1 **Habitual aerobic exercise increases plasma pentraxin 3 levels in**

2 **middle-aged and elderly women**

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1   **ABSTRACT**

2   Chronic inflammation that occurs with aging is one of the risk factors for

3   cardiovascular disease. Regular exercise may prevent cardiovascular morbidity by

4   decreasing chronic systematic inflammation. Additionally, excess inflammation can be

5   reduced by the anti-inflammatory protein pentraxin 3 (PTX3). Thus, both habitual

6   exercise and PTX3 have an anti-inflammatory effect. However, it is unclear whether

7   regular exercise leads to increased plasma PTX3 concentration. In the present study,

8   we investigated the effects of regular aerobic exercise on plasma PTX3 concentration

9   in middle-aged and elderly women. Twenty-two postmenopausal women (60 ± 6 yrs)

10 were randomly divided evenly into 2 groups (i.e., exercise intervention and control).

11 Subjects in the exercise group completed 2 months of regular aerobic exercise

12 training (walking and cycling, 30-45min, 3-5 days/wk). Before and after the

13 intervention, we evaluated plasma PTX3 concentration, peak oxygen uptake

14 ($\dot{V}O_2$peak), blood chemistry, and arterial distensibility (carotid arterial compliance and

15 β-stiffness) in all participants. There were no significant differences in baseline

16 parameters between the 2 groups. Plasma PTX3 concentration was significantly

17 increased in the exercise group after the intervention ($P < 0.05$). HDL cholesterol,

18 $\dot{V}O_2$peak, and arterial compliance were also significantly increased ($P < 0.05$), while

19 β-stiffness was markedly decreased ($P < 0.01$) after the intervention. On the other

20 hand, there was no change in the parameters tested in the control group. This study

21 demonstrates that regular aerobic exercise increases plasma PTX3 concentration

22 with improvement of HDL cholesterol, $\dot{V}O_2$peak, and arterial distensibility in

23 postmenopausal women.

24

25 **Key words:** Aging, Inflammation, Exercise

26

1   **INTRODUCTION**

2   Cardiovascular disease is the leading cause of death worldwide. Systemic

3   inflammatory factors increase with age, which lead to an increased risk of

4   cardiovascular disease in middle-aged and elderly individuals (Kritchevsky et al.

5   2005). Inflammatory factors can be classified as pro-inflammatory or

6   anti-inflammatory, depending on their function. Several pro-inflammatory cytokines

7   increase gradually with aging, causing an imbalance in the levels of pro- and

8   anti-inflammatory factors. This is of particular concern for middle-aged and elderly

9   women, whose risk of cardiovascular disease increases during menopause due to

10   declining estrogen levels, in addition to the increase in systemic inflammation due to

11   aging (Reckelhoff. 2006). Therefore, it is important for postmenopausal middle-aged

12   and elderly women to modulate the balance of systemic inflammatory factors to

13   reduce the risk of cardiovascular disease.

14   Physical inactivity, as well as aging, may induce systemic inflammation. It has

15   been suggested that regular physical activity may contribute to lower levels of

16   pro-inflammatory and higher levels of anti-inflammatory factors (Mathur and

17   Pedersen. 2008). Furthermore, exercise can decrease several kinds of

18   pro-inflammatory factors in elderly individuals (Roubenoff. 2007). Thus, regular

19   exercise may decrease excess inflammation due to aging and/or physical inactivity,

20   reducing the risk of cardiovascular disease in elderly individuals.

21   Pentraxin 3 (PTX3) may be a biomarker of cardiovascular events, in that it

22   increases with the progression of atherosclerosis (Rolph et al. 2002). Recently, PTX3

23   has been established as an anti-inflammatory protein in atherosclerosis (Norata et al.

24   2010). In previous studies using PTX3-deficient or PTX3-over expressing mice, it has

25   been shown that PTX3 has an anti-inflammatory and/or anti-atherosclerotic role (Dias

26   et al. 2001;Norata et al. 2008). It has been reported that a bout of endurance exercise

1　or resistance exercise increases plasma PTX3 concentrations in young healthy men

2　(Nakajima et al. 2010). We demonstrated that plasma PTX3 concentrations in young

3　endurance-trained men were higher than age-matched sedentary controls (Miyaki et

4　al. 2011). On the other hand, it has been reported that plasma PTX3 concentrations

5　decreased with exercise training in patients undergoing cardiac rehabilitation (Fukuda

6　et al. 2011). However, it is unclear whether aerobic exercise training affects circulating

7　PTX3 concentration in healthy middle-aged and elderly individuals. The purpose of

8　the present study was to investigate the effect of an 8-week aerobic exercise

9　intervention on plasma PTX3 concentration in healthy postmenopausal middle-aged

10　and elderly women.

11

12　**METHODS**

13　***Subjects.*** Twenty-two sedentary or recreationally active (e.g., stretching

14　exercise, yoga or golf) postmenopausal women participated in the study (age: 52–77

15　years). Prior to enrollment onto the study, participants answered a questionnaire

16　about their lifestyle and postmenopausal condition. Individuals taking

17　anti-inflammatory, anti-hypertensive, or steroidal drugs and hormone replacement

18　were excluded. Subjects were nonsmokers, nonobese, and free of overt

19　cardiovascular disease, as assessed by medical history and physical examination

20　and all were at least 2 years post menopause. Subjects were randomly assigned to

21　the exercise or control group before all measurements. The control group requested

22　to maintain normal habits. This study was reviewed and approved by the Institutional

23　Review Board at the University of Tsukuba. All potential risks and procedures of the

24　study were explained to the subjects, who provided a written informed consent to

25　participate in the study. Before and after the intervention, all measurements

26　(excluding peak oxygen consumption) were performed between 8 a.m. and 12 p.m.

1   after an overnight fast. Subjects were studied under supine resting conditions in a

2   quiet, temperature-controlled room (24–26°C). All measurements were performed

3   after a resting period of at least 20 min.

4

5   ***Exercise Intervention****.* Subjects in the exercise group underwent aerobic

6   exercise training 3–5 days/wk (more than 3 supervised sessions, and additional

7   home-based training) for 8 weeks. Initially, subjects performed walking or cycling

8   30–40 min/day, at a relatively low intensity (at least 60% of their individually

9   determined maximal heart rate). As their exercise tolerance improved, the intensity

10   and duration of aerobic exercise were increased to 40–45 min/day, at an intensity of

11   70–75% of maximal heart rate. We instructed walking for 30-45 min/day at an

12   intensity of 60-75% of maximal heart rate as additional home-based training. There

13   were no adverse events with the exercise intervention.

14

15   ***Peak Oxygen Consumption****.* To determine peak oxygen consumption

16   ($\dot{V}O_2$peak) all subjects underwent an incremental cycle exercise test (2 min at 40 W

17   with 20 W increases every 2 min) on a cycle ergometer (75XL, COMBI wellness,

18   Tokyo). Throughout this test, heart rate, ratings of perceived exertion (Borg scale)

19   and breath by breath oxygen consumption and carbon dioxide production were

20   monitored (AE300S; Minato Medical Science, Osaka, Japan). The values of peak

21   oxygen uptake were accepted if subjects met at least two of the following criteria: a

22   $\dot{V}O_2$ plateau (<150 mL O_2 per minute with an increased work rate), highest respiratory

23   exchange ratio > 1.15, peak heart rate within 5 beats of the age-predicted maximum

24   (220 - age), rating of perceived exertion > 19, or extreme fatigue such that the

25   pedaling rate on the bicycle ergometer was < 50 rpm.

26

1   ***Plasma Pentraxin 3 Concentration.*** Plasma pentraxin 3 (PTX3) was

2   measured as previously described (Miyaki et al. 2011). Briefly, each blood sample

3   was placed in a chilled tube containing ethylenediaminetetraacetic acid (2 mg/mL)

4   and centrifuged at 2000 *g* for 15 min at 4°C. The plasma was stored at -80°C until

5   analysis. Plasma concentrations of PTX3 were determined using a commercial

6   enzyme-linked immunosorbent assay (ELISA) kit (Quantikine DPTX 30; R&D

7   Systems Inc., Minneapolis, USA). The PTX3 assay was carried out according to the

8   manufacturer's instructions. The intra-assay coefficients of variation in plasma PTX3

9   levels were 2.2 % in this study.

10

11   ***Blood Biochemistry.*** Serum concentrations of total cholesterol (TC), high-density

12   lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C),

13   triglycerides (TG), and fasting plasma concentrations of glucose (FBG) were

14   determined using standard enzymatic techniques. TC and TG concentrations were

15   determined by the cholesterol dehydrogenase and glycerol kinase methods,

16   respectively (Allain et al. 1974; Kohlmeler et al. 1984). LDL-C and HDL-C

17   concentrations were measured by a direct method (Finley et al. 1978; Yamashita et al.

18   2008). The FBG concentration was assayed by the hexokinase and

19   glucose-6-phosphate dehydrogenase methods (Slein et al. 1963).

20

21   ***Blood Pressure.*** The supine systolic blood pressure, diastolic blood

22   pressure, mean arterial pressure, and heart rate (HR) were recorded from the left arm

23   using a semi-automated device (form PWV/ABI; Colin Medical Technology, Komaki,

24   Japan).

25

26   ***Carotid Artery Distensibility.*** Dynamic arterial compliance was

1 noninvasively determined using a combination of ultrasonography of the common

2 carotid artery and simultaneous applanation tonometry of the contralateral carotid

3 artery, as previously described (Miyaki et al. 2009). Briefly, subjects were examined in

4 the supine position under quiet resting conditions. The diameter of the common

5 carotid artery was measured using images from an ultrasound machine (EnVisor;

6 Koninklijke Philips Electronics, Eindhoven, the Netherlands) equipped with a

7 high-resolution (7.5 MHz) linear array transducer. Pressure wave forms of the left

8 common carotid artery were recorded with an applanation tonometry device (form

9 PWV/ABI) and calibrated by equating the mean arterial and diastolic blood pressure

10 values of the carotid artery to those of the brachial artery.

11 The β-stiffness index provides an index of arterial compliance adjusted for

12 distending pressure, as previously described (Miyaki et al. 2009). Briefly, the

13 β-stiffness index was calculated using the equation $\beta = \ln(P_s/P_d)/([D_s - D_d]/D_d)$,

14 where D_s and D_d are the maximum and minimum inner arterial diameters, and P_s

15 and P_d are the highest and lowest blood pressures, respectively. All image analyses

16 were performed by the same investigator.

17

18 ***Statistical Analysis.*** Data are expressed as means ± SD. Statistical analysis

19 was performed by a 2-way (time-by-group) ANOVA with repeated measures to

20 determine the effects of the 2 different interventions. For intergroup comparisons, an

21 unpaired student's t test was used. *P* < 0.05 was accepted as significant.

22

23 **RESULTS**

24 All 11 participants in the exercise group completed the 8-week aerobic

25 exercise training (at least 3 days per week). Table 1 summarizes the characteristics of

26 the subject groups before and after the intervention. There were no significant

1   differences at baseline in age, height, weight, BMI, TG, TC, LDL-C, FBG and

2   $\dot{V}O_2$peak between the 2 groups. HDL-C and $\dot{V}O_2$peak were significantly increased in

3   the exercise group after the 8-week aerobic exercise program. On the other hand,

4   there were no significant differences in the parameters tested before and after the

5   intervention in the control group (Table 1). Before the intervention, there was no

6   difference in the baseline plasma PTX3 concentration between the groups. After the

7   intervention, plasma PTX3 concentration in the exercise group significantly increased

8   (Figure 1). There was no significant change in plasma PTX3 concentration in the

9   control group. Table 2 shows the hemodynamic changes before and after the

10   intervention. In the exercise group, there were no significant differences in blood

11   pressure and HR before and after the intervention. On the other hand, arterial

12   compliance significantly increased and β-stiffness significantly decreased in the

13   exercise group after the aerobic exercise intervention. The control group showed no

14   significant changes in hemodynamic parameters before and after the intervention

15   (Table 2). In all participants, the percent changes in plasma PTX3 concentration and

16   those in arterial compliance be positively correlated (R = 0.40, P = 0.07).

17

18   **DISCUSSION**

19   It is recognized that an imbalance of inflammatory factors (i.e., increasing

20   pro-inflammatory and decreasing anti-inflammatory substances) due to physical

21   inactivity is a risk factor for cardiovascular disease in middle-aged and elderly

22   individuals (Mathur and Pedersen. 2008). Habitual aerobic exercise is thought to

23   have an anti-inflammatory effect. Regular exercise promotes an increase in

24   anti-inflammatory factors (interleukin-1 and interleukin-10) in the circulation (Petersen

25   and Pedersen. 2005). Furthermore, long-term aerobic exercise can decrease

26   systemic pro-inflammatory factors (C-reactive protein and tumor necrosis

1   factor-alpha) in middle-aged and elderly individuals (Mathur and Pedersen. 2008).

2   We previously reported that plasma PTX3 concentrations in young endurance-trained

3   men were significantly higher than age-matched sedentary controls (Miyaki et al.

4   2011). However, the effect of habitual aerobic exercise on systemic PTX3 levels in

5   middle-aged and elderly individuals remained unclear. In the present study, we

6   demonstrated that plasma PTX3 concentration in healthy sedentary or recreationally

7   active postmenopausal middle-aged and elderly women was significantly increased

8   after an 8-week moderate aerobic exercise intervention. Thus, habitual aerobic

9   exercise increased plasma PTX3 concentration possibly as an anti-inflammatory

10 effect in healthy postmenopausal middle-aged and elderly women.

11   Elevated systemic inflammation is known to be one of the mechanisms that

12 promotes cardiovascular diseases (Ross. 1993). Circulating levels of inflammatory

13 factors are a useful clinical diagnostic tool to measure the state of cardiovascular

14 disease (Petersen and Pedersen. 2005; Wilund. 2007). PTX3 may be a biomarker of

15 cardiovascular events, in that it increases with the progression of atherosclerosis

16 (Rolph et al. 2002). Importantly, PTX3 is mainly produced from cells composing the

17 arterial wall (i.e., endothelial cells, smooth muscle cells, and macrophages) at the

18 local part of atherosclerotic lesion (Savchenko et al. 2008). It has been suggested

19 that PTX3 modulates the balance of pro- and anti-inflammatory factors (Norata et al.

20 2009). Compared to wild-type mice, PTX3-overexpressing mice are protected against

21 a lethal dose of lipopolysaccharide (Dias et al. 2001). This may indicate that PTX3

22 controls excess pro-inflammatory reaction and defend the body against the lethal

23 damage. Furthermore, PTX3-deficient mice fed a western-diet have a greater area of

24 inflammatory atherosclerosis in the aorta than controls (Norata et al. 2008). It has

25 been demonstrated that ischemic-reperfusion treatment in PTX3-deficient mice at the

26 aortic arch significantly increases apoptosis in cardiovascular tissue. Such an

1   increase in apoptotic cells can be abrogated by administration of exogenous PTX3

2   (Salio et al. 2008). Recently, it has been postulated that elevation of PTX3 during

3   cardiovascular disease may be a compensatory response by the body and serve to

4   protect it from inflammation (Norata et al. 2010). Thus, these results suggest that

5   PTX3 is an anti-inflammatory protein and has an important protective role in

6   cardiovascular inflammation. In the present study, 8-week aerobic exercise training

7   caused not only an increase in plasma PTX3 concentration, but also increased

8   arterial distensibility and HDL-C in postmenopausal women. Decreases in arterial

9   distensibility and HDL-C are characteristic of aging, and are known risk factors for

10   cardiovascular disease morbidity (Chan et al. 1999; Safar and London. 2000). The

11   percent change in plasma PTX3 concentration and that in arterial compliance tended

12   to have positive correlation ($R = 0.40$, $P = 0.07$). Taken together, our results suggest

13   that an increase in PTX3 by moderate aerobic exercise training may contribute to the

14   cardiovascular protective effect in healthy postmenopausal middle-aged and elderly

15   women.

16   The present study has the following study limitations. We demonstrated that

17   plasma PTX3 concentrations increased after aerobic exercise training. However, the

18   increase in plasma PTX3 concentration was very small. At this stage, it is unclear

19   whether such a small increase in PTX3 has a clinical significance. Furthermore, there

20   is one recent study on exercise training in patients undergoing cardiac rehabilitation

21   that found that plasma PTX3 concentrations decreased with exercise training

22   (Fukuda et al. 2011). In the present study, exercise training increased plasma PTX3

23   concentration in healthy middle-aged and elderly subjects. The difference in subjects

24   (i.e., patients with cardiovascular disease or healthy individuals) may affect the

25   response of plasma PTX3 concentrations during exercise training. However, we can

26   not explain the reason for the contradictory results between these studies at present.

1   Moreover, it is unclear the mechanism/s behind increases in plasma PTX3

2   concentration after aerobic exercise training in postmenopausal middle-aged and

3   elderly women. Further study is needed to reveal the mechanism underlying increase

4   in plasma PTX3 concentration after aerobic exercise training.

5   In conclusion, 8 weeks of moderate aerobic exercise training increased

6   plasma PTX3 concentration in healthy postmenopausal middle-aged and elderly

7   women, and this may partly participate in the mechanism underlying aerobic exercise

8   training-induced anti-inflammatory effect and cardiovascular protection.

9

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6   **Conflicts of interest:** The authors have no financial, consultant, institutional, or other

7   relationships that might lead to bias or a conflict of interest.

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19

1   **FIGURE LEGENDS**

2   **Figure 1.** Plasma PTX3 concentration before and after the 8-week intervention. Data

3   are expressed as mean ± SD.

4

Table 1 Selected subject characteristics before and after the intervention

	Exercise		Control	
	Pre	Post	Pre	Post
Age, yrs	60 ± 6	-	60 ± 7	-
Height, m	1.53 ± 4.2	-	1.54 ± 6.2	-
Weight, kg	52.0 ± 5.5	52.0 ± 5.7	53.1 ± 7.1	53.4 ± 6.8
BMI, kg/m^2	22.2 ± 2.0	22.2 ± 2.1	22.4 ± 2.6	22.5 ± 2.4
TC, mmol/l	5.79 ± 0.83	5.97 ± 0.7	5.72 ± 0.52	5.87 ± 0.62
TG, mmol/l	1.17 ± 0.45	1.06 ± 0.48	1.24 ± 0.59	1.37 ± 0.58
HDL-C, mmol/l	1.68 ± 0.28	1.86 ± 0.31*	1.68 ± 0.39	1.68 ± 0.34
LDL-C, mmol/l	3.57 ± 0.72	3.67 ± 0.62	3.47 ± 0.39	3.49 ± 0.44
FBG, mmol/l	5.00 ± 0.44	5.17 ± 0.33	4.89 ± 0.44	4.94 ± 0.39
$\dot{V}O_2$peak , ml/min/kg	24.7 ± 4.6	26.8 ± 5.4*	23.1 ± 4.8	22.1 ± 3.6

Data are expressed as means ± SD. TC , total cholesterol; TG, triglyceride; HDL-C, hdl cholesterol; LDL-C; ldl cholesterol; FBG, fasting blood glucose; $\dot{V}O_2$peak, peak oxygen consumption. *$P < 0.05$ vs. before the intervention.

Table. 2 Hemodynamic changes before and after the intervention

	Exercise		Control	
	Pre	Post	Pre	Post
SBP, mmHg	97 ± 12	100 ± 15	107 ± 14	110 ± 13
DBP, mmHg	63 ± 6	62 ± 7	68 ± 9	69 ± 6
HR, bpm	58 ± 5	56 ± 4	64 ± 8	63 ± 8
CAC, mm^2/mmHg	0.083 ± 0.019	0.092 ± 0.023*	0.091 ± 0.019	0.090 ± 0.020
β-stiffness	8.72 ± 2.05	7.76 ± 1.97**	7.58 ± 1.34	7.71 ± 1.51

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.)