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Habitual aerobic exercise increases plasma pentraxin 3 levels in middle-aged and elderly women

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Running title: Aerobic exercise and pentraxin 3

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ABSTRACT

Chronic inflammation that occurs with aging is one of the risk factors for cardiovascular disease. Regular exercise may prevent cardiovascular morbidity by decreasing chronic systematic inflammation. Additionally, excess inflammation can be reduced by the anti-inflammatory protein pentraxin 3 (PTX3). Thus, both habitual exercise and PTX3 have an anti-inflammatory effect. However, it is unclear whether regular exercise leads to increased plasma PTX3 concentration. In the present study, we investigated the effects of regular aerobic exercise on plasma PTX3 concentration in middle-aged and elderly women. Twenty-two postmenopausal women (60 ± 6 yrs) were randomly divided evenly into 2 groups (i.e., exercise intervention and control). Subjects in the exercise group completed 2 months of regular aerobic exercise training (walking and cycling, 30-45min, 3-5 days/wk). Before and after the intervention, we evaluated plasma PTX3 concentration, peak oxygen uptake (VO2peak), blood chemistry, and arterial distensibility (carotid arterial compliance and β-stiffness) in all participants. There were no significant differences in baseline parameters between the 2 groups. Plasma PTX3 concentration was significantly increased in the exercise group after the intervention (P < 0.05). HDL cholesterol, VO2peak, and arterial compliance were also significantly increased (P < 0.05), while β-stiffness was markedly decreased (P < 0.01) after the intervention. On the other hand, there was no change in the parameters tested in the control group. This study demonstrates that regular aerobic exercise increases plasma PTX3 concentration with improvement of HDL cholesterol, VO2peak, and arterial distensibility in postmenopausal women.

Key words: Aging, Inflammation, Exercise
INTRODUCTION

Cardiovascular disease is the leading cause of death worldwide. Systemic inflammatory factors increase with age, which lead to an increased risk of cardiovascular disease in middle-aged and elderly individuals (Kritchevsky et al. 2005). Inflammatory factors can be classified as pro-inflammatory or anti-inflammatory, depending on their function. Several pro-inflammatory cytokines increase gradually with aging, causing an imbalance in the levels of pro- and anti-inflammatory factors. This is of particular concern for middle-aged and elderly women, whose risk of cardiovascular disease increases during menopause due to declining estrogen levels, in addition to the increase in systemic inflammation due to aging (Reckelhoff. 2006). Therefore, it is important for postmenopausal middle-aged and elderly women to modulate the balance of systemic inflammatory factors to reduce the risk of cardiovascular disease.

Physical inactivity, as well as aging, may induce systemic inflammation. It has been suggested that regular physical activity may contribute to lower levels of pro-inflammatory and higher levels of anti-inflammatory factors (Mathur and Pedersen. 2008). Furthermore, exercise can decrease several kinds of pro-inflammatory factors in elderly individuals (Roubenoff. 2007). Thus, regular exercise may decrease excess inflammation due to aging and/or physical inactivity, reducing the risk of cardiovascular disease in elderly individuals.

Pentraxin 3 (PTX3) may be a biomarker of cardiovascular events, in that it increases with the progression of atherosclerosis (Rolph et al. 2002). Recently, PTX3 has been established as an anti-inflammatory protein in atherosclerosis (Norata et al. 2010). In previous studies using PTX3-deficient or PTX3-over expressing mice, it has been shown that PTX3 has an anti-inflammatory and/or anti-atherosclerotic role (Dias et al. 2001;Norata et al. 2008). It has been reported that a bout of endurance exercise
or resistance exercise increases plasma PTX3 concentrations in young healthy men (Nakajima et al. 2010). We demonstrated that plasma PTX3 concentrations in young endurance-trained men were higher than age-matched sedentary controls (Miyaki et al. 2011). On the other hand, it has been reported that plasma PTX3 concentrations decreased with exercise training in patients undergoing cardiac rehabilitation (Fukuda et al. 2011). However, it is unclear whether aerobic exercise training affects circulating PTX3 concentration in healthy middle-aged and elderly individuals. The purpose of the present study was to investigate the effect of an 8-week aerobic exercise intervention on plasma PTX3 concentration in healthy postmenopausal middle-aged and elderly women.

METHODS

Subjects. Twenty-two sedentary or recreationally active (e.g., stretching exercise, yoga or golf) postmenopausal women participated in the study (age: 52–77 years). Prior to enrollment onto the study, participants answered a questionnaire about their lifestyle and postmenopausal condition. Individuals taking anti-inflammatory, anti-hypertensive, or steroidal drugs and hormone replacement were excluded. Subjects were nonsmokers, nonobese, and free of overt cardiovascular disease, as assessed by medical history and physical examination and all were at least 2 years post menopause. Subjects were randomly assigned to the exercise or control group before all measurements. The control group requested to maintain normal habits. This study was reviewed and approved by the Institutional Review Board at the University of Tsukuba. All potential risks and procedures of the study were explained to the subjects, who provided a written informed consent to participate in the study. Before and after the intervention, all measurements (excluding peak oxygen consumption) were performed between 8 a.m. and 12 p.m.
after an overnight fast. Subjects were studied under supine resting conditions in a quiet, temperature-controlled room (24–26°C). All measurements were performed after a resting period of at least 20 min.

**Exercise Intervention.** Subjects in the exercise group underwent aerobic exercise training 3–5 days/wk (more than 3 supervised sessions, and additional home-based training) for 8 weeks. Initially, subjects performed walking or cycling 30–40 min/day, at a relatively low intensity (at least 60% of their individually determined maximal heart rate). As their exercise tolerance improved, the intensity and duration of aerobic exercise were increased to 40–45 min/day, at an intensity of 70–75% of maximal heart rate. We instructed walking for 30-45 min/day at an intensity of 60-75% of maximal heart rate as additional home-based training. There were no adverse events with the exercise intervention.

**Peak Oxygen Consumption.** To determine peak oxygen consumption (VO$_2$peak) all subjects underwent an incremental cycle exercise test (2 min at 40 W with 20 W increases every 2 min) on a cycle ergometer (75XL, COMBI wellness, Tokyo). Throughout this test, heart rate, ratings of perceived exertion (Borg scale) and breath by breath oxygen consumption and carbon dioxide production were monitored (AE300S; Minato Medical Science, Osaka, Japan). The values of peak oxygen uptake were accepted if subjects met at least two of the following criteria: a VO$_2$ plateau (<150 mL O$_2$ per minute with an increased work rate), highest respiratory exchange ratio > 1.15, peak heart rate within 5 beats of the age-predicted maximum (220 - age), rating of perceived exertion > 19, or extreme fatigue such that the pedaling rate on the bicycle ergometer was < 50 rpm.
**Plasma Pentraxin 3 Concentration.** Plasma pentraxin 3 (PTX3) was measured as previously described (Miyaki et al. 2011). Briefly, each blood sample was placed in a chilled tube containing ethylenediaminetetraacetic acid (2 mg/mL) and centrifuged at 2000 g for 15 min at 4°C. The plasma was stored at -80°C until analysis. Plasma concentrations of PTX3 were determined using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Quantikine DPTX 30; R&D Systems Inc., Minneapolis, USA). The PTX3 assay was carried out according to the manufacturer’s instructions. The intra-assay coefficients of variation in plasma PTX3 levels were 2.2 % in this study.

**Blood Biochemistry.** Serum concentrations of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides (TG), and fasting plasma concentrations of glucose (FBG) were determined using standard enzymatic techniques. TC and TG concentrations were determined by the cholesterol dehydrogenase and glycerol kinase methods, respectively (Allain et al. 1974; Kohlmeler et al. 1984). LDL-C and HDL-C concentrations were measured by a direct method (Finley et al. 1978; Yamashita et al. 2008). The FBG concentration was assayed by the hexokinase and glucose-6-phosphate dehydrogenase methods (Slein et al. 1963).

**Blood Pressure.** The supine systolic blood pressure, diastolic blood pressure, mean arterial pressure, and heart rate (HR) were recorded from the left arm using a semi-automated device (form PWV/ABI; Colin Medical Technology, Komaki, Japan).

**Carotid Artery Distensibility.** Dynamic arterial compliance was
noninvasively determined using a combination of ultrasonography of the common
carotid artery and simultaneous applanation tonometry of the contralateral carotid
artery, as previously described (Miyaki et al. 2009). Briefly, subjects were examined in
the supine position under quiet resting conditions. The diameter of the common
carotid artery was measured using images from an ultrasound machine (EnVisor;
Koninklijke Philips Electronics, Eindhoven, the Netherlands) equipped with a
high-resolution (7.5 MHz) linear array transducer. Pressure wave forms of the left
common carotid artery were recorded with an applanation tonometry device (form
PWV/ABI) and calibrated by equating the mean arterial and diastolic blood pressure
values of the carotid artery to those of the brachial artery.

The $\beta$-stiffness index provides an index of arterial compliance adjusted for
distending pressure, as previously described (Miyaki et al. 2009). Briefly, the
$\beta$-stiffness index was calculated using the equation $\beta = \ln (P_s/P_d)/([D_s - D_d]/D_d)$,
where $D_s$ and $D_d$ are the maximum and minimum inner arterial diameters, and $P_s$
and $P_d$ are the highest and lowest blood pressures, respectively. All image analyses
were performed by the same investigator.

**Statistical Analysis.** Data are expressed as means ± SD. Statistical analysis
was performed by a 2-way (time-by-group) ANOVA with repeated measures to
determine the effects of the 2 different interventions. For intergroup comparisons, an
unpaired student's t test was used. $P < 0.05$ was accepted as significant.

**RESULTS**

All 11 participants in the exercise group completed the 8-week aerobic
exercise training (at least 3 days per week). Table 1 summarizes the characteristics of
the subject groups before and after the intervention. There were no significant
differences at baseline in age, height, weight, BMI, TG, TC, LDL-C, FBG and 
\( \dot{V}O_2 \text{peak} \) between the 2 groups. HDL-C and \( \dot{V}O_2 \text{peak} \) were significantly increased in 
the exercise group after the 8-week aerobic exercise program. On the other hand, 
there were no significant differences in the parameters tested before and after the 
intervention in the control group (Table 1). Before the intervention, there was no 
difference in the baseline plasma PTX3 concentration between the groups. After the 
intervention, plasma PTX3 concentration in the exercise group significantly increased 
(Figure 1). There was no significant change in plasma PTX3 concentration in the 
control group. Table 2 shows the hemodynamic changes before and after the 
intervention. In the exercise group, there were no significant differences in blood 
pressure and HR before and after the intervention. On the other hand, arterial 
compliance significantly increased and \( \beta \)-stiffness significantly decreased in the 
exercise group after the aerobic exercise intervention. The control group showed no 
significant changes in hemodynamic parameters before and after the intervention 
(Table 2). In all participants, the percent changes in plasma PTX3 concentration and 
those in arterial compliance be positively correlated (\( R = 0.40, P = 0.07 \)).

**DISCUSSION**

It is recognized that an imbalance of inflammatory factors (i.e., increasing 
pro-inflammatory and decreasing anti-inflammatory substances) due to physical 
inactivity is a risk factor for cardiovascular disease in middle-aged and elderly 
individuals (Mathur and Pedersen. 2008). Habitual aerobic exercise is thought to 
have an anti-inflammatory effect. Regular exercise promotes an increase in 
anti-inflammatory factors (interleukin-1 and interleukin-10) in the circulation (Petersen 
and Pedersen. 2005). Furthermore, long-term aerobic exercise can decrease 
 systemic pro-inflammatory factors (C-reactive protein and tumor necrosis.
factor-alpha) in middle-aged and elderly individuals (Mathur and Pedersen. 2008).

We previously reported that plasma PTX3 concentrations in young endurance-trained men were significantly higher than age-matched sedentary controls (Miyaki et al. 2011). However, the effect of habitual aerobic exercise on systemic PTX3 levels in middle-aged and elderly individuals remained unclear. In the present study, we demonstrated that plasma PTX3 concentration in healthy sedentary or recreationally active postmenopausal middle-aged and elderly women was significantly increased after an 8-week moderate aerobic exercise intervention. Thus, habitual aerobic exercise increased plasma PTX3 concentration possibly as an anti-inflammatory effect in healthy postmenopausal middle-aged and elderly women.

Elevated systemic inflammation is known to be one of the mechanisms that promotes cardiovascular diseases (Ross. 1993). Circulating levels of inflammatory factors are a useful clinical diagnostic tool to measure the state of cardiovascular disease (Petersen and Pedersen. 2005; Wilund. 2007). PTX3 may be a biomarker of cardiovascular events, in that it increases with the progression of atherosclerosis (Rolph et al. 2002). Importantly, PTX3 is mainly produced from cells composing the arterial wall (i.e., endothelial cells, smooth muscle cells, and macrophages) at the local part of atherosclerotic lesion (Savchenko et al. 2008). It has been suggested that PTX3 modulates the balance of pro- and anti-inflammatory factors (Norata et al. 2009). Compared to wild-type mice, PTX3-overexpressing mice are protected against a lethal dose of lipopolysaccharide (Dias et al. 2001). This may indicate that PTX3 controls excess pro-inflammatory reaction and defend the body against the lethal damage. Furthermore, PTX3-deficient mice fed a western-diet have a greater area of inflammatory atherosclerosis in the aorta than controls (Norata et al. 2008). It has been demonstrated that ischemic-reperfusion treatment in PTX3-deficient mice at the aortic arch significantly increases apoptosis in cardiovascular tissue. Such an
increase in apoptotic cells can be abrogated by administration of exogenous PTX3 (Salio et al. 2008). Recently, it has been postulated that elevation of PTX3 during cardiovascular disease may be a compensatory response by the body and serve to protect it from inflammation (Norata et al. 2010). Thus, these results suggest that PTX3 is an anti-inflammatory protein and has an important protective role in cardiovascular inflammation. In the present study, 8-week aerobic exercise training caused not only an increase in plasma PTX3 concentration, but also increased arterial distensibility and HDL-C in postmenopausal women. Decreases in arterial distensibility and HDL-C are characteristic of aging, and are known risk factors for cardiovascular disease morbidity (Chan et al. 1999; Safar and London. 2000). The percent change in plasma PTX3 concentration and that in arterial compliance tended to have positive correlation ($R = 0.40, P = 0.07$). Taken together, our results suggest that an increase in PTX3 by moderate aerobic exercise training may contribute to the cardiovascular protective effect in healthy postmenopausal middle-aged and elderly women.

The present study has the following study limitations. We demonstrated that plasma PTX3 concentrations increased after aerobic exercise training. However, the increase in plasma PTX3 concentration was very small. At this stage, it is unclear whether such a small increase in PTX3 has a clinical significance. Furthermore, there is one recent study on exercise training in patients undergoing cardiac rehabilitation that found that plasma PTX3 concentrations decreased with exercise training (Fukuda et al. 2011). In the present study, exercise training increased plasma PTX3 concentration in healthy middle-aged and elderly subjects. The difference in subjects (i.e., patients with cardiovascular disease or healthy individuals) may affect the response of plasma PTX3 concentrations during exercise training. However, we can not explain the reason for the contradictory results between these studies at present.
Moreover, it is unclear the mechanism/s behind increases in plasma PTX3 concentration after aerobic exercise training in postmenopausal middle-aged and elderly women. Further study is needed to reveal the mechanism underlying increase in plasma PTX3 concentration after aerobic exercise training.

In conclusion, 8 weeks of moderate aerobic exercise training increased plasma PTX3 concentration in healthy postmenopausal middle-aged and elderly women, and this may partly participate in the mechanism underlying aerobic exercise training-induced anti-inflammatory effect and cardiovascular protection.
ACKNOWLEDGEMENTS

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Conflicts of interest: The authors have no financial, consultant, institutional, or other relationships that might lead to bias or a conflict of interest.
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FIGURE LEGENDS

Figure 1. Plasma PTX3 concentration before and after the 8-week intervention. Data are expressed as mean ± SD.
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<thead>
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<th>Exercise</th>
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<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>Age, yrs</td>
<td>60 ± 6</td>
<td>-</td>
<td>60 ± 7</td>
<td>-</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.53 ± 4.2</td>
<td>-</td>
<td>1.54 ± 6.2</td>
<td>-</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>52.0 ± 5.5</td>
<td>52.0 ± 5.7</td>
<td>53.1 ± 7.1</td>
<td>53.4 ± 6.8</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>22.2 ± 2.0</td>
<td>22.2 ± 2.1</td>
<td>22.4 ± 2.6</td>
<td>22.5 ± 2.4</td>
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<td>TC, mmol/l</td>
<td>5.79 ± 0.83</td>
<td>5.97 ± 0.7</td>
<td>5.72 ± 0.52</td>
<td>5.87 ± 0.62</td>
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<td>TG, mmol/l</td>
<td>1.17 ± 0.45</td>
<td>1.06 ± 0.48</td>
<td>1.24 ± 0.59</td>
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<td>HDL-C, mmol/l</td>
<td>1.68 ± 0.28</td>
<td>1.86 ± 0.31*</td>
<td>1.68 ± 0.39</td>
<td>1.68 ± 0.34</td>
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<td>LDL-C, mmol/l</td>
<td>3.57 ± 0.72</td>
<td>3.67 ± 0.62</td>
<td>3.47 ± 0.39</td>
<td>3.49 ± 0.44</td>
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<tr>
<td>FBG, mmol/l</td>
<td>5.00 ± 0.44</td>
<td>5.17 ± 0.33</td>
<td>4.89 ± 0.44</td>
<td>4.94 ± 0.39</td>
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<tr>
<td>VO₂peak, ml/min/kg</td>
<td>24.7 ± 4.6</td>
<td>26.8 ± 5.4*</td>
<td>23.1 ± 4.8</td>
<td>22.1 ± 3.6</td>
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Data are expressed as means ± SD. TC, total cholesterol; TG, triglyceride; HDL-C, hdl cholesterol; LDL-C; ldl cholesterol; FBG, fasting blood glucose; VO₂peak, peak oxygen consumption. *P < 0.05 vs. before the intervention.
Table 2: Hemodynamic changes before and after the intervention

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<td>Pre</td>
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<tr>
<td>SBP, mmHg</td>
<td>97 ± 12</td>
<td>100 ± 15</td>
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<td>110 ± 13</td>
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<td>DBP, mmHg</td>
<td>63 ± 6</td>
<td>62 ± 7</td>
<td>68 ± 9</td>
<td>69 ± 6</td>
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<td>HR, bpm</td>
<td>58 ± 5</td>
<td>56 ± 4</td>
<td>64 ± 8</td>
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<tr>
<td>CAC, mm²/mmHg</td>
<td>0.083 ± 0.019</td>
<td>0.092 ± 0.023*</td>
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<td>β-stiffness</td>
<td>8.72 ± 2.05</td>
<td>7.76 ± 1.97**</td>
<td>7.58 ± 1.34</td>
<td>7.71 ± 1.51</td>
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Data are expressed as means ± SD. SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; CAC, carotid arterial compliance. *P < 0.05, **P < 0.01 vs. before the intervention.
Figure 1 (Miyaki et al.)

Plasma PTX3 concentrations (ng/ml)

Exercise vs. Control

Pre vs. Post

P < 0.05