Aerobic Exercise Training Decreases Plasma Asymmetric Dimethylarginine Concentrations With Increase in Arterial Compliance in Postmenopausal Women

Koichiro Tanahashi, Nobuhiko Akazawa, Asako Miyaki, Youngju Choi, Song-Gyu Ra, Tomoko Matsubara, Hiroshi Kumagai, Satoshi Oikawa, and Seiji Maeda

BACKGROUND
Asymmetric dimethylarginine (ADMA), an endogenous inhibitor of nitric oxide synthase, increase with advancing age and menopausal status. ADMA plays a role in endothelial dysfunction and increasing arterial stiffness. Regular aerobic exercise has a positive effect on arterial function (i.e., arterial compliance). This study investigated the effects of aerobic exercise training on plasma ADMA concentrations and arterial compliance in postmenopausal women.

METHODS
Thirty postmenopausal women were divided into 2 groups: an exercise group (n = 20) and a control group (n = 10). Subjects in the exercise group completed 12 weeks of aerobic exercise training (65%–80% of maximal heart rate, 40–60 min/day, 3–6 days/week). Before and after each intervention, plasma ADMA concentrations and carotid arterial compliance were measured in all participants.

RESULTS
The baseline plasma ADMA concentrations, carotid arterial compliance, and most other key dependent variables did not differ between the 2 groups. In the exercise group, carotid arterial compliance significantly increased after exercise intervention (P < 0.01), and plasma ADMA concentrations significantly decreased (P < 0.05). In addition, changes in carotid arterial compliance after the exercise intervention were inversely correlated with changes in plasma ADMA concentrations (r = −0.367; P < 0.05).

CONCLUSIONS
We demonstrated that aerobic exercise training significantly decreased plasma ADMA concentrations with increase in carotid arterial compliance in postmenopausal women. These results suggest that reduction in ADMA may play an important role in the aerobic exercise training–induced increase in arterial compliance.

Keywords: aerobic exercise training; aging; arterial compliance; asymmetric dimethylarginine (ADMA); blood pressure; hypertension.

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Arterial compliance plays an important role in the vascular function (i.e., arterial buffering function of the pulsation of blood pressure and flow). Reduction in central arterial compliance is an independent risk factor for cardiovascular disease. Central arterial compliance decreases with advancing age. In addition, postmenopausal women have lower central arterial compliance than premenopausal women. We and other researchers have shown that regular aerobic exercise increases central arterial compliance in healthy, postmenopausal women. However, the precise mechanisms underlying the aerobic exercise training–induced increase in central arterial compliance are unclear.

Endothelium-derived nitric oxide (NO) is the most potent endogenous vasodilator. Endogenous NO contributes to the regulation of vascular tone, arterial compliance, and arterial stiffness. NO is synthesized from L-arginine by nitric oxide synthase (NOS) in the endothelium. Asymmetric dimethylarginine (ADMA) is an endogenous competitive inhibitor of NOS. An increase in ADMA is associated with impairment of NO synthesis. Furthermore, plasma concentrations of ADMA increase in elderly people and in postmenopausal women. However, the effect of aerobic exercise training on ADMA in healthy, postmenopausal women has not been investigated.

The purpose of this study was to examine the effects of aerobic exercise training on central arterial compliance and plasma ADMA concentrations. We hypothesized that the decrease in plasma ADMA concentrations induced by aerobic exercise training would contribute to increase in arterial compliance. In this study, we measured plasma ADMA concentrations and carotid arterial compliance before and after a 12-week aerobic exercise training intervention in postmenopausal women.

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METHODS

Subjects

Thirty apparently healthy, sedentary, postmenopausal women volunteered to participate. They were randomly assigned to the exercise group (n = 20) and the control group (n = 10) by use of a lottery system. Subjects were nonsmokers, nonobese, and free of cardiovascular disease, as assessed by medical history. None of the subjects were receiving cardiovascular-acting medications or hormone replacement therapy. All potential risks were explained to the study participants, and they provided written informed consent for participation in the study. All of the procedures were reviewed and approved by the Ethics Committee of the University of Tsukuba.

Experimental protocol

All experiments were performed in the morning after a 12-hour overnight fast. Subjects abstained from alcohol and caffeine for at least 12 hours and did not exercise for at least 24 hours before beginning the experiment to avoid the potential acute effects of exercise. Measurements were obtained in a quiet, temperature-controlled room (24–26°C). After a resting period of at least 20 minutes, carotid arterial compliance, carotid artery intima-media thickness (IMT), and arterial blood pressure were measured, and a blood sample was drawn to determine the plasma ADMA concentrations. After these procedures, oxygen uptake at the ventilatory threshold (VO2VT) was measured during an incremental cycle ergometer exercise.

Exercise training

Subjects in the exercise group were trained in aerobic exercise for >3 days per week (2–3 supervised sessions and additional home-based training) for 12 weeks. Initially, subjects cycled or walked 30 minutes per day at a relatively low intensity (60% of their individually determined maximal heart rate). As their exercise tolerance improved, the intensity (60% of their individually determined maximal heart rate) was defined as the distance between the lumen and intima of the far wall to the media–adventitia of the near wall. Pulsatile changes in the common carotid artery diameter were analyzed 1–2 cm proximal to its bifurcation. The diameter of the arterial lumen at minimal diastolic relaxation and maximal systolic expansion was measured at 3 points per frame, and the points were then averaged. Carotid arterial pressure waveforms were obtained with arterial application tonometry using an array of 15 micro-piezoresistive transducers (form PWV/ABI; Colin Medical Technology, Komaki, Japan); these waveforms were calibrated by equating the carotid mean arterial pressure and diastolic blood pressure to the brachial mean arterial pressure and diastolic blood pressure. Each parameter was averaged over 10–15 continuous beats and statistically analyzed. Arterial compliance was obtained using the following equation:

\[
\frac{D_1 - D_2}{D_2} = \frac{1}{2}\frac{P_1 - P_2}{\pi D_2},
\]

where \(D_1\) and \(D_2\) are the maximal and minimum arterial diameters, respectively, and \(P_1\) and \(P_2\) are the highest and lowest blood pressures, respectively. The day-to-day coefficient of variations for carotid arterial compliance measurement was 5.6 ± 2.8%.

Carotid artery IMT. Carotid artery IMT was measured from the images derived from the same ultrasound machine (En Visor, Koninklijke Philips Electronics) as previously described17. Carotid IMT was defined as distance from the leading edge of the lumen–intima interface. Lumen diameter was defined as the distance between the lumen and intima and a near-wall boundary, corresponding to the interface of the adventitia and media. These measurements were made at end diastole. At least 10 measurements of IMT were taken at each segment, and the mean values were used for analysis.

Plasma ADMA concentrations. Each blood sample was placed in a chilled tube containing ethylenediaminetetraacetic acid (2 mg/ml) and centrifuged at 2000 x g for 15 minutes at 4°C. The plasma was stored at –80°C until analysis. Plasma concentrations of ADMA were determined using a commercial enzyme-linked immunosorbent assay kit (Immundagnostik AG, Bensheim, Germany). The ADMA assay was performed according to the manufacturer’s instructions. The intra-assay coefficient of variation ranged 5.8%–7.9%. Standards and controls provided with the kits were used in measurements.

Aerobic capacity. VO2VT was measured during the incremental cycle ergometer exercise by using online computer-assisted circuit spirometry (AE300S; Minato Medical Science, Osaka, Japan). Before and after the intervention, all subjects underwent an incremental cycle exercise test (2 minutes at 20 W, followed by 10-W increases every 1 minute) until they felt exhausted or reached 85% of their age-predicted maximal heart rate. Each individual VO2VT was calculated using regression analysis of the slopes of carbon

Carotid arterial compliance. Subjects were studied in the supine position under quiet resting conditions. The common carotid artery was imaged using B-mode ultrasound (En Visor; Koninklijke Philips Electronics, Eindhoven, the Netherlands), equipped with a high-resolution linear-array transducer (7.5 MHz). Diameters were measured from the intima of the far wall to the media–adventitia of the near wall. Pulsatile changes in the common carotid artery diameter were analyzed 1–2 cm proximal to its bifurcation. The diameter of the arterial lumen at minimal diastolic relaxation and maximal systolic expansion was measured at 3 points per frame, and the points were then averaged. Carotid arterial pressure waveforms were obtained with arterial application tonometry using an array of 15 micro-piezoresistive transducers (form PWV/ABI; Colin Medical Technology, Komaki, Japan); these waveforms were calibrated by equating the carotid mean arterial pressure and diastolic blood pressure to the brachial mean arterial pressure and diastolic blood pressure. Each parameter was averaged over 10–15 continuous beats and statistically analyzed. Arterial compliance was obtained using the following equation:

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dioxide production, oxygen uptake, and the minute-ventilation plot.\textsuperscript{18}

**Statistical analyses**

Data are expressed as mean $\pm$ SD. On the basis of the Kolmogorov–Smirnov test for normality, all parameters were normally distributed. A 2-way analysis of variance with repeated measures was performed to identify a group (Exercise or Control) $\times$ time (before or after) interaction or main effect. When indicated by a significant main effect or interaction, specific mean comparisons were performed by paired Student $t$ test to identify significant differences within each intervention. For intergroup comparisons, an unpaired Student $t$ test was used. Univariable correlation analyses were used to determine the relations between variables of interest. The partial correlation coefficients were adjusted with parameters that a priori potentially associated with the variable of interest: age. Statistical significance was set at $P < 0.05$ for comparisons.

**RESULTS**

In the exercise group, the average frequency of the exercise training was 4.7 $\pm$ 1.2 days/week (supervised exercise training was 3.5 $\pm$ 0.9 days/week, and additional home-based training was 1.3 $\pm$ 0.8 days/week). The average time of the exercise training was 47.8 $\pm$ 17.4 minutes per day. The average of the physical activity during intervention was significantly higher in the exercise group compared with the control group (298 $\pm$ 103 kcal/day vs. 173 $\pm$ 87 kcal/day; $P < 0.01$).

Table 1 shows the characteristics of the study participants. There were no differences in age, height, body weight, body mass index, systolic blood pressure, diastolic blood pressure, carotid artery IMT, and $\text{VO}_{2\text{VT}}$ between the groups at the start of the study. Body weight decreased significantly after intervention in the exercise group ($P < 0.05$). There were no significant changes in body mass index, systolic blood pressure, diastolic blood pressure, and carotid artery IMT in either of the groups. The $\text{VO}_{2\text{VT}}$ in the exercise group increased significantly after the intervention ($P < 0.01$). There were no differences in body weight, body mass index, systolic blood pressure, diastolic blood pressure, and carotid artery IMT between the groups at after the intervention. $\text{VO}_{2\text{VT}}$ at after the intervention was significantly higher in the exercise group compared with the control group ($P < 0.01$).

There was no difference in baseline carotid arterial compliance between the exercise group and the control group (Figure 1). After the 12-week exercise intervention, carotid arterial compliance increased significantly in the exercise group ($P < 0.01$). There was no significant change in carotid arterial compliance in the control group. There were no differences in carotid arterial compliance between the groups after the intervention. There was no difference in baseline plasma ADMA concentrations between the exercise group and the control group (Figure 2). After the 12-week exercise intervention, plasma ADMA concentrations decreased significantly in the exercise group ($P < 0.05$). There was no significant change in the plasma ADMA concentrations in the control group. Plasma ADMA concentrations after the intervention was significantly lower in the exercise group compared with the control group ($P < 0.05$). As shown in Figure 3, the change in carotid arterial compliance after the intervention was inversely correlated with the change in plasma ADMA concentrations ($r = -0.367$; $P < 0.05$). After adjusting for age, the association of change in carotid arterial compliance and change in plasma ADMA concentrations was confirmed ($\beta = -0.374$; $P < 0.05$).

**DISCUSSION**

The major findings of this study were as follows: aerobic exercise training significantly increased carotid arterial compliance and significantly decreased plasma ADMA concentrations in postmenopausal women. This is the first study to

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Data are expressed as means $\pm$ SD.

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; IMT, intima-media thickness; SBP, systolic blood pressure; $\text{VO}_{2\text{VT}}$, oxygen uptake at the ventilatory threshold.

*$P < 0.05$ vs. exercise group before intervention; **$P < 0.01$ vs. exercise group before intervention; ***$P < 0.01$ vs. control group before intervention.
Tanahashi et al. show that aerobic exercise training decreases plasma ADMA concentrations in healthy, postmenopausal women and that the change in ADMA after the intervention correlated inversely with the change in arterial compliance. Therefore, the aerobic exercise training–induced decrease in plasma ADMA concentrations may contribute to the increase in arterial compliance.

ADMA is an endogenous competitive inhibitor of NOS and affects vascular function and structure. Several studies have shown a relationship between an increase in circulating ADMA levels and cardiovascular disease. Elevated plasma ADMA concentrations have been reported in patients with coronary artery disease, chronic heart failure, and hypertrophic cardiomyopathy. In addition to reports of elevated ADMA in cardiovascular disease, a previous study reported that aging and menopause cause elevations in circulating ADMA levels. In this study, we showed that aerobic exercise training decreased plasma ADMA concentrations in postmenopausal women. Therefore, our findings suggest that the aerobic exercise training–induced decrease in plasma ADMA concentrations may reduce the risk of cardiovascular disease in postmenopausal women.

In this study, we showed that aerobic exercise training decreased plasma ADMA concentrations with increase in carotid arterial compliance in postmenopausal women. ADMA inhibits endogenous NOS and impairs NO synthesis. Hence, a reduction in ADMA is associated with an increase in NO synthesis. NO has been reported to play a role in the regulation of arterial compliance and arterial stiffness. Our previous studies have demonstrated that regular aerobic exercise training increases NO production in young men and elderly women. Taken together, these findings show that aerobic exercise training decreases plasma ADMA concentrations in healthy, postmenopausal women and that the change in ADMA after the intervention correlated inversely with the change in arterial compliance. Therefore, the aerobic exercise training–induced decrease in plasma ADMA concentrations may contribute to the increase in arterial compliance.

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**Figure 1.** Carotid arterial compliance before and after intervention in postmenopausal women. Data are reported as means ± SD.

**Figure 2.** Plasma ADMA concentrations before and after intervention in postmenopausal women. Data are reported as means ± SD.
suggested that the increase in NO production caused by the aerobic exercise training–induced reduction in ADMA may increase central arterial compliance.

It has been reported that an increase in ADMA is associated with increase in carotid artery IMT. In this study, 12 weeks aerobic exercise training decreased plasma ADMA concentrations but did not change carotid artery IMT. Spence et al. reported that carotid artery IMT decreased after 24 weeks of aerobic exercise training. In contrast with 24 weeks of exercise training, 8–12 weeks of aerobic exercise training could not induce decrease in carotid artery IMT. Our results are consistent with these results. On the other hand, carotid arterial compliance has been reported to begin to increase after 8–12 weeks of aerobic exercise training, which suggests that exercise training–induced improvement of carotid arterial compliance occurs in the relatively short term. Therefore, our results that aerobic exercise training increases carotid arterial compliance but does not change carotid artery IMT may be accounted for by the relatively short exercise training period.

The mechanisms underlying the decrease in plasma ADMA concentrations caused by aerobic exercise training remain to be elucidated. ADMA is influenced by oxidative stress. Previous studies have demonstrated that antioxidant therapies such as medications or supplements decrease plasma ADMA concentrations. Aerobic exercise training also reduces oxidative stress; therefore, the aerobic exercise training–induced reduction in oxidative stress may contribute to the decrease in plasma ADMA concentrations as well. Moreover, dimethylarginine dimethylaminohydrolase is involved in the breakdown of ADMA. A recent study reported that aerobic exercise training enhanced the mRNA gene expression of dimethylarginine dimethylaminohydrolase. Therefore, the decrease in plasma ADMA concentrations induced by aerobic exercise training may be further mediated by an increase in dimethylarginine dimethylaminohydrolase. Further studies are necessary to elucidate the mechanisms underlying the decrease in plasma ADMA concentrations caused by aerobic exercise training.

There are several limitations of this study that should be emphasized. First, this study included a relatively small sample size because of the following rigorous screening criteria: healthy, postmenopausal women who were nonsmokers, nonobese, free of cardiovascular disease, and taking no medications or supplements. The statistical power provided by a post hoc power analysis was not always high enough (i.e., \( \alpha > 0.80 \)). Thus, the findings of this study cannot be generalizable to other populations. Further studies should be warranted in a larger sample size and different populations, such as elderly men and age-matched populations with high body mass index. Second, in this study, we showed that aerobic exercise training decreased plasma ADMA concentrations with an increase in carotid arterial compliance in postmenopausal women. However, it should be noted that we cannot determine cause and effect between carotid arterial compliance and plasma ADMA concentrations with aerobic exercise training, because our data was performed with only correlation. Third, carotid–femoral pulse wave velocity is known to be the gold standard to assess vascular function. However, we did not measure carotid–femoral pulse wave velocity in this study. It has been reported that carotid arterial compliance is inversely correlated to carotid–femoral pulse wave velocity. In addition, decrease in carotid arterial compliance has been identified as an independent risk factor for future cardiovascular disease. Therefore, we measured carotid arterial compliance as vascular function. Fourth, a previous study reported that ADMA is associated with insulin sensitivity. However, we did not measure insulin sensitivity in this study. Further studies are needed to clarify the relationship changes in ADMA and insulin sensitivity with exercise training.

In conclusion, this study demonstrated that aerobic exercise training increased carotid arterial compliance and decreased plasma ADMA concentrations in postmenopausal women.
women. In addition, the changes in arterial compliance after the intervention correlated with the change in plasma ADMA concentrations. These findings suggest that exercise training increases central arterial compliance through a reduction in ADMA.

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DISCLOSURES

The authors declared no conflict of interest.

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