in the training group. However, muscle strength (evaluated by one repetition maximum) was not different from that of the control group after the intervention, so the muscle strength appears not to be different at the time when the plasma data were collected. This may invalidate any interpretation, and we consider this as a study limitation.

In conclusion, the present study showed that, in young men, the high-intensity resistance training of four-week periods caused a significant reduction of the AC (indicator of arterial distensibility) without changing the resting circulating vasoconstrictors (ET-1, Ang II, and NE) levels. The decrease in the AC represents an increase

in the arterial stiffness. Despite of no alteration of the resting circulating ET-1 levels, the present data cannot exclude a possibility that, at the basal condition after the four-week exercise training, the tissue/local ET-1 concentrations around the vessels may be increased and hence involved in a reduction of AC induced by the four-week resistance training without changing the resting circulating ET-1 levels in plasma. Moreover, because a single bout of resistance exercise (concentric exercise) causes a transient increase in circulating ET-1 in humans (Okamoto *et al.* 2008), it seems to be likely that a repeated increase in the tissue ET-1 around the vessels in the present training group might cause a repeated over-stimulation of the ET receptors (ET_A and ET_B) during the four-week intervention and hence might affect a vascular structure and might result in contributing to a decrease in the AC in the training group *via* its strong vasoconstrictive/vasoproliferative actions of ET-1. Taken together, the present results suggest that circulating plasma vasoconstrictors (ET-1, etc.) are not involved in a reduction in the AC by the high-intensity resistance training in young men.

Conflict of Interest

There is no conflict of interest.

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