Systemic Oxidative Stress is Associated With Visceral Fat Accumulation and the Metabolic Syndrome

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Background The metabolic syndrome (MetS) is a major target for prevention of atherosclerotic cardiovascular diseases and visceral fat accumulation is an underlying component of MetS. The aim of this study was to investigate the association of systemic oxidative stress with visceral fat accumulation and MetS.

Methods and Results The study group consisted of Japanese men ($n=44$; 51.2 ± 11.4 years) and women ($n=61$; 55.4 ± 13.4 years). Urinary 8-epi-prostaglandin F₂ (8-epi-PGF₂) concentration, a biomarker of systemic oxidative stress, was significantly high in the subjects with MetS. As the urinary concentration of 8-epi-PGF₂ increased, the number of criteria for MetS were significantly met (abdominal obesity, hypertriglyceridemia, low high-density lipoprotein-cholesterol, hypertension, and high fasting glucose). Among parameters associated with MetS, the correlation coefficient of visceral fat area (VFA) with urinary 8-epi-PGF₂ concentration was the highest ($r=0.636$, $p<0.0001$). In non-obese subjects, the correlation coefficient of VFA with urinary 8-epi-PGF₂ concentration was higher ($r=0.728$, $p<0.0001$), although there was no significant correlation between subcutaneous fat area and urinary 8-epi-PGF₂. Stepwise multiple regression analysis identified VFA as the strongest and independent determinant of urinary 8-epi-PGF₂ ($p<0.0001$) followed by adiponectin ($p=0.0212$) and, high sensitive C-reactive protein ($p=0.0365$).

Conclusions Systemic oxidative stress, as measured by urinary 8-epi-PGF₂, is strongly associated with visceral fat accumulation and MetS. (Circ J 2006; 70: 1437–1442)

Key Words: Metabolic syndrome; Systemic oxidative stress; Urinary 8-epi-PGF₂; Visceral fat accumulation

The metabolic syndrome (MetS), a cluster of glucose intolerance, hypertension, and dyslipidemia with visceral fat accumulation, is a common health problem in industrialized countries and its management is a major target for the prevention of atherosclerotic cardiovascular diseases.^{1–4} There is evidence for the role of obesity, particularly abdominal or intra-abdominal visceral fat accumulation, in promoting the development of metabolic diseases including glucose intolerance, dyslipidemia, hypertension, and atherosclerosis.^{5–9} Adipose tissue was previously considered an energy-storage organ, but numerous studies have demonstrated recently that adipose tissue produces and secretes a variety of biologically active molecules, conceptualized as adipocytokines,^{1,4,10–14} such as plasminogen activator inhibitor-1 (PAI-1),¹⁰ tumor necrosis factor (TNF)- α ,¹¹ resistin,¹² leptin¹³ and adiponectin.¹⁴ Importantly, dysregulated production of adipocytokines in obesity is involved in the development of MetS.^{1,4,10–15} Increased production of

PAI-1 and TNF- α from accumulated fat causes thrombosis, and atherosclerosis, respectively.^{10,16,17} Adiponectin exhibits anti-atherogenic^{18–20} and insulin-sensitizing properties.^{21,22} Hypoadiponectinemia, associated with visceral fat area (VFA), is considered to play an important role in the development of coronary artery disease and type 2 diabetes in MetS.^{23–25}

Oxidative stress has been implicated in the pathogenesis of atherosclerosis.^{26–30} Reactive oxygen species (ROS) resulting from NADPH oxidase activation in cardiovascular cells are involved in the adhesion and migration of monocyte/macrophages, proliferation of vascular smooth muscle cells and fibroblasts, and remodeling of the extracellular matrix, leading to atherosclerotic cardiovascular diseases.²⁶ Recently, our group demonstrated that, in obese mice, production of ROS increased selectively in accumulated fat but not in muscle, liver, and aorta. The increase in fat ROS was associated with augmented expression of NADPH oxidase and decreased expression of antioxidative enzymes such as Cu,Zn-superoxide dismutase and catalase in adipose tissue.³¹ These results suggest that obesity per se can induce systemic ROS. The same study also demonstrated that fat ROS can trigger the dysregulation of adipocytokines, such as decreased adiponectin and increased PAI-1 and TNF- α , which would lead to the development of MetS. Treatment with NADPH oxidase inhibitor ameliorated adipocytokine dysregulation and improved the metabolic disorders. Therefore, fat ROS are considered a suitable therapeutic target and a biomarker for MetS. However, the association between systemic ROS and fat distribution, especially visceral fat

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Table 1 Clinical Characteristics of the Study Subjects

	MetS (–) (n=69)	MetS (+) (n=36)	p value
Age (years)	53.3±13.7	54.2±10.6	NS
BMI (kg/m ²)	25.8±5.0	28.7±3.9	0.0034
Waist circumference (cm)	91.0±13.1	96.9±6.6	0.0128
VFA (cm ²)	115.6±71.6	183.7±94.6	0.0012
SFA (cm ²)	171.2±94.0	200.9±87.8	NS
Systolic blood pressure (mmHg)	124.5±15.7	144.0±21.2	<0.0001
Diastolic blood pressure (mmHg)	71.2±11.0	84.5±9.9	<0.0001
Total cholesterol (mmol/L)	5.21±0.94	5.90±1.04	0.0009
Triglyceride (mmol/L)	1.36±0.81	2.58±1.40	<0.0001
HDL-cholesterol (mmol/L)	1.49±0.41	1.30±0.28	0.0147
Fasting plasma glucose (mmol/L)	5.67±1.52	6.98±1.57	<0.0001
Hemoglobin A1c (%)	5.58±0.94	6.41±1.19	0.0002
Adiponectin (μg/ml)	6.73±3.31	5.11±2.20	0.0263
hsCRP (mg/L)	1.80±3.13	3.11±3.77	0.0017
Smoking (%)	15	11	NS
Urinary 8-epi-PGF2 (pg/mg Cr)	234.9±134.7	309.4±112.3	0.0054

Data are mean ± SD.

MetS, metabolic syndrome (Japanese criteria); NS, not significant; BMI, body mass index; VFA, visceral fat area; SFA, subcutaneous fat area; HDL, high-density lipoprotein; hsCRP, high-sensitive C-reactive protein; 8-epi-PGF2, 8-epi-prostaglandin F2.

accumulation, which plays a key role in MetS, has not been defined in human subjects.

In the present study, we investigated the correlations between systemic oxidative stress, measured by urinary 8-epi-PGF2 concentration, and various conditions associated with MetS.

Methods

Subjects

Among 261 Japanese adults who visited the university hospital for a health checkup between 2003 and 2005, 105 [44 men (mean age ± SD, 51.2 ± 11.4 years) and 61 women (55.4 ± 13.4 years)] were recruited into the study. Subjects were excluded if they did not agree to participate, had a history of diabetes mellitus, cardiovascular or cerebrovascular disease, hepatic and/or renal disease, or were being treated with antihypertensive or antihyperlipidemic medications. Interested participants visited our laboratories by self-referral or by recommendation for further check-up after local center screening. The study protocol complied with the Guidelines of the Ethical Committees of Osaka University and University of the Ryukyus. Informed consent was given by all subjects.

Anthropometry and Abdominal Fat Distribution

Anthropometric measurements (height, weight and waist circumference (WC)) were performed in a standing position. Body mass index (BMI) was calculated as weight divided by the square of height (kg/m²). WC at the umbilical level was measured with a non-stretchable tape in the late exhalation phase while standing.³² Systolic and diastolic blood pressures were measured in the sitting position to the nearest mmHg. Abdominal fat distribution was determined using computed tomography (CT) while the subjects were supine.³³ Ordinary CT parameters were used, specifically 120 kV and 200 mA, as well as a slice thickness of 5 mm, a scanning time of 2 s, and a field of view of 400 mm. The subcutaneous fat area (SFA) and intra-abdominal VFA were measured at the level of the umbilicus and determined by a standardized method with CT numbers. Briefly, a region of interest of the subcutaneous fat layer was defined by tracing its contour

on each scan, and the attenuation range of CT numbers (in Hounsfield units) for fat tissue was calculated. A histogram for fat tissue was computed on the basis of mean attenuation ± 2SD. Total and intraperitoneal tissue with attenuation within the mean ± 2SD were considered to be the total fat area (TFA) and VFA, and the SFA was defined by subtracting the VFA from the TFA. Smoking was assessed using a smoking index, defined cigarettes/day × years.

Laboratory Measurements

Blood was drawn after an overnight fast and plasma concentrations of adiponectin were measured by a sandwich enzyme-linked immunosorbent assay (ELISA) system (Adiponectin ELISA Kit, Otsuka Pharmaceutical Co, Tokushima, Japan).¹⁵ Plasma glucose concentrations were determined by glucose oxidase method. The value of hemoglobin A1c (HbA1c) was determined by high-performance liquid chromatography. Serum total cholesterol and triglyceride concentrations were determined by enzymatic methods. High-density lipoprotein (HDL)-cholesterol was also measured by enzymatic method after heparin and calcium precipitation. Serum concentration of C-reactive protein (CRP) was measured with high-sensitive CRP (hsCRP) assay (Denka Seiken). A single urine sample was subjected to analysis of systemic oxidative stress using urinary 8-epi-prostaglandin F2 (8-epi-PGF2) as the marker³⁴ in an enzyme immunoassay kit (Assay Design Inc), as described previously.^{31,35,36} The urinary concentration of 8-epi-PGF2 was indexed to that of urinary creatinine (Cr) and expressed in pg/mg Cr.

Definition of MetS

MetS was defined according to the 2005 guidelines of the Japanese Society of Internal Medicine or to the American Heart Association/National Heart, Lung, and Blood Institute (AHA/NHLBI) criteria.^{37,38} In the Japanese guideline, which is similar to the IDF criteria,^{39,40} subjects with MetS must have: abdominal obesity (defined as WC ≥ 85 cm in men or ≥ 90 cm in women⁴¹), and plus any 2 of the following 3 factors: (1) dyslipidemia: hypertriglyceridemia (serum triglyceride concentration ≥ 150 mg/dl [1.69 mmol/L]) and/or low HDL-cholesterol (serum concentration < 40 mg/dl

Fig 1. Urinary 8-epi-prostaglandin F2 (8-epi-PGF2) concentration and metabolic syndrome (MetS). (a) Urinary 8-epi-PGF2 concentrations in subjects with or without MetS. (Left panel) MetS (-): n=69, MetS (+): n=36, diagnosed by the Japanese criteria; (Right panel) MetS (-): n=55, MetS (+): n=50, diagnosed by the American Heart Association/National Heart, Lung, and Blood Institute (AHA/NHLBI) criteria. Data are mean $\pm$ SEM. * p <0.01 by Student's t-test. (b) Prevalence of MetS according to the category of urinary 8-epi-PGF2 concentration (see text for details). Analysis using the Japanese criteria (Left panel) or the AHA/NHLBI criteria (Right panel). (c) The number of components of MetS (abdominal obesity, hypertriglyceridemia, low high-density lipoprotein-cholesterol, hypertension, and high fasting glucose) in each category of urinary 8-epi-PGF2 concentration. Data are mean $\pm$ SEM. * p <0.05, ** p <0.01, *** p <0.001 vs category 1 by Kruskal-Wallis test and Scheffe's test.

[1.04 mmol/L]); (2) hypertension: systolic blood pressure $\geq$ 130 mmHg and/or diastolic blood pressure $\geq$ 85 mmHg; (3) high fasting glucose: serum glucose concentration $\geq$ 110 mg/dl (6.1 mmol/L). In the present study, abdominal obesity was defined WC $\geq$ 85 cm in men or $\geq$ 90 cm in women as reported for Japanese population.⁴¹

Statistical Analysis

All statistical analyses were performed with Stat View-J 5.0 (Statistical Analysis System Inc, Cary, NC, USA). Because plasma adiponectin concentrations, hsCRP, and serum triglyceride concentrations did not show a Gaussian distribution, these 3 parameters were log-transformed before analysis. We compared clinical characteristics in the subjects with or without MetS (Table 1) using the Student's t-test. Only the prevalence of smokers was analyzed by the chi-square test. The subjects were then categorized into 4 groups according to the urinary concentrations of 8-epi-PGF2. The prevalence of the MetS in each category was analyzed by Mann-Whitney U-test. Comparisons of the mean values of components of the MetS in each category were analyzed by the Kruskal-Wallis test and Scheffe's test. Pearson's correlation coefficient was used to examine the association between urinary 8-epi-PGF2 concentration and various parameters of MetS. To identify the parameters of

MetS that contribute significantly to urinary 8-epi-PGF2 concentration, stepwise multiple regression analysis was conducted. Parameters with an F-value $>$ 4.0 were entered into the regression analysis as independent variables. A p-value less than 0.05 denoted a statistically significant difference.

Results

The basic anthropometric and metabolic characteristics of the subjects enrolled in this study are presented in Table 1. Based on the Japanese criteria, 34% had MetS (Table 1, 40% of the enrolled men and 29% of the enrolled women), whereas according to the AHA/NHLBI criteria, 47% of the enrolled subjects had MetS (52% of men and 44% of women). As expected, between the subjects with and without MetS according to Japanese criteria, there were significant differences in several parameters related to MetS, including BMI, WC, VFA, systolic and diastolic blood pressures, total cholesterol, triglyceride, HDL-cholesterol, fasting plasma glucose, HbA1c, adiponectin, and hsCRP (Table 1). Urinary concentration of 8-epi-PGF2 was significantly higher in subjects diagnosed with MetS (either definition) than in those without (Table 1, Fig 1a). There was no difference between male and female subjects

Table 2 Correlation Coefficients of the Relationships Between Urinary 8-epi-PGF2 Concentration and Various Parameters of the MetS

	<i>r</i>	<i>p</i> value
Age	0.228	0.0193
BMI	0.585	<0.0001
Waist circumference	0.559	<0.0001
VFA	0.636	<0.0001
SFA	0.493	<0.0001
Systolic blood pressure	0.327	0.0006
Diastolic blood pressure	0.118	NS
Total cholesterol	0.181	NS
Triglyceride	0.403	<0.0001
HDL-cholesterol	-0.198	0.0425
Fasting plasma glucose	0.127	NS
Hemoglobin A1c	0.207	0.0337
Adiponectin	-0.464	<0.0001
hsCRP	0.529	<0.0001
Smoking index	0.205	0.0354

Smoking index is expressed as cigarettes/day×years.

Abbreviations see in Table 1.

in the urinary concentrations of 8-epi-PGF2 (men: 260±107.7 pg/mg Cr; women: 260.7±147.8 pg/mg Cr).

We categorized the subjects into quartiles according to the urinary 8-epi-PGF2 concentration (category 1 (n=25): 40.0–145.0 pg/mg Cr; category 2 (n=25): 150.0–224.0 pg/mg Cr; category 3 (n=28): 231.0–331.0 pg/mg Cr; category 4 (n=27): 332.0–710.7 pg/mg Cr). The prevalence of MetS (both definitions) significantly increased with increased 8-epi-PGF2 urinary concentration (Japanese criteria: $p=0.0014$; AHA/NHLBI criteria: $p=0.0018$, Mann-Whitney U-test) (Fig 1b). Furthermore, the number of components of the MetS (abdominal obesity, hypertriglyceridemia, low HDL-cholesterol, hypertension, and high fasting glucose) significantly increased with increased 8-epi-PGF2 urinary concentration (Fig 1c). We further investigated the relationship between urinary 8-epi-PGF2 concentration and selected conditions associated with MetS and the coefficients are listed in Table 2.

Urinary 8-epi-PGF2 concentration correlated positively with age, and of the anthropometric parameters, there was a significant correlation with BMI, WC, VFA, and SFA. Urinary 8-epi-PGF2 concentration correlated positively with systolic blood pressure, HbA1c, triglycerides, and smoking index and negatively with HDL-cholesterol, but not with fasting plasma glucose, diastolic blood pressure, or total cholesterol. Plasma adiponectin and hsCRP concentrations correlated significantly with urinary 8-epi-PGF2 concentration. The coefficient for the correlation of VFA with urinary 8-epi-PGF2 concentration was the highest among all parameters analyzed ($r=0.636$, $p<0.0001$, Table 2). Furthermore, among non-obese subjects, the correlation

Fig 2. Association of urinary 8-epi-prostaglandin F2 (8-epi-PGF2) concentration with visceral fat area, subcutaneous fat area or body mass index (BMI) in the non-obese subjects (BMI <25, n=38). (a) Correlation between urinary 8-epi-PGF2 concentration and visceral fat area. (b) Association of urinary 8-epi-PGF2 concentration with subcutaneous fat area. (c) Association of urinary 8-epi-PGF2 concentration with BMI. NS, not significant.

Table 3 Stepwise Multiple Regression With Urinary 8-epi-PGF2 Concentration as the Dependent Variable

Independent variable	Regression coefficient	Standard error	Standardized regression coefficient	<i>p</i> value
VFA	0.779	0.176	0.467	<0.0001
Adiponectin	-170.914	72.091	-0.238	0.0212
hsCRP	57.866	27.012	0.223	0.0365
Intercept	296.788	65.849		<0.0001

Adjusted R²: 0.520.

Not accepted variables (F value < 4.0) were age, systolic blood pressure, hemoglobin A1c, triglyceride, HDL-cholesterol, and smoking index.

Abbreviations see in Table 1.

coefficient of VFA with urinary 8-epi-PGF2 concentration was higher (VFA: $r=0.728$, $p<0.0001$, Fig 2a) than that in all subjects ($r=0.636$, Table 2). There was no significant correlation between SFA or BMI and urinary 8-epi-PGF2 concentration in non-obese subjects (SFA: $r=0.035$, not significant (NS), Fig 2b; BMI: $r=0.251$, NS, Fig 2c).

To examine if the positive correlation between VFA and urinary 8-epi-PGF2 concentration was independent of other clinical factors associated with MetS, including age, smoking index, systolic blood pressure, HbA1c, triglyceride, HDL-cholesterol, hsCRP, and adiponectin, stepwise multiple regression analyses were performed. As shown in Table 3, VFA was the strongest and independent determinant of urinary 8-epi-PGF2 concentration ($p<0.0001$) followed by adiponectin ($p=0.0212$) and hsCRP ($p=0.0365$).

Discussion

The major findings of the present cross-sectional study indicate that the systemic oxidative stress increased in the subjects with MetS, and was strongly correlated with visceral fat accumulation.

Plasma levels of adipocytokines are dysregulated in visceral fat accumulation, including inflammatory adipocytokines, such as interleukin-6, TNF α , and CRP, and this chronic low-grade inflammation in MetS is involved in the pathogenesis of atherosclerotic cardiovascular diseases.^{42–45} Hypoadiponectinemia is also located upstream of the pathogenesis of metabolic diseases and atherosclerosis.^{23–25} However, the precise molecular mechanism of adipocytokine dysregulation has not been fully elucidated. Using animal models, we recently reported that plasma H₂O₂, a hazardous ROS, is already augmented in the obese and non-diabetic state, and that increased levels of ROS primarily originate from accumulated fat, not from liver, muscle or aorta.³¹ That same study also demonstrated that increased ROS in obesity caused dysregulation of adipocytokine production, including decreased adiponectin and increased PAI-1 and TNF α , and that treatment with antioxidants in vivo redressed the adipocytokine dysregulation, resulting in improvement of glucose intolerance, hypertriglyceridemia, and fatty liver in obese mice.³¹ These findings suggest that increased fat ROS in obesity is a candidate upstream factor for adipocytokine dysregulation leading to MetS.

The Framingham study revealed that BMI was highly associated with systemic oxidative stress.⁴⁶ However, whether ROS are linked more to visceral or to subcutaneous fat accumulation is still unknown. In the present study, we measured VFA and SFA by the CT cross-sectional method.³³ The urinary 8-epi-PGF2 concentration was more strongly associated with visceral fat accumulation than with subcutaneous fat in all subjects enrolled in this study (Table 2). In the non-obese subjects, VFA was more significantly correlated with urinary 8-epi-PGF2 than in all subjects, although there was no significant correlation between SFA or BMI and urinary 8-epi-PGF2 concentration (Fig 2). Moreover, stepwise multiple regression analysis indicated that visceral fat accumulation was an independent indicator of systemic oxidative stress, whereas glucose intolerance, hypertension, dyslipidemia, or aging was not. Thus, these results suggest that systemic oxidative stress, represented by the urinary 8-epi-PGF2 concentration, is strongly associated with visceral fat accumulation leading to MetS. Very recently, it was reported that there was no increase in the urinary 8-epi-PGF2 concentration in otherwise healthy

63-year-old men in Sweden with MetS,⁴⁷ which might be explained by the age of the subjects. The average age of the subjects in that study was almost 10 years older than that in the present study. Further investigation is required to verify an age-related association between systemic oxidative stress and MetS.

Smoking has been reported to be associated with systemic oxidative stress,^{46,48,49} and in the present study, the urinary 8-epi-PGF2 concentration of smokers ($n=15$) was higher than that of non-smokers ($n=90$) ($p=0.0640$), and the smoking index (cigarettes/day $\times$ years) significantly correlated with urinary 8-epi-PGF2 concentration ($r=0.205$, $p=0.0354$) as shown in Table 2. The smoking index was not found to be an independent determinant of urinary 8-epi-PGF2 concentration in the present study, which may be related to the small number of smokers enrolled in this study. Among the non-smokers only, VFA was also the strongest and independent determinant of urinary 8-epi-PGF2 concentration (data not shown).

Study Limitations

First, this is a cross-sectional study of a limited number of subjects. Second, the study group comprised Japanese men and women who visited hospital for a health checkup, including participants coming by self-referral or by recommendation from local center screening. Therefore, the subjects included a higher ratio of subjects diagnosed with MetS as compared to the general population.⁵⁰

Conclusion

We have demonstrated that systemic oxidative stress, measured by urinary 8-epi-PGF2 concentration, is closely associated with visceral fat accumulation and MetS. It is important to investigate in a future prospective study whether a reduction of visceral fat by life-style intervention is associated with decreased systemic oxidative stress. It is suggested that oxidative stress, especially fat ROS, could be an important therapeutic target for future intervention strategies for visceral fat accumulation and MetS.

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