Original Article

Is Pentraxin 3 Involved in Obesity-Induced Decrease in Arterial Distensibility?

Asako Miyaki¹, Seiji Maeda¹, Mutsuko Yoshizawa¹, Maiko Misono¹, Hiroyuki Sasai¹, Nobutake Shimojo², Kiyoji Tanaka¹, and Ryuichi Ajisaka¹

¹Division of Sports Medicine, Graduate School of Comprehensive Human Sciences, University of Tsukuba, Ibaraki, Japan

²Division of Cardiovascular Medicine, Graduate School of Comprehensive Human Sciences, University of Tsukuba, Ibaraki, Japan

Aim: Obesity is a strong risk factor for cardiovascular morbidity and mortality. In addition, decreased central arterial distensibility is recognized as an independent risk factor for cardiovascular disease (CVD). Obese subjects exhibit low arterial distensibility; however, the mechanism responsible for the decrease in arterial distensibility in obese subjects has not yet been elucidated. Pentraxin 3 (PTX3), a recently identified member of the pentraxin family of proteins, is produced in areas of atherosclerosis. A recent study has revealed that the PTX3 level may indicate the vascular inflammatory status. The aim of this study was to investigate plasma PTX3 concentrations and arterial distensibility in obese subjects.

Methods: Eleven obese men (age: 44 ± 2 years, body mass index [BMI]: 32 ± 1 kg/m²) and 14 non-obese men (age: 42 ± 2 years, BMI: 26 ± 1 kg/m²) participated in this study. We measured arterial compliance (using simultaneous B-mode ultrasound and arterial applanation tonometry of the common carotid artery); β -stiffness index, an index of arterial compliance adjusted for distending pressure; and plasma PTX3 concentrations.

Results: Arterial compliance was significantly lower and the β -stiffness index was significantly higher in obese men than in non-obese men. Plasma PTX3 concentration was markedly higher in obese than non-obese men.

Conclusions: Obese men have lower arterial distensibility and higher circulating PTX3 levels than non-obese men; therefore, higher PTX3 levels and decreased arterial distensibility coexist in obese men. The high PTX3 concentrations in obese men may be involved in the mechanism underlying the obesity-induced decrease in arterial distensibility.

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Key words; PTX3, Obesity, Vascular inflammation

Introduction

Globally, cardiovascular diseases (CVDs) are the leading cause of mortality. Obesity, defined as body mass index (BMI ≥ 30), is recognized as an independent and strong risk factor for CVD^{1, 2}. Further, reduction in central arterial distensibility has been implicated in the pathophysiology of CVD and iden-

tified as a strong and independent risk factor for CVD^{3, 4}. In cross-sectional studies, obese subjects have been found to have lower degrees of arterial distensibility than non-obese subjects^{5, 6}; however, the mechanism responsible for the decrease in arterial distensibility in obese subjects has not been elucidated.

Obesity is characterized by a chronic systemic inflammatory state⁷. Chronic inflammation is indicated by elevated plasma levels of C-reactive protein (CRP)^{8, 9}, tumor necrosis factor- α (TNF- α)¹⁰, monocyte chemoattractant protein-1 (MCP-1)¹¹, and adhesion molecules¹² and has been implicated in the progression of atherosclerosis. Recently, pentraxin 3 (PTX3) has been identified as a new inflammatory

Address for correspondence: Seiji Maeda, Division of Sports Medicine, Graduate School of Comprehensive Human Sciences, University of Tsukuba, Tsukuba, Ibaraki 305-8577, Japan
E-mail: smaeda@tara.tsukuba.ac.jp

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substance¹³). PTX3 is a member of the pentraxin protein family and is mainly produced in atherosclerotic vessels¹⁴⁻¹⁶. Furthermore, it has been reported that plasma PTX3 concentrations were higher in patients with heart failure and that these concentrations varied according to the severity of disease^{13, 17}; therefore, plasma PTX3 levels may indicate vascular inflammation.

In obese subjects, both chronic systemic inflammation and decreased arterial distensibility are serious risk factors, because both of these factors are involved in the pathophysiology of CVDs; however, the relationship between vascular inflammation and decreased arterial distensibility in obesity remains unclear. Since the plasma PTX3 level may indicate vascular inflammation, we hypothesized that higher PTX3 levels in obese subjects are involved in the mechanism underlying the obesity-induced decrease in arterial distensibility. The aim of this study was to investigate plasma PTX3 concentration and arterial distensibility in obese men. To test our hypothesis, we measured carotid arterial distensibility (on the basis of arterial compliance and β -stiffness index) and plasma PTX3 concentrations in obese and non-obese men.

Methods

Subjects

Eleven obese men (age, 44 ± 2 years; BMI, 32 ± 1 kg/m²; obesity group) and 14 non-obese men (age, 42 ± 2 years; BMI, 26 ± 1 kg/m²; control group) participated in this study. Obesity is internationally defined as BMI ≥ 30 kg/m² by WHO. In this study, normal (BMI $18 < 25$ kg/m²) and overweight (BMI $25 < 30$ kg/m²) subjects were included in the non-obese (control) group. White *et al.*¹⁸ reported that subjects with BMI > 25 kg/m² (BMI $25 < 30$ kg/m²) were included in non-obese subjects; therefore, we adopted BMI ≥ 30 kg/m² as obese men in this study. All subjects were free of the signs, symptoms, and history of any overt chronic disease. None of the participants had an active lifestyle (regular exercise), and none were currently smokers or taking any medications. All measurements were obtained after abstinence from caffeine and an overnight fast. Subjects were examined in the supine position in a quiet, temperature-controlled room (24–26°C). All measurements were acquired after a resting period of at least 20 min.

This study was reviewed and approved by the institutional review board at the University of Tsukuba. The study conformed to the principles outlined in the Helsinki Declaration. All potential risks and procedures involved in the study were explained to the

subjects, and written informed consent to participate in the study was obtained from all subjects.

Anthropometric Variables

Body weight was measured to the nearest 0.1 kg using a digital scale. Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer. BMI was calculated as weight (in kilograms) divided by the square of the height (in metres). Waist circumference was measured to the nearest 0.1 cm at the level of the umbilicus with the subjects in the standing position.

Blood Pressure

Systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean arterial pressure (MAP) in the supine position were recorded from the left arm using a semi-automated device (formPWV/ABI; Colin Medical Technology, Komaki, Japan). Pulse pressure (PP) was calculated as the SBP minus DBP. Heart rate (HR) was measured with the subjects in the supine position using a semi-automated device (formPWV/ABI).

Carotid Artery Compliance

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. 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. Longitudinal images of the cephalic portion of the common carotid artery were obtained 1–2 cm proximal to the carotid bulb, with the transducer placed at a 90° angle to the vessel such that the proximal and distal wall boundaries were clearly discernible. These images were recorded on a computer for subsequent offline analysis. Computer images were analyzed using image-analysis software. All image analyses were performed by the same investigator. Intima-media thickness (IMT) of the far wall was evaluated as the distance between the lumen-intima interface and the media-adventitia interface in ten frozen basal diastolic frames. The IMT measurement was obtained from three contiguous sites at 1-mm intervals in each frame, and the average of thirty measurements was used for analyses. Time points that corresponded with maximum systolic expansion and the basal (minimum) diastolic relaxation of carotid artery were selected to measure vascular diameter. Subse-

quently, the distances (or diameter) between the distal boundaries of the vessel wall, corresponding to the boundary of the tunica adventitia and tunica media, were measured.

To characterize central arterial compliance as comprehensively as possible, 2 different measures—namely, arterial compliance¹⁹⁾ and β -stiffness index²⁰⁾—were used. Cross-sectional compliance was calculated on the basis of the change in the cross-sectional area (dA), local pulse pressure (dP), arterial diameter (D) and the change in arterial diameter during the heart cycle (dD), using the following formula: Cross-sectional compliance = dA/dP. dA was calculated as $dA = \pi \cdot [(D + dD)/2]^2 - \pi \cdot (D/2)^2$. Pressure wave forms of the left common carotid artery were recorded with an applanation tonometry device (formPWV/ABI) and calibrated by equating the mean arterial and diastolic blood pressure values of the carotid artery to those of the brachial artery²⁰⁾.

The β -stiffness index is an index of arterial compliance adjusted for distending pressure and 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 arterial diameters, and P_s and P_d are the highest and lowest blood pressures, respectively²⁰⁾.

Blood Biochemistry

Each blood sample was placed in a chilled tube containing ethylenediaminetetraacetic acid (EDTA) (2 mg/mL), which was then centrifuged at 2,000 g for 15 min at 4°C. The plasma thus obtained was stored at -20°C until assayed. Plasma concentrations of PTX3 were determined using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Quantakine DPTX 30; R&D Systems Inc., Minneapolis, USA). Serum concentrations of total cholesterol (TCHO), high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), and triglycerides (TG), as well as the plasma concentrations of glucose (BG), were determined using standard enzymatic techniques.

Statistical Analysis

Data are expressed as the means $\pm$ SE. To evaluate statistical differences between the control (BMI <30) and obesity (BMI $\geq$ 30) groups, Student's *t*-test for unpaired values was used. The relationship between body weight and plasma PTX3 concentrations was analyzed using Pearson's correlation. Moreover, to study whether there was an independent relationship between plasma PTX3 levels and body weight, stepwise regression analysis was used. In the model, we took plasma PTX3 levels as the dependent variable,

Table 1. Characteristics of obese (BMI $\geq$ 30) and non-obese (BMI <30) men

	Non-obese men	Obese men
Age, years	42 $\pm$ 2	44 $\pm$ 2
Height, m	1.73 $\pm$ 0.02	1.72 $\pm$ 0.02
Body weight, kg	78.2 $\pm$ 2.4	94.4 $\pm$ 1.5**
Body mass index, kg/m ²	26.0 $\pm$ 0.7	31.9 $\pm$ 0.8**
Waist circumference, cm	92.6 $\pm$ 2.1	104 $\pm$ 1.5**
Total cholesterol, mg/dL	194 $\pm$ 8	210 $\pm$ 9
Triglycerid, mg/dL	140 $\pm$ 23	165 $\pm$ 20
HDL-cholesterol, mg/dL	50 $\pm$ 3	53 $\pm$ 3
LDL-cholesterol, mg/dL	118 $\pm$ 8	124 $\pm$ 6
Fasting blood glucose, mg/dL	97 $\pm$ 2	106 $\pm$ 9
Intima-media thickness, mm	0.49 $\pm$ 0.03	0.55 $\pm$ 0.05

Data are expressed as the means $\pm$ SE. Significant difference obese men vs. non-obese men, ***p* < 0.01.

Table 2. Hemodynamics of obese (BMI $\geq$ 30) and non-obese men (BMI <30)

	Non-obese men	Obese men
Systolic blood pressure, mmHg	131 $\pm$ 6	134 $\pm$ 3
Diastolic blood pressure, mmHg	87 $\pm$ 4	94 $\pm$ 3
Mean arterial pressure, mmHg	99 $\pm$ 4	101 $\pm$ 2
Pulse pressure, mmHg	44 $\pm$ 3	40 $\pm$ 4
Heart rate, bpm	62 $\pm$ 2	63 $\pm$ 2

Data are expressed as the means $\pm$ SE.

and age, body weight, BMI, SBP, DBP, MAP, PP, HR, arterial compliance, β -stiffness, waist circumference, HDLC, TG, LDL, and BG as independent variables. *p* < 0.05 was accepted as significant.

Results

Table 1 show the characteristics of obese and control (non-obese) subjects. Body weight, BMI and waist circumference were significantly greater in the obese group than in the control group; however, no significant differences were observed between the control group and obesity group in terms of height, serum concentrations of TCHO, LDL, HDL, and TG, plasma BG concentration, and IMT (**Table 1**). **Table 2** shows the haemodynamics in the obesity and control groups. No significant differences were detected between groups with regard to SBP, DBP, MAP, PP, and HR (**Table 2**). Arterial compliance was significantly lower in the obesity group than in the control group (**Fig. 1A**). The β -stiffness index was significantly higher in the obesity group than in the control

Fig. 1. Arterial compliance (A) and β -stiffness index (B) in obese men and non-obese men (controls).

Data are expressed as the means $\pm$ SE.

group (**Fig. 1B**). The plasma PTX3 concentration was markedly higher in the obesity group than in the control group (**Fig. 2**). A significant positive correlation was noted between the plasma PTX3 concentration and body weight ($r=0.47$, $p>0.05$). In a stepwise regression model using plasma PTX3 levels as the dependent variable, the independent predictor was only body weight ($R^2=0.22$; $p<0.05$).

Discussion

In the present study, we measured plasma PTX3 concentrations and arterial distensibility in obese men. Carotid arterial compliance was significantly lower and the β -stiffness index was obviously higher in obese than non-obese men. Thus, arterial distensibility sig-

Fig. 2. Plasma pentraxin 3 (PTX3) concentrations in obese men and non-obese men (controls).

Data are expressed as the means $\pm$ SE.

nificantly decreased due to obesity. We also found that the plasma PTX3 concentration was markedly higher in obese than non-obese control subjects. Furthermore, the relationship of body weight to plasma PTX3 concentration was linear; therefore, the increase in plasma PTX3 concentration in obese men may be involved in the mechanism underlying the obesity-induced decrease in arterial distensibility.

Obesity is strongly associated with CVD^{1,2}. Further, reduced arterial distensibility has been identified as an independent risk factor for CVD^{3,4}. It has been reported that obese subjects are characterized by lower aortic elasticity⁵ and higher aortic pulse-wave velocity (PWV)⁶ (a traditional index of arterial stiffness) than age-matched non-obese subjects. In the present study, we showed that carotid arterial compliance is significantly lower and β -stiffness index is significantly higher in obese than non-obese men; thus, the results of previous and the present study clearly show that arterial distensibility significantly decreases in obesity.

Obesity is characterized by a chronic systemic inflammatory condition^{7, 21, 22}. Previous studies have reported that the levels of inflammatory markers, such as CRP, interleukin (IL)-6, TNF- α , IL-1, MCP-1, adiponectin, and adhesion molecules (inter-cellular adhesion molecule (ICAM)-1, vascular cell adhesion molecule (VCAM)-1, P-selectin and E-selectin), increase in obesity, thereby contributing to the development of CVD^{8-12, 23, 24}. In particular, the level of CRP is widely used for clinically monitoring the course of CVD²⁵. It has been reported that, even in healthy individuals, the level of CRP can be used as an indicator of future CVD risk^{25, 26}; however, because CRP is synthesized and secreted in the liver, the level of CRP

cannot directly indicate vascular inflammation. Thus, CRP values are of limited use in the estimation of vascular inflammation.

Chronic inflammation, foci of macrophages and T-lymphocytes, proliferation and migration of smooth muscle cells, matrix formation and neovascularization, are processes leading to atherosclerosis²⁷⁾. Ross proposed the 'chronic endothelial injury hypothesis', according to which all arterial wall elements are closely related in the pathogenesis of atherosclerosis due to inflammatory factors^{27, 28)}. Several previous investigations demonstrated that pro-inflammatory cytokines and CRP are involved in the acceleration of aortic atherosclerosis and decreased arterial distensibility^{29, 30)}. Furthermore, it has been confirmed that some anti-inflammatory drugs decrease aortic stiffness and help to prevent cardiovascular diseases^{31, 32)}. Recently, PTX3, a pentraxin, was identified as an inflammatory protein¹³⁾. Some reports have revealed that, in humans, PTX3 is produced in areas of atherosclerosis and may contribute to the pathogenesis of atherosclerosis^{16, 33, 34)}. It has been shown that endothelial cells, macrophages and smooth muscle cells involved in atherosclerosis produce PTX3^{16, 35)}; therefore, levels of PTX3 may directly indicate the status of vascular inflammation. It has been reported that circulating PTX3 concentrations were higher in patients with heart failure and that these concentrations varied depending on the severity of CVD^{13, 17, 36, 37)}; however, it has not yet been confirmed if the levels of circulating PTX3 in obese subjects are higher than in non-obese subjects. Our present study revealed that the plasma PTX3 concentration was higher in obese than non-obese men. We have also demonstrated that carotid arterial compliance was lower and the β -stiffness index was higher in obese than non-obese men. These results suggest that, in obese subjects, PTX3 levels increase as arterial distensibility decreases; therefore, we propose that the increase in PTX3 in obese men may be involved, at least in part, in the mechanism underlying the obesity-induced decrease in arterial distensibility.

There are several limitations of this study that should be emphasized. First, BMI is not exactly associated with fat mass, which is a good indicator of obesity; however, we did not measure fat mass in this study. Second, a significant positive correlation between plasma PTX3 levels and body weight may not always indicate that a higher plasma PTX3 level in obese subjects could result in lower carotid arterial compliance and a higher β -stiffness index. We have analyzed the relation between plasma PTX3 levels and carotid arterial compliance or the β -stiffness index. There were

no significant correlations between the plasma PTX3 level and arterial compliance or the β -stiffness index. Although PTX3 is produced by various tissues and cells, such as vascular endothelial cells and macrophages, we measured plasma levels of PTX3. It is possible that there is a correlation between the arterial tissue PTX3 level and arterial distensibility; however, the precise roles of PTX3 in obesity-related arterial stiffening remain to be elucidated. Third, although carotid arterial compliance was lower and the β -stiffness index was higher in obese than non-obese men, there was no significant difference in IMT between non-obese and obese men. Obese subjects in the present study were free of signs, symptoms, and a history of any overt chronic disease, which may explain the lack of difference in IMT between non-obese and obese men.

In conclusion, the present study was the first to reveal that circulating PTX3 levels are markedly higher in obese than non-obese men. We have also demonstrated that arterial distensibility is decreased in obese men, as indicated by the lower carotid arterial compliance and higher β -stiffness index in obese than non-obese men; therefore, higher PTX3 levels and decreased arterial distensibility coexist in obese men. Taken together, higher PTX3 levels in obese men may be involved in the mechanism underlying the obesity-induced decrease in arterial distensibility.

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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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