

1 **Plasma pentraxin3 concentration increases in**
2 **endurance-trained men**

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5 Asako Miyaki¹⁾, Seiji Maeda¹⁾, Takeshi Otsuki²⁾, and Ryuichi Ajisaka¹⁾
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8 ¹⁾Division of Sports Medicine, Graduate School of Comprehensive Human Sciences,
9 University of Tsukuba, Tsukuba, Ibaraki, Japan, ²⁾ Graduate School of Health and Sport
10 Sciences, Ryutsu Keizai University, Ryugasaki, Ibaraki, Japan
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14 **Running title:** Exercise training and PTX3
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20 **Address for Correspondence:**

21 Seiji Maeda, Ph.D.

22 Division of Sports Medicine

23 Graduate School of Comprehensive Human Sciences

24 University of Tsukuba

25 Tsukuba, Ibaraki 305-8577

26 Japan

27 (TEL) +81 29-853-2683

28 (FAX) +81 29-853-2986

29 E-mail: maeda@taiiku.tsukuba.ac.jp
30

1 **ABSTRACT**

2 **Background:** Pentraxin3 (PTX3), which is mainly produced by endothelial cells,
3 macrophages, and smooth muscle cells in the atherosclerotic region, has a
4 cardioprotective effect. Endurance exercise training has also been known to offer
5 cardioprotection. However, the effect of regular endurance exercise on PTX3 is
6 unknown. This study aimed to investigate whether plasma PTX3 concentrations
7 increase in endurance-trained men. Ten young endurance-trained men and 12 age-
8 and gender-matched sedentary controls participated in this study. **Methods:** We
9 measured plasma PTX3 concentrations of the participants in each group. We also
10 determined systemic arterial compliance (SAC) by using simultaneous M-mode
11 ultrasound and arterial applanation tonometry of the common carotid artery and used
12 high-density lipoprotein cholesterol (HDLC) as an index of cardioprotective effect.

13 **Results:** Maximal oxygen uptake was significantly higher in the endurance-trained
14 men than in the sedentary controls. SAC and HDLC were significantly higher in the
15 endurance-trained men than in the sedentary controls (SAC: 1.74 ± 0.11 vs. $1.41 \pm$
16 0.09 ml/mmHg; $p < 0.05$, HDLC: 70 ± 5 vs. 57 ± 4 mg/dl; $p < 0.05$). Plasma PTX3
17 concentrations were markedly higher in the endurance-trained men than in the
18 sedentary controls (0.93 ± 0.11 vs. 0.68 ± 0.06 ng/ml; $p < 0.05$). Relationships
19 between plasma PTX3 concentrations and SAC and HDLC were linear. **Conclusion:**
20 This is the first study revealing that endurance-trained individuals had higher levels of
21 circulating PTX3 than sedentary controls. PTX3 may play a partial role in endurance
22 exercise training-induced cardioprotection.

23 **Keywords:** endurance training, cardioprotection, systemic arterial compliance,
24 high-density lipoprotein cholesterol

1 INTRODUCTION

2 *Paragraph Number 1* It is generally accepted that an increase in regular
3 physical activity, especially habitual endurance exercise, reduces cardiovascular risk
4 factors (7, 15, 16). Endurance exercise training induces increase in both high-density
5 lipoprotein cholesterol (HDL) (2, 30) and arterial compliance (5, 6). Increased HDL
6 and arterial compliance have been recognized as having beneficial cardioprotective
7 effects (20, 29).

8 *Paragraph Number 2* Recently, pentraxin 3 (PTX3), which is mainly
9 produced by endothelial cells, macrophages and smooth muscle cells in the
10 atherosclerotic region (21, 24), has been identified as a substance playing an
11 important role in cardioprotection and atheroprotection. It has been reported in mice
12 that after transient ischemia in the left anterior descending coronary artery, the area
13 of necrotic heart tissue expanded in PTX3-deficient mice compared to that in the
14 control mice (25), suggesting that PTX3 can prevent ischemic tissue from necrotizing.
15 Furthermore, a previous study demonstrated that PTX3 heals vascular injury via
16 activation of tissue factor (17). Recently, it has been revealed that the mice lacking
17 PTX3 promotes vascular inflammatory response and atherosclerosis (19). These
18 findings suggest that PTX3 has cardioprotective and atheroprotective effects.

19 *Paragraph Number 3* Since PTX3 is implicated in cardioprotection, it is
20 reasonable to hypothesize that PTX3 participates in the mechanisms underlying
21 endurance exercise training-induced cardioprotective effect. However, the
22 relationship between plasma PTX3 concentrations and exercise training-induced
23 cardioprotective effect remains unclear. We hypothesized that endurance trained

1 individuals have higher levels of plasma PTX3 than sedentary controls and this
2 increase in PTX3 would partly participate in the mechanism underlying endurance
3 exercise training-induced cardioprotection. To test our hypothesis, we measured
4 plasma PTX3 concentrations; plasma HDLC concentrations; and systemic arterial
5 compliance (SAC) in endurance-trained men. We measured HDLC and SAC as
6 indices of endurance exercise training-induced cardioprotective effect.

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8 **METHODS**

9 *Paragraph Number 4* **Subjects.** All participants in this study were Japanese.
10 Ten young endurance-trained men (19-26 years) and 12 age- and gender-matched
11 sedentary controls (19-25 years) participated in this study. All of endurance-trained
12 men's careers were longer than 2 years. The training mainly consisted of some kind
13 of running training, such as long-distance running and interval training, and which
14 volume and intensity were 5.5 ± 0.3 sessions/wk (2.4 ± 0.3 h/session) and the rating
15 of 15–17 in the Borg's scale (i.e., hard-very hard). On the other hand, control men had
16 a sedentary lifestyle (no regular physical activity) for at least 2 years. All subjects
17 were free of signs, symptoms, and history of any overt chronic diseases. None of the
18 participants had a history of smoking, and none were currently taking any medications.
19 Additionally, none of the subjects were NSAIDs or aspirin users. Before all
20 measurements, the subjects refrained from alcohol consumption and intense physical
21 activity (exercise) for 24 h and fasted overnight (12 h), without water. All
22 measurements were performed after a resting period of at least 20 min at a constant
23 room temperature (25°C).

24 *Paragraph Number 5* This study was reviewed and approved by the
25 institutional review board at the University of Tsukuba. The study conformed to the

1 principles outlined in the Helsinki Declaration. All potential risks and procedures
2 involved in the study were explained to the subjects, and written informed consent to
3 participate in the study was obtained from all subjects.

4 *Paragraph Number 6 **Maximal Oxygen Uptake.*** The maximal oxygen
5 uptake was determined during incremental cycling to exhaustion (3 min at 80 W, with
6 a 30-W increase every 3 min) by monitoring breath-by-breath oxygen consumption
7 and carbon dioxide production (AE280S; Minato Medical Science, Osaka, Japan),
8 heart rate, and ratings of perceived exertion (Borg scale). The values of maximal
9 oxygen uptake were accepted if subjects met at least 2 of the following criteria: a $\dot{V}O_2$
10 plateau (<150 ml O_2 /min with an increased work rate), highest respiratory exchange
11 ratio >1.15, peak heart rate within 5 beats of the age-predicted maximum (220 minus
12 the age in years), rating of perceived exertion >19, or extreme fatigue such that the
13 pedaling rate on the bicycle ergometer was <50 rpm.

14 *Paragraph Number 7 **SAC.*** SAC was measured by carotid artery
15 applanation tonometry and Doppler echocardiography as described previously (22).
16 Briefly, carotid artery pressure waveforms were obtained by applanation tonometry
17 (formPWV/ABI; Colin Medical Technology, Komaki, Japan) after a resting period of at
18 least 20 min. At the time of waveform recording, brachial arterial systolic, diastolic,
19 and mean blood pressure (SBP, DBP, and MBP, respectively) were measured by
20 oscillometry (form PWV/ABI; Colin Medical Technology). The pressure signal
21 obtained by tonometry was calibrated by equating the carotid MBP and DBP to
22 brachial artery values. SAC was calculated as follows: $SAC = Ad/(dP \times R)$, where Ad
23 is the area under an arbitrary portion of the diastolic pressure waveform, dP is the
24 pressure change in this portion, and R is systemic vascular resistance given as MBP
25 divided by mean blood flow. The calculation of SAC is based on the assumption that
26 the diastolic pressure decay is a mono-exponential function of time. Mean blood flow

1 was obtained using a Doppler echocardiographic system (EnVisor; Koninklijke Philips
2 Electronics, Eindhoven, Netherlands) as described previously by our laboratory (22).
3 The insertion point of the aortic valve tips at the end of diastole was defined by
4 two-dimensional imaging in the parasternal long-axis view with a 3.5-MHz transducer,
5 and the M-mode echocardiogram at that level was recorded with the computer.
6 Doppler ultrasonographic flow velocity curves in the ascending aorta were
7 simultaneously obtained using a 1.9-MHz probe held in the suprasternal notch. Mean
8 blood flow was calculated as a product of the aortic cross-sectional area and the
9 mean flow velocity (ImageJ; National Institutes of Health, Bethesda, MD).

10 *Paragraph Number 8 Plasma PTX3 Concentration.* All the blood samples
11 were obtained from the antecubital vein with using a 21-gauge needle. Each blood
12 sample was placed in a chilled tube containing ethylenediaminetetraacetic acid
13 (EDTA) (2 mg/mL) and was then centrifuged at 2,000 g for 15 min at 4°C. The plasma
14 was stored at –80°C until the assay. Plasma concentrations of PTX3 were determined
15 using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Quantakine
16 DPTX 30; R&D Systems Inc., Minneapolis, USA). The PTX3 assay was carried out
17 according to the manufacturer's instructions. Briefly, standard or plasma samples
18 assayed in duplicate, and 20 µl of which were added to microtiter plate wells coated
19 with a monoclonal antibody specific for PTX3, followed by incubation at room
20 temperature for 2 hours. The wells were then washed 4 times with a buffered
21 surfactant solution, and thereafter, 200 µl of anti-PTX3 polyclonal antibody
22 conjugated to alkaline phosphatase were added to each well and incubation for 2
23 hours at room temperature. After appropriate washing, 200 µl of substrate solution
24 were added to each well and incubated again for 30 min at room temperature. The
25 reaction was then stopped by the addition of 2N sulfuric acid to the wells, and
26 absorbance was measured at 450 nm with corrections set at 540 nm using a

1 microplate reader. The values of plasma PTX3 levels were extrapolated from a curve
2 drawn using standard PTX3. The intra- and inter-assay coefficients of variation were
3 3.8% and 6.1%, respectively (values provided by Quantakine DPTX 30; R&D
4 Systems Inc.). The intra-assay coefficient of variation in this study was 5.6%. No
5 significant cross-reactivity or interference with other factors related to PTX3 or other
6 cytokines was observed (information provided by Quantakine DPTX 30; R&D
7 Systems Inc.).

8 *Paragraph Number 9 **Blood Biochemistry.*** The serum concentrations of
9 total cholesterol (TC), low-density lipoprotein cholesterol (LDLC), HDLC, and
10 triglycerides (TG) and the plasma concentrations of glucose (BG) were determined
11 using standard enzymatic techniques. Briefly, TG and TC concentrations were
12 determined by the cholesterol dehydrogenase and glycerol kinase methods,
13 respectively (1, 13). LDLC and HDLC concentrations were measured by a direct
14 method (9, 31). The BG concentration was assayed by the hexokinase and
15 glucose-6-phosphate dehydrogenase methods (26).

16 *Paragraph Number 10 **Statistical Analysis.*** Student's *t* test for unpaired
17 values was used to evaluate the statistical differences between the endurance-trained
18 men and the sedentary controls. Relationships between SAC or HDLC and plasma
19 PTX3 concentrations were analyzed using Pearson's correlation. Data were
20 expressed as means \pm SE. Values of $P < 0.05$ were accepted as significant.

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22 **RESULTS**

23 *Paragraph Number 11* Table 1 summarizes the characteristics of the
24 endurance-trained men and the sedentary controls. There were no significant
25 differences in age, height, weight, BMI, TG, TC, LDLC, and BG between the

1 endurance-trained men and the sedentary controls. Table 2 shows the
2 hemodynamics in the endurance-trained men and the sedentary controls. Diastolic
3 blood pressure and resting heart rate were significantly lower in the
4 endurance-trained men than in the sedentary controls. There was no significant
5 difference in systolic blood pressure and pulse pressure between the two groups.
6 Maximal oxygen uptake was higher in the endurance-trained men than in the
7 sedentary controls (Table 1). HDLC in the endurance-trained men was markedly
8 higher than the sedentary controls (Fig. 1). SAC was significantly higher in the
9 endurance-trained men than in the sedentary controls (Fig. 2). Figure 3 shows
10 plasma PTX3 concentrations in the two groups. Plasma PTX3 concentrations were
11 higher in the endurance-trained men than in the sedentary controls. The relationships
12 between plasma PTX3 concentrations and HDLC and SAC were linear (Fig. 4).
13 However, no significant relationship was detected between maximal oxygen uptake
14 and plasma PTX3 concentrations. In the trained group, we found a significant positive
15 correlation between plasma PTX3 concentrations and HDLC (Fig. 5). However, there
16 was no relation between plasma PTX3 concentrations and SAC in the trained group
17 (Fig. 5). In the sedentary controls, plasma PTX3 concentrations were not related to
18 HDLC or SAC (Fig. 5).

19

20 **DISCUSSION**

21 *Paragraph Number 12* In the present study, we determined plasma PTX3
22 concentrations in endurance-trained men. It was first demonstrated that plasma PTX3
23 concentrations were markedly higher in the endurance-trained men than in the
24 sedentary controls. The endurance-trained men also showed clearly higher maximal
25 oxygen uptake, HDLC, and SAC than the sedentary controls. Furthermore, the

1 relationships between plasma PTX3 concentrations and HDLC and SAC were linear.
2 An increase in PTX3 may play a role in the endurance exercise training-induced
3 increase in HDLC and SAC, i.e., the cardioprotective effects induced by exercise
4 training.

5 *Paragraph Number 13* PTX3 is mainly produced by endothelial cells,
6 macrophages, and smooth muscle cells in the local atherosclerotic region (21, 24).
7 However, the role of PTX3 in the cardiovascular system is unclear. Circulating PTX3
8 concentrations were reported to increase in patients with cardiovascular disease (23,
9 27). Napoleone et al. (17) reported that PTX3 could repair vascular wounds by
10 promoting activation of tissue factor. Peri et al. (23) demonstrated that PTX3 was
11 produced from dying cardiomyocytes but not from necrotic cells in patients with acute
12 myocardial infarction. Recently, it has been demonstrated that PTX3 functions at the
13 crossway between pro-inflammatory and anti-inflammatory stimuli to balance the over
14 activation of a pro-inflammatory, pro-atherogenic cascade (19). Namely, the
15 increased levels of PTX3 in cardiovascular disease could reflect a protective
16 physiological response (19). Salio et al. (25) demonstrated that after acute myocardial
17 infarction, the exacerbated heart tissue area in PTX3-deficient mice had expanded
18 compared to that in the control mice. Thus, PTX3 plays a role of repair in
19 cardiovascular injury. Moreover, the recent report showed that the double-knockout
20 mice lacking PTX3 and apolipoprotein E (ApoE) gene developed larger
21 atherosclerosis than the mice lacking only ApoE (19). Taken together, it is thought
22 that PTX3 has a cardioprotective and atheroprotective effects.

23 *Paragraph Number 14* The benefit of habitual endurance exercise is
24 recognized as a lifestyle modification worldwide. In epidemiological studies,

1 physically inactive subjects were reported have significantly higher risks of
2 cardiovascular disease, and mortality rates in these subjects were reported to be high
3 (7, 15, 16). Endurance exercise training produces beneficial cardioprotective effects.
4 Increased HDLC and arterial compliance have been recognized as beneficial
5 cardioprotective effects (20, 29). Habitual endurance exercise induces the increase in
6 HDLC and SAC (2, 5, 6, 30). In the present study, HDLC, SAC, and plasma PTX3
7 concentrations were significantly higher in the endurance-trained men than in the
8 sedentary controls. Furthermore, we demonstrated that there was a significant
9 positive correlation between plasma PTX3 concentrations and SAC or HDLC. These
10 findings suggest that endurance-trained men have beneficial cardioprotective effects
11 and PTX3 may partly participate in the mechanism underlying endurance exercise
12 training-induced cardioprotective effect.

13 *Paragraph Number 15* It is known that high physical activity and/or
14 endurance exercise training is effective for good health. On the other hand, exercise
15 causes increase in inflammatory factors in various tissues, such as circulating blood,
16 fat, and skeletal muscle (3, 10, 12, 18). A previous study reported that PTX3 is
17 expressed and secreted in vascular walls as a result of the inflammatory response
18 (11). Furthermore, it has been reported that PTX3 is produced via the myeloid
19 differentiation protein 88-interleukin-1 receptor [MyD88-IL1R] pathway, which induces
20 initial factors for starting inflammatory response (e.g., nuclear factor-kappa B [NF- κ B])
21 (25). MyD88 is also known as a necessary factor for vascular remodeling (28). Tumor
22 necrosis factor- α (TNF- α) and interleukin-1 β (IL-1 β) are known as triggers of PTX3
23 production (4, 14). On the other hand, anti-atherogenic IL-10 stimulates PTX3
24 production from dendric cells and monocytes (8). Additionally, PTX3 inhibits
25 pro-atherogenic cytokines interferon- γ production (21). Norata et al. (19) recently

1 reported that PTX3 is a molecule for finely tuning vascular inflammatory response by
2 both pro- and anti-inflammatory factors. Thus, PTX3 is modulated by both
3 pro-atherogenic and anti-atherogenic factors. PTX3 may be a necessary substance
4 for maintaining vascular homeostasis. Taken together, PTX3 participates in a part of
5 inflammation and plays a role in cardioprotection and atheroprotection. However, the
6 precise roles of PTX3 remain to be elucidated.

7 *Paragraph Number 16* There are several limitations of this study that should
8 be emphasized. First, this was a cross-sectional study. Therefore, the results
9 suggesting a role for PTX3 in cardioprotection are preliminary. These findings need to
10 be confirmed in a longitudinal study. Second, the small sample size is clearly one of
11 the limitations of this study. We have demonstrated that plasma PTX3 concentrations,
12 HDLC, SAC and maximal oxygen uptake were increased in endurance-trained men.
13 Furthermore, the relationships between plasma PTX3 concentrations and HDLC and
14 SAC were linear. However, there was no relation between plasma PTX3
15 concentrations and maximal oxygen uptake. This may be the influence of a small
16 sample size in the present study. Furthermore, the subjects in this study were young
17 Japanese men. Therefore, these results may not generalize to other populations.

18 *Paragraph Number 17* In conclusion, the present study revealed for the first
19 time that circulating PTX3 concentrations are markedly higher in endurance-trained
20 men than in sedentary controls. We also demonstrated that SAC and HDLC, which
21 are cardioprotective factors, were elevated by the regular endurance exercise. It is
22 possible that PTX3 may partly participate in the mechanism underlying endurance
23 exercise training-induced cardioprotection.

1 **ACKNOWLEDGMENTS**

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3 Research 21300234 and 21650179 and Grant-in-Aid for JSPS Fellows 21-692 from
4 Japan Society for the Promotion of Science. And, the results of the present study do
5 not constitute endorsement by ACSM

6

7 **CONFLICTS OF INTEREST**

8 *Paragraph Number 18* The authors have no financial, consultant, institutional, or
9 other relationships that might lead to bias or a conflict of interest.

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1 **FIGURE LEGENDS**

2 **Figure 1.** High-density lipoprotein cholesterol (HDLC) in endurance-trained men and
3 in sedentary controls. Data are expressed as means \pm SE.

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5 **Figure 2.** Systemic arterial compliance (SAC) in endurance-trained men and in
6 sedentary controls. Data are expressed as means \pm SE.

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8 **Figure 3.** Plasma pentraxin 3 (PTX3) concentrations in endurance-trained men and in
9 sedentary controls. Data are expressed as means \pm SE.

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11 **Figure 4.** Relationships between plasma PTX3 concentrations and HDLC (A) and
12 SAC (B) were linear. Endurance-trained men (Δ) and sedentary controls (\circ) are
13 shown.

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15 **Figure 5.** Relationships between plasma PTX3 concentrations and HDLC (A) and
16 SAC (B) in endurance-trained men and between plasma PTX3 concentrations and
17 HDLC (C) and SAC (D) in sedentary controls.

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Table. 1 Characteristics of sedentary control men and endurance-trained men.

	Sedentary	Endurance
Age, years	20.8 ± 0.8	20.7 ± 0.6
Height, cm	173.2 ± 1.5	173.3 ± 1.9
Weight, kg	66.3 ± 2.1	62.5 ± 1.9
BMI, kg/m ²	22.1 ± 0.6	20.8 ± 0.4
TG, mg/dL	75 ± 16	72 ± 14
TC, mg/dL	178 ± 9	183 ± 9
LDLC, mg/dL	107 ± 10	101 ± 8
BG, mg/dL	88 ± 3	86 ± 2
Maximal oxygen uptake, ml/min/kg	44.7 ± 1.0	60.3 ± 0.8

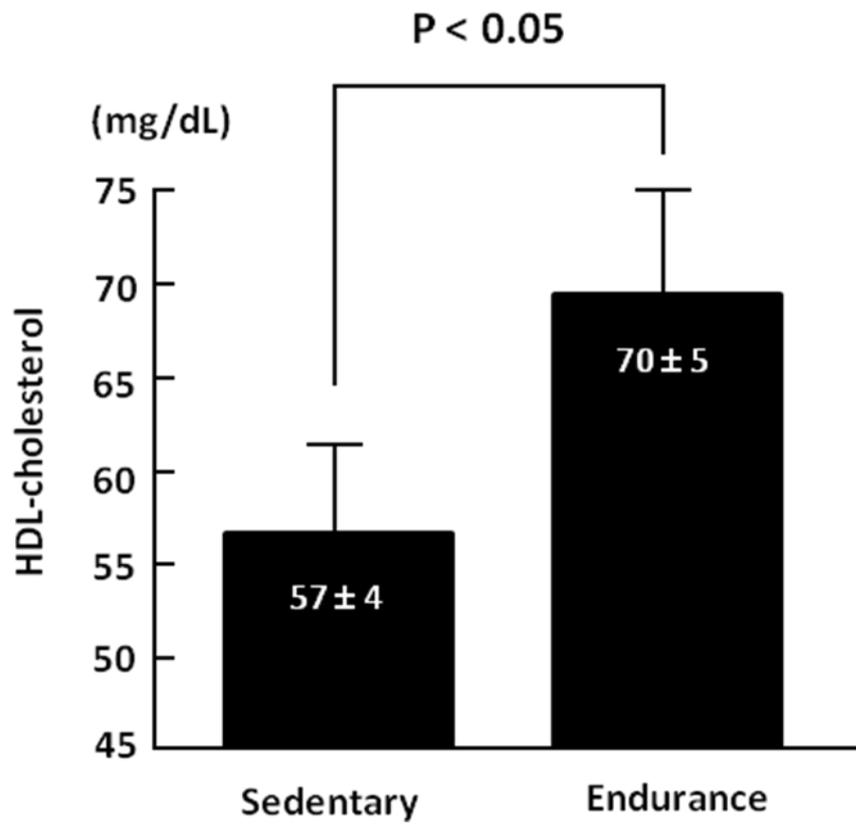
Data are expressed as means ± SE. Sedntary, sedentary control men; Endurance, endurance-trained men.

Table. 2 Hemodynamics of sedentary control men and endurance-trained men

	Sedentary	Endurance
Systolic blood pressure, mmHg	118 ± 3	114 ± 3
Diastolic blood pressure, mmHg	65 ± 2	59 ± 1**
Pulse pressure, mmHg	53 ± 1	55 ± 2
Heart rate, bpm	63 ± 3	53 ± 3*

Data are expressed as means ± SE. * $P < 0.05$ vs. Sedentary, ** $P < 0.01$ vs. Sedentary

Figure 1 (Miyaki et al.)



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Figure 2 (Miyaki et al.)

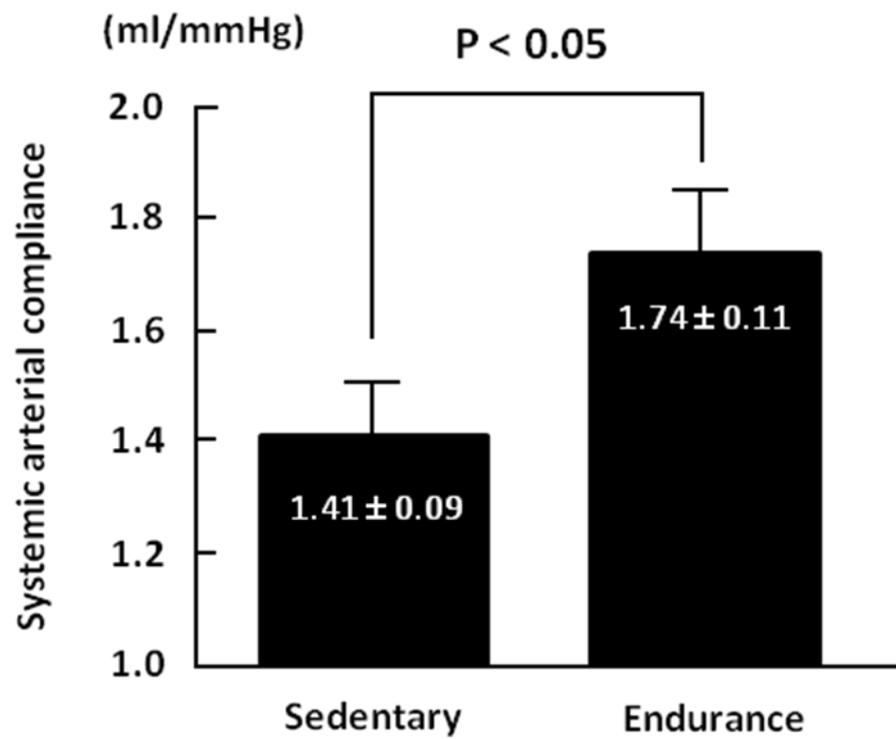
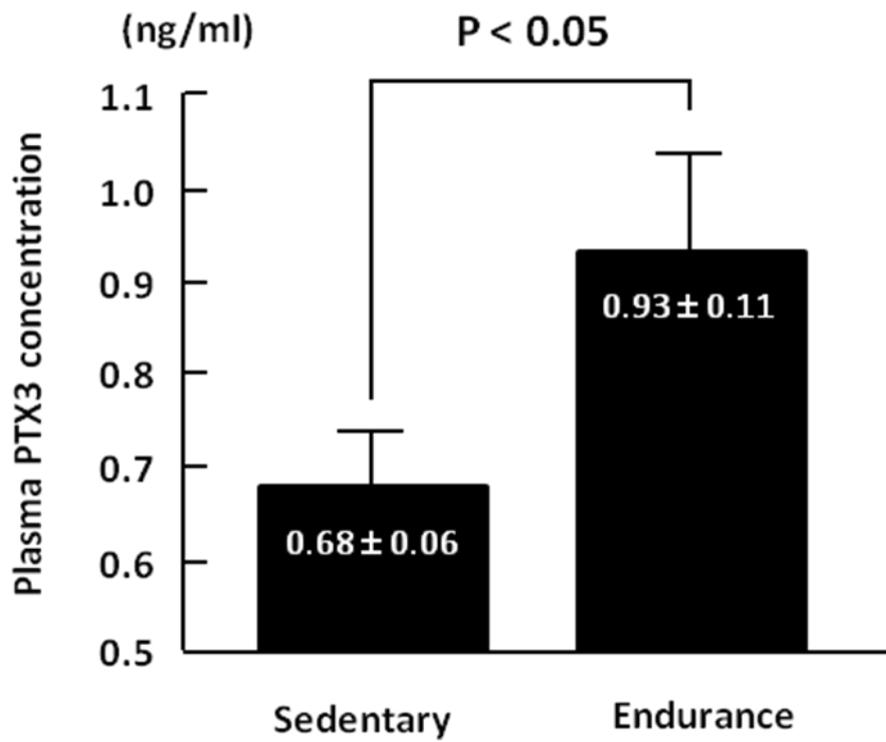
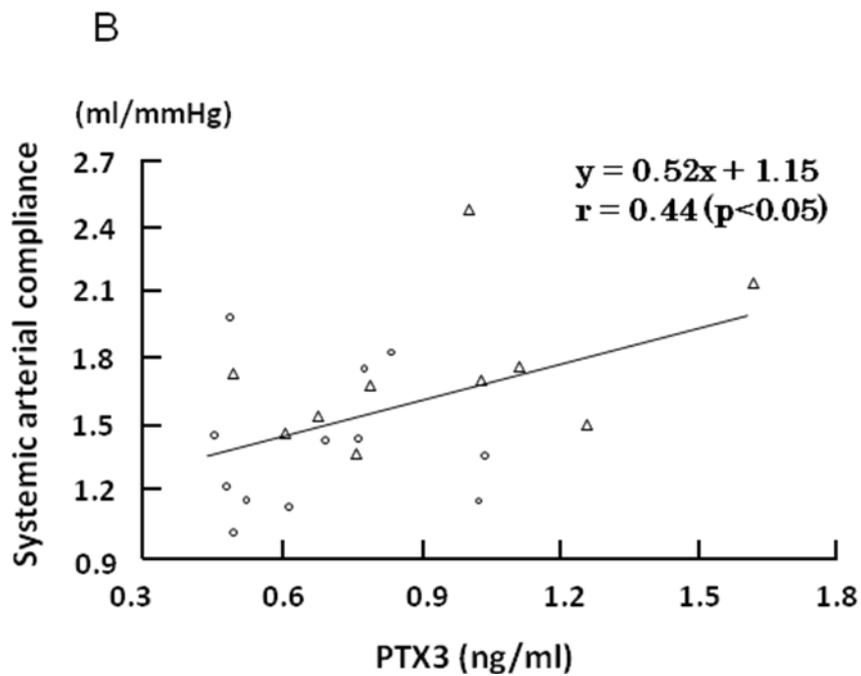
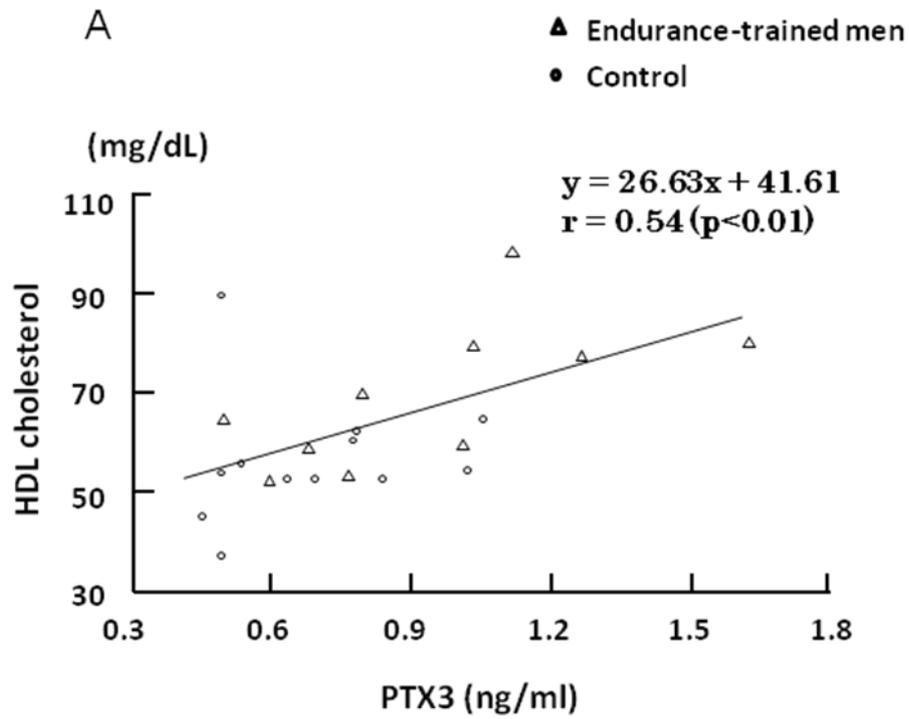


Figure 3 (Miyaki et al.)



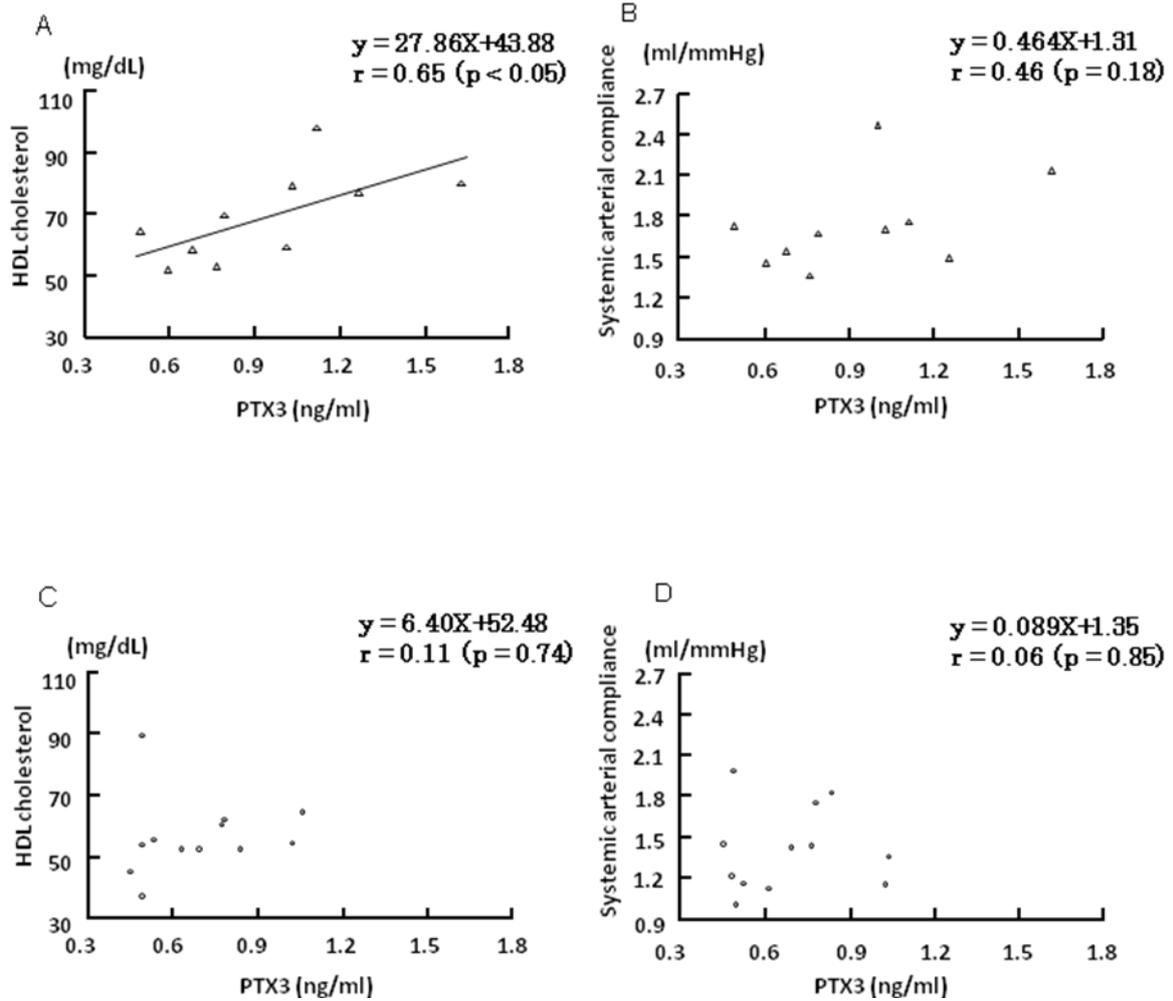
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Figure 4 (Miyaki et al.)



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Figure 5 (Miyaki et al.)



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