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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 F2α (8-epi-PGF2α) concentration, a biomarker of systemic oxidative stress, was significantly high in the subjects with MetS. As the urinary concentration of 8-epi-PGF2α 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-PGF2α concentration was the highest ($r=0.636$, $p<0.0001$). In non-obese subjects, the correlation coefficient of VFA with urinary 8-epi-PGF2α concentration was higher ($r=0.728$, $p<0.0001$), although there was no significant correlation between subcutaneous fat area and urinary 8-epi-PGF2α. Stepwise multiple regression analysis identified VFA as the strongest and independent determinant of urinary 8-epi-PGF2α ($p<0.0001$) 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-PGF2α, 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-PGF2α; 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] leptin[13] 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-PGF2α (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-PGF2α, 8-epi-prostaglandin F2α.

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-PGF2α 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/m^2). 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 F2α (8-epi-PGF2α) as the marker[34] in an enzyme immunoassay kit (Assay Design Inc), as described previously.[31,35,36] The urinary concentration of 8-epi-PGF2α 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 women[41]), 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

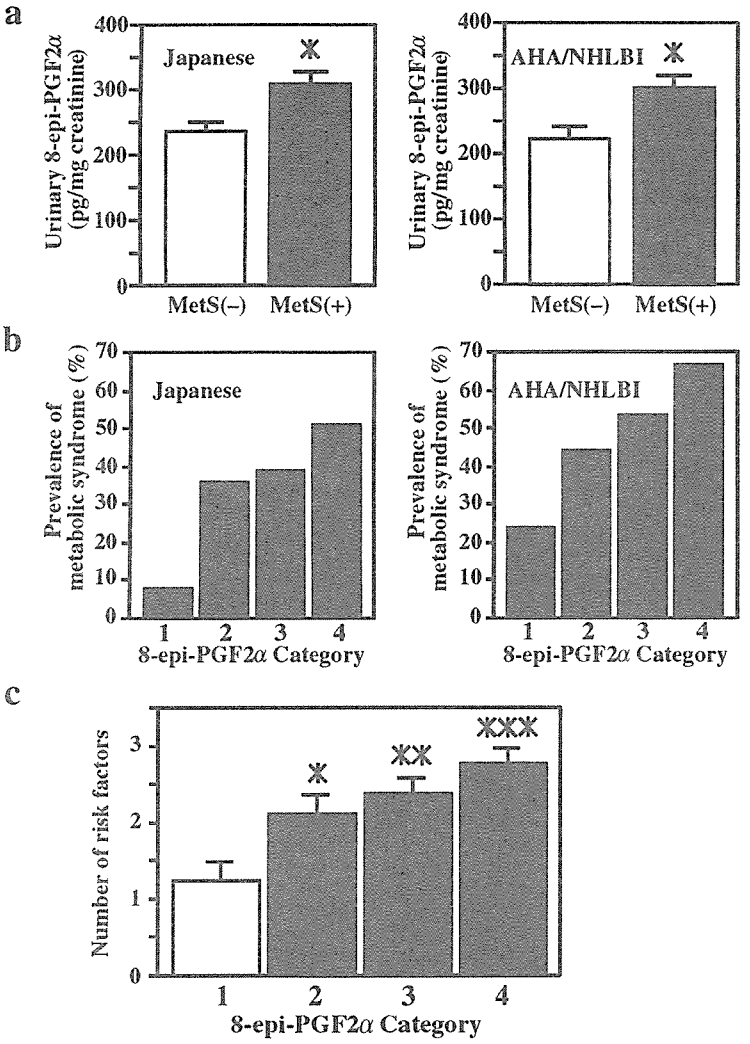

Fig 1. Urinary 8-epi-prostaglandin F2α (8-epi-PGF2α) concentration and metabolic syndrome (MetS). (a) Urinary 8-epi-PGF2α concentrations in subjects with or without MetS. (Left panel) MetS (−): n=69, MetS (+): n=36, diagnosed by the Japanese criteria; (Right panel) MetS (−): n=55, MetS (+): n=50, diagnosed by the American Heart Association/National Heart, Lung, and Blood Institute (AHA/NHLBI) criteria. Data are mean±SEM. *p<0.01 by Student's t-test. (b) Prevalence of MetS according to the category of urinary 8-epi-PGF2α 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-PGF2α concentration. Data are mean±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 ≥130 mmHg and/or diastolic blood pressure ≥85 mmHg; (3) high fasting glucose: serum glucose concentration ≥110 mg/dl (6.1 mmol/L). In the present study, abdominal obesity was defined WC ≥85 cm in men or ≥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-PGF2α. 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-PGF2α concentration and various parameters of MetS. To identify the parameters of MetS that contribute significantly to urinary 8-epi-PGF2α 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-PGF2α 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-PGF2α Concentration and Various Parameters of the MetS

	r	p 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-PGF2α (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-PGF2α 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-PGF2α 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-PGF2α urinary concentration (Fig 1c). We further investigated the relationship between urinary 8-epi-PGF2α concentration and selected conditions associated with MetS and the coefficients are listed in Table 2.

Urinary 8-epi-PGF2α concentration correlated positively with age, and of the anthropometric parameters, there was a significant correlation with BMI, WC, VFA, and SFA. Urinary 8-epi-PGF2α 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-PGF2α concentration. The coefficient for the correlation of VFA with urinary 8-epi-PGF2α concentration was the highest among all parameters analyzed (r=0.636, p<0.0001, Table 2). Furthermore, among non-obese subjects, the correlation

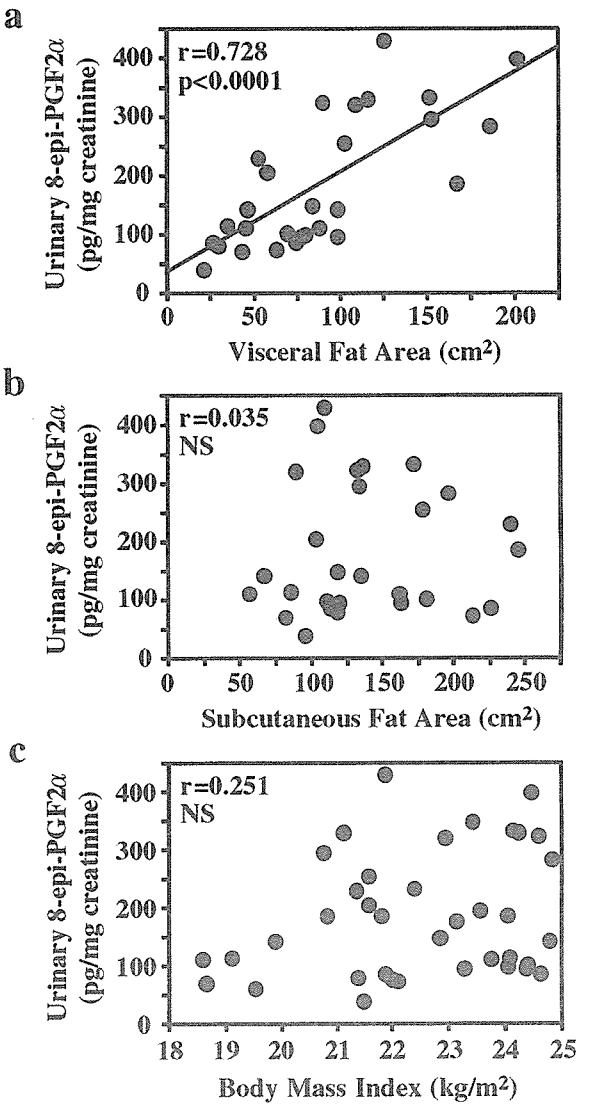

Fig 2. Association of urinary 8-epi-prostaglandin F2α (8-epi-PGF2α) concentration with visceral fat area, subcutaneous fat area or body mass index (BMI) in the non-obese subjects (BMI <25, n=38). (a) Correlation between urinary 8-epi-PGF2α concentration and visceral fat area. (b) Association of urinary 8-epi-PGF2α concentration with subcutaneous fat area. (c) Association of urinary 8-epi-PGF2α concentration with BMI. NS, not significant.

Table 3 Stepwise Multiple Regression With Urinary 8-epi-PGF2α Concentration as the Dependent Variable

Independent variable	Regression coefficient	Standard error	Standardized regression coefficient	p 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 R^2: 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-PGF2α 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-PGF2α 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-PGF2α 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-PGF2α 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, TNFα, 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 H_2O_2, 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-PGF2α 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-PGF2α than in all subjects, although there was no significant correlation between SFA or BMI and urinary 8-epi-PGF2α 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-PGF2α 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-PGF2α 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-PGF2α concentration of smokers (n=15) was higher than that of non-smokers (n=90) (p=0.0640), and the smoking index (cigarettes/day×years) significantly correlated with urinary 8-epi-PGF2α 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-PGF2α 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-PGF2α 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-PGF2α 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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Associations of Adiponectin Levels With Incident Impaired Glucose Metabolism and Type 2 Diabetes in Older Men and Women

The Hoorn Study

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OBJECTIVE — Adiponectin is an adipose tissue–derived protein. Low levels are associated with obesity, insulin resistance, and type 2 diabetes. Our objective was to investigate the prospective association between adiponectin levels and the 6.4-year risk of type 2 diabetes and of impaired glucose metabolism (IGM).

RESEARCH DESIGN AND METHODS — The Hoorn Study is a cohort study among Caucasians, aged 50–75 years. BMI, waist-to-hip ratio (WHR), fasting glucose, 2-h glucose, triglycerides, HDL cholesterol, LDL cholesterol, alanine aminotransferase, leptin, and adiponectin were measured at baseline. Lifestyle (alcohol intake, smoking, and physical activity) was assessed by questionnaires. After a mean follow-up of 6.4 years, glucose tolerance was assessed by a 75-g oral glucose tolerance test. Analyses were performed in 1,264 subjects (584 men and 680 women) without type 2 diabetes at baseline. For analyses of incident IGM, 239 subjects with IGM at baseline and/or type 2 diabetes at follow-up were excluded.

RESULTS — Age- and lifestyle-adjusted odds ratios and 95% CIs comparing highest with lowest adiponectin quartile were 0.52 (0.23–1.18) in men and 0.15 (0.06–0.39) in women for type 2 diabetes and 0.90 (0.51–1.61) and 0.28 (0.16–0.48) for IGM, respectively. The risks were only slightly reduced after adjustment for WHR and leptin as markers of (abdominal) adiposity. Adjustment for baseline fasting and postload glucose levels (potential mediators) substantially diminished these inverse associations with type 2 diabetes (0.79 [0.32–1.91] and 0.62 [0.21–1.81]) and with IGM (1.20 [0.61–2.35] and 0.48 [0.26–0.90]), respectively.

CONCLUSIONS — A high adiponectin level was strongly associated with a lower risk of IGM and type 2 diabetes, particularly in women. These results suggest that adiponectin is involved in the pathophysiology linking obesity to type 2 diabetes.

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The pathophysiology linking obesity to type 2 diabetes is not completely understood, but adipokines are thought to be involved (1). Adiponectin is a recently discovered protein that seems to be exclusively secreted by adipocytes and is the most abundant adipose tissue–derived protein (2,3). In contrast to other adipokines (such as leptin and interleukin-6) that are often elevated in obese subjects, adiponectin is reduced (3–6).

In animal studies, adiponectin has been shown to have insulin-sensitizing properties. Adiponectin knockout mice are insulin resistant in a gene-dose fashion (7,8). Overexpression of adiponectin prevented diabetes in transgenic mice (9), and administration of adiponectin reversed insulin resistance in various mouse models of obesity and diabetes (10). Adiponectin increased insulin action via effects on hepatic glucose production and by increasing fat oxidation and lowering circulating free fatty acids (10–13).

In humans, several cross-sectional studies showed that adiponectin correlates negatively with measures of insulin resistance and type 2 diabetes, but cause or consequence cannot be distinguished. Results from a few prospective studies suggest that a low adiponectin level is predictive of insulin resistance or diabetes in Pima Indians (14), Asian Indians (15), and Japanese subjects (16,17). Some of these studies are rather small (14,15) or had a short follow-up period (15,17). To our knowledge, the only prospective study in Caucasians was a nested case-control study based on self-reported diabetes after a follow-up of 2–3 years (18). All of these previous prospective studies were performed in relatively young subjects.

The objective of the present study was to investigate the association between adiponectin and subsequent 6.4-year incidence of abnormal glucose metabolism in a large population-based cohort of older men and women.

RESEARCH DESIGN AND METHODS

— The Hoorn Study is a population-based cohort study among 2,484 Caucasians (aged 50–75 years) that was performed in 1989–1991 and has been described previously (19). In 1996–1998, a follow-up examination took place. Of the 2,484 subjects, 150 subjects had died and 108 subjects had moved out

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Abbreviations: ALT, alanine aminotransferase; GFR, glomerular filtration rate; HMW, high molecular weight; IGM, impaired glucose metabolism; WHR, waist-to-hip ratio.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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Table 1—*Baseline characteristics of men by quartiles of adiponectin*

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P_{trend}	P_{trend}*
n	145	151	143	145		
Adiponectin (μg/ml)	5.66 ± 0.89	7.92 ± 0.65	10.30 ± 0.77	20.41 ± 6.75	<0.001	<0.001
Age (years)	57.4 ± 5.9	59.7 ± 6.5	60.5 ± 7.2	62.4 ± 7.0	<0.001	—
Anthropometry						
BMI (kg/m^2)	26.6 ± 2.8	26.2 ± 2.4	26.1 ± 3.6	25.1 ± 2.5	<0.001	<0.001
Waist circumference (cm)	95.6 ± 8.4	94.7 ± 7.5	94.0 ± 9.0	91.9 ± 8.1	<0.001	<0.001
Hip circumference (cm)	101.1 ± 5.7	100.3 ± 4.7	100.1 ± 5.1	99.3 ± 5.0	0.002	0.003
WHR	0.94 ± 0.05	0.94 ± 0.05	0.93 ± 0.07	0.93 ± 0.06	0.004	<0.001
Metabolic variables						
Fasting glucose (mmol/l)	5.48 ± 0.50	5.54 ± 0.51	5.41 ± 0.48	5.40 ± 0.50	0.046	0.013
2-h glucose (mmol/l)	5.39 ± 1.63	5.35 ± 1.70	4.83 ± 1.48	5.03 ± 1.51	0.006	<0.001
LDL cholesterol (mmol/l)	4.44 ± 0.93	4.52 ± 1.03	4.46 ± 0.93	4.53 ± 0.95	0.583	0.575
HDL cholesterol (mmol/l)	1.16 ± 0.31	1.14 ± 0.25	1.18 ± 0.24	1.33 ± 0.36	<0.001	<0.001
Triglycerides (mmol/l)	1.4 (1.1–2.2)	1.5 (1.1–2.1)	1.4 (1.0–1.7)	1.2 (1.0–1.7)	<0.001	<0.001
Systolic blood pressure (mmHg)	133.8 ± 18.8	132.0 ± 16.7	130.3 ± 16.3	134.2 ± 18.6	0.951	0.025
Diastolic blood pressure (mmHg)	85.0 ± 9.2	83.1 ± 9.6	81.7 ± 8.7	82.4 ± 10.7	0.011	0.013
Leptin (μg/l)	3.29 (1.73–5.59)	3.51 (1.79–5.74)	3.13 (1.95–4.71)	2.43 (1.36–4.94)	0.002	0.001
ALT activity (units/l)	16.6 ± 10.9	14.2 ± 8.3	13.3 ± 6.2	11.9 ± 5.4	0.000	0.000
GFR (ml/min per 1.73 m^2)	74.7 ± 12.3	71.1 ± 10.0	70.9 ± 11.7	69.7 ± 12.7	0.001	0.668
Lifestyle factors						
Smoking (% yes)	31.7	39.7	40.6	32.4	0.881	0.696
Habitual physical activity (h/day)	2.8 (1.6–4.4)	2.9 (1.7–5.0)	3.0 (1.9–5.1)	3.1 (2.0–5.0)	0.217	0.878
Sports (% yes)	34.5	29.1	30.1	25.5	0.127	0.865
Alcohol (% yes)	90.3	87.1	84.2	85.8	0.199	0.601
Alcohol (g/day)						
0	9.7	12.9	15.8	14.2	0.199	0.601
<10	39.6	43.5	43.9	43.3	0.539	0.603
10–30	34.0	32.7	30.9	34.8	0.980	0.662
≥30	16.7	10.9	9.4	7.8	0.018	0.046

Data are means ± SD or median (interquartile range) unless otherwise indicated. *P_{trend} adjusted for age.

of Hoorn. Another 140 subjects were not invited because of logistic reasons. Of the remaining 2,086 subjects, 1,513 (72.5%) participated. At both examinations, an extensive physical examination and an oral glucose tolerance test were performed.

For the present analyses, from the baseline population (n = 2,484) we excluded subjects with missing baseline data (n = 184) and subjects who already had type 2 diabetes (either newly detected or previously diagnosed) at baseline (n = 224). From the remaining 2,076 subjects, 1,264 persons had follow-up data available on glucose tolerance status and were included for prospective analyses. Informed consent was obtained from all participants, and ethical approval was obtained from the Ethical Review Committee of the VU University Medical Center.

Adiponectin

Baseline adiponectin was determined in 2004 in spare plasma samples that had been stored at −80°C and had never been thawed before. Adiponectin was determined by a latex turbidometric immunoassay (Otsuka Pharmaceutical). The inter- and intra-assay coefficients of variation were <2.0 and <3.1%, respectively.

Glucose metabolism

Fasting glucose and 2-h postload glucose levels after a 75-g oral glucose tolerance test were measured by the glucose dehydrogenase method (Merck, Darmstadt, Germany) at baseline and by the hexokinase method (Boehringer-Mannheim, Mannheim, Germany) at follow-up. Glucose levels were used to classify subjects according to the 1999 World Health Organization criteria into normal glucose metabolism, impaired glucose metabolism (IGM) (impaired fasting glucose and/or impaired glucose tolerance), or type 2 diabetes.

Additional measurements

Serum lipids and lipoproteins were determined by enzymatic techniques (Boehringer-Mannheim). Serum leptin concentrations (micrograms per liter) were determined by a radioimmunoassay. Serum alanine aminotransferase (ALT) enzyme activity was measured according to the method of the International Federation of Clinical Chemistry of 1985 and expressed as units per liter (20). The serum creatinine level was determined to calculate the glomerular filtration rate (GFR) by the Cockcroft-Gault formula in milliliters per minute per 1.73 m^2 body surface area. BMI (weight in kilograms divided by the square of height in meters) and waist-to-hip ratio (WHR) were calculated. Blood pressure (millimeters of mercury) was measured in duplicate by means of a random-zero sphygmomanometer (Hawksley-Gelma, Lancing, U.K.). Information on lifestyle factors was obtained by questionnaires. Habitual

Table 2—*Baseline characteristics of women by quartiles of adiponectin*

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P_{trend}	P_{trend}*
n	170	170	170	170		
Adiponectin (μg/ml)	8.48 ± 1.74	12.67 ± 1.03	16.60 ± 1.26	24.78 ± 5.72	<0.001	<0.001
Age (years)	59.1 ± 6.4	59.5 ± 6.7	60.2 ± 6.6	62.2 ± 7.2	<0.001	—
Anthropometry						
BMI (kg/m^2)	27.3 ± 3.3	26.8 ± 3.8	26.2 ± 3.3	25.7 ± 3.1	<0.001	<0.001
Waist circumference (cm)	88.5 ± 9.3	87.2 ± 10.4	85.4 ± 9.5	82.3 ± 9.0	<0.001	<0.001
Hip circumference (cm)	103.1 ± 6.9	103.6 ± 7.4	102.7 ± 6.4	102.3 ± 6.8	0.185	0.045
WHR	0.86 ± 0.07	0.84 ± 0.07	0.83 ± 0.07	0.80 ± 0.07	<0.001	<0.001
Metabolic variables						
Fasting glucose (mmol/l)	5.46 ± 0.50	5.32 ± 0.48	5.27 ± 0.54	5.13 ± 0.51	<0.001	<0.001
2 h glucose (mmol/l)	6.18 ± 1.72	5.42 ± 1.38	5.28 ± 1.44	5.07 ± 1.33	<0.001	<0.001
LDL cholesterol (mmol/l)	4.81 ± 1.19	4.60 ± 1.03	4.67 ± 1.07	4.51 ± 1.00	0.023	0.012
HDL cholesterol (mmol/l)	1.30 ± 0.32	1.46 ± 0.34	1.54 ± 0.32	1.66 ± 0.37	<0.001	<0.001
Triglycerides (mmol/l)	1.7 (1.2–2.2)	1.3 (1.0–1.7)	1.2 (0.9–1.5)	1.0 (0.8–1.3)	<0.001	<0.001
Systolic blood pressure (mmHg)	134.0 ± 20.9	132.2 ± 20.1	132.0 ± 20.5	130.0 ± 19.4	0.082	<0.001
Diastolic blood pressure (mmHg)	81.6 ± 10.0	80.7 ± 9.7	79.9 ± 9.7	78.9 ± 10.7	0.010	0.006
Leptin (μg/l)	16.44 (10.14–27.30)	12.13 (7.75–22.78)	14.82 (8.40–25.02)	10.86 (6.43–18.90)	<0.001	<0.001
ALT activity (units/l)	10.9 ± 4.9	10.4 ± 4.3	11.2 ± 6.6	10.3 ± 4.6	0.582	0.762
GFR (ml/min per 1.73 m^2)	73.1 ± 12.4	73.0 ± 12.0	70.6 ± 11.0	69.3 ± 10.6	0.001	0.149
Lifestyle factors						
Smoking (% yes)	35.7	27.8	27.1	16.5	<0.001	0.001
Habitual physical activity (h/day)	4.4 (3.3–6.0)	4.7 (3.3–6.3)	4.6 (3.4–5.9)	4.1 (3.0–5.7)	0.210	0.451
Sports (% yes)	28.4	33.5	39.4	38.8	0.024	0.004
Alcohol (% yes)	58.3	64.5	61.8	61.7	0.664	0.232
Alcohol (g/day)						
0	41.7	35.5	38.2	38.3	0.674	0.232
<10	37.5	43.8	44.1	46.1	0.126	0.079
10–30	18.5	18.3	10.0	14.4	0.097	0.242
≥30	2.4	2.4	7.6	1.2	0.771	0.506

Data are means ± SD or median (interquartile range) unless otherwise indicated. *P_{trend} adjusted for age.

physical activity (hours per day) included sports, bicycling, gardening, walking, odd jobs, and housekeeping. An additional variable was created that only included performing sports (yes/no).

Statistical analyses

Baseline characteristics are presented for men and women separately according to sex-specific quartiles of adiponectin. To test for trend over the adiponectin quartiles, linear (for continuous dependent variables) or logistic (for dichotomous dependent variables) regression analyses were performed, modeling the categorical variable of adiponectin quartiles as a continuous independent variable. Variables with a skewed distribution were log normal transformed for these analyses.

For prospective analyses, we first plotted the incidence density of type 2 diabetes (number of cases per person-years) against mean adiponectin within each sex-specific quartile of adiponectin. Logistic regression analyses were then performed to calculate the risk (odds ratios [ORs] with 95% CIs) of developing type 2 diabetes or developing IGM during a mean follow-up of 6.4 years. The lowest adiponectin quartile was the reference group. In separate analyses, the continuous adiponectin variable was used as independent variable. In this case, the OR was expressed per sex-specific SD of adiponectin. For the analyses on the incidence of IGM, subjects with IGM (n = 189) at baseline and/or with type 2 diabetes at follow-up (n = 118) were excluded (leaving 1,025 subjects for analyses). Effect modification by sex was examined by adding an interaction term (adiponectin quartiles × sex) to the regression models. Because there was significant interaction by sex (P = 0.051 for type 2 diabetes and P = 0.002 for IGM), the analyses were subsequently performed and reported separately for men and women. Further adjustments were made for age, sex, lifestyle factors, anthropometric variables, leptin, baseline fasting and postload glucose, lipids, ALT, and GFR by adding these variables to the regression models. All statistical analyses were performed using SPSS (version 11.0 for Windows; SPSS, Chicago, IL).

RESULTS— Baseline characteristics are shown in Table 1 (men) and Table 2 (women). At baseline, after adjustment for age, a low adiponectin level was strongly associated with unfavorable measures of anthropometric body composition and measures of glucose (except for HbA_{1c} in men) and lipid metabolism (except for LDL cholesterol in men). A low adiponectin level was also associated with high leptin levels and with high systolic and diastolic blood pressure. In women, smoking was associated with

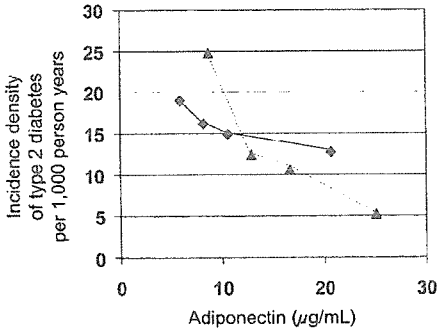

Figure 1—*Incidence density of type 2 diabetes within sex-specific adiponectin quartiles, after a mean follow-up of 6.4 years. ◆, men; ▲, women.*

lower adiponectin levels, and participating in sports was associated with higher adiponectin levels.

The mean follow-up duration was 6.4 ± 0.5 years for both sexes. Figure 1 shows the crude association between adiponectin levels and the incidence density of type 2 diabetes. High adiponectin levels were associated with a lower incidence of type 2 diabetes, and this association appeared stronger in women than in men. After adjustment for age and lifestyle, higher adiponectin levels at baseline were associated with a lower risk of development of type 2 diabetes, although not statistically significant in men (Table 3, model 1). Adjustment for leptin, as a marker for body fat, and WHR weakened the associations, particularly in women (model 2). Adjustment for waist and hip separately or for waist circumference alone instead of WHR or additional adjustment for BMI yielded similar results (not shown). Adjustment for glucose levels, particularly postload glucose, substantially weakened the associations (model 3). Additional adjustment for triglycerides also diminished the associations (model 4), whereas adjustment for LDL cholesterol did not (not shown). Adjustment for HDL cholesterol instead of triglycerides diminished the strength of the associations (ORs [95% CI] in quartile 2, 3, and 4 were 0.66 [0.29–1.52], 0.95 [0.40–2.25], and 0.75 [0.30–1.90] in men and 0.98 [0.44–2.19], 0.75 [0.31–1.80], and 0.71 [0.23–2.17] in women, respectively), but not if triglycerides were already entered into the model (data not shown). Adjustment for ALT or GFR (instead of triglycerides in model 4) did not change the associations (not shown). If only subjects with normal glucose tolerance were selected (subjects with IGM at baseline also excluded) the risk of development of diabetes associated with low adiponectin levels became stronger: the OR (95% CI) for the highest compared with the lowest adiponectin quartile was 0.45 (0.14–1.47) in men and 0.17 (0.03–0.83) in women, after adjustment for age, lifestyle, and WHR.

The association between adiponectin levels and the incidence of IGM (Table 4) had a pattern similar to that for the association with type 2 diabetes in women, but the association of a high adiponectin level with a lower risk of IGM was not observed in men. We also calculated the risk for the incidence of impaired fasting glucose and impaired glucose tolerance separately. After adjustment for age, lifestyle, and WHR, the ORs (95% CI) for highest compared with lowest adiponectin quartile were 1.15 (0.60–2.20) in men and 0.54 (0.28–1.03) in women for impaired fasting glucose and 0.92 (0.34–2.44) in men and 0.28 (0.12–0.68) in women for impaired glucose tolerance.

CONCLUSIONS— Our main finding is that a lower adiponectin level is associated with a higher risk of developing type 2 diabetes after a mean follow-up of 6.4 years in an elderly Caucasian population. This association was largely explained by fasting and postload glucose levels. In women with a normal glucose metabolism at baseline, a low adiponectin level was associated with an increased risk of developing IGM (pre-diabetic state), but this association was not observed in men. To our knowledge, our study is the first prospective cohort study in elderly Caucasian men and women.

Baseline adiponectin independently predicted subsequent type 2 diabetes during 6.4 years of follow-up in our population. Together with the observation that weight reduction affected adiponectin (5), but that adiponectin did not predict weight change (17), our results support the hypothesis that adiponectin may play an important role in the pathogenesis of abnormal glucose metabolism. It is suggested that, possibly as a result of a low adiponectin level, liver fat accumulation plays a key role in the development of metabolic disturbances (21). However, ALT, which is used as a marker of liver fat accumulation, was inversely associated with adiponectin in men but not in women. Additional analyses revealed that an inverse association between ALT and adiponectin existed in the entire population of women, but it disappeared once

Table 3—*Risk of developing type 2 diabetes in 6.4 years associated with adiponectin*

	Adiponectin				
	Quartile 1	Quartile 2	Quartile 3	Quartile 4	Continuous, per SD
Men					
Cases (%)	12.4 (18/145)	10.6 (16/151)	9.8 (14/143)	8.3 (12/145)	10.3 (60/584)
Model 1	1.0	0.77 (0.37–1.61)	0.67 (0.31–1.46)	0.52 (0.23–1.18)	0.83 (0.62–1.12)
Model 2	1.0	0.79 (0.38–1.67)	0.71 (0.33–1.55)	0.61 (0.27–1.40)	0.89 (0.66–1.20)
Model 3	1.0	0.66 (0.29–1.52)	0.96 (0.41–2.27)	0.79 (0.32–1.91)	1.01 (0.74–1.39)
Model 4	1.0	0.67 (0.29–1.54)	0.98 (0.41–2.33)	0.80 (0.33–1.95)	1.02 (0.75–1.40)
Women					
Cases (%)	15.8 (27/170)	8.2 (14/170)	7.2 (11/170)	3.5 (6/170)	8.5 (58/680)
Model 1	1.0	0.48 (0.24–0.97)	0.37 (0.18–0.97)	0.15 (0.06–0.39)	0.42 (0.28–0.62)
Model 2	1.0	0.58 (0.28–1.20)	0.47 (0.22–1.01)	0.27 (0.10–0.73)	0.55 (0.36–0.83)
Model 3	1.0	0.91 (0.41–2.00)	0.68 (0.29–1.61)	0.62 (0.21–1.81)	0.78 (0.52–1.18)
Model 4	1.0	0.97 (0.43–2.17)	0.75 (0.31–1.80)	0.69 (0.23–2.07)	0.82 (0.54–1.26)

Data are OR (95% CI) unless otherwise indicated. Model 1: adjusted for age and lifestyle (smoking and sports). Model 2: model 1 plus adjusted for leptin (log normal transformed) and WHR. Model 3: model 2 plus adjusted for fasting and postload glucose. Model 4: model 3 plus adjusted for triglycerides (log normal transformed).

Table 4—*Risk of developing IGM in 6.4 years associated with adiponectin (excluding subjects who already had IGM at baseline or developed type 2 diabetes at follow-up)*

	Adiponectin				
	Quartile 1	Quartile 2	Quartile 3	Quartile 4	Continuous, per SD
Men					
Cases (%)	32.4 (35/108)	36.5 (42/115)	29.9 (35/117)	34.4 (41/119)	33.3 (153/459)
Model 1	1.0	1.08 (0.62–1.90)	0.80 (0.45–1.43)	0.90 (0.51–1.61)	0.93 (0.76–1.14)
Model 2	1.0	1.15 (0.65–2.03)	0.89 (0.49–1.60)	1.11 (0.61–2.02)	0.99 (0.80–1.22)
Model 3	1.0	1.06 (0.56–2.01)	1.06 (0.54–2.07)	1.20 (0.61–2.35)	1.01 (0.79–1.28)
Model 4	1.0	1.05 (0.55–2.00)	1.11 (0.57–2.16)	1.25 (0.63–2.46)	1.03 (0.81–1.31)
Women					
Cases (%)	45 (54/120)	27.8 (40/144)	27.1 (39/144)	20.9 (33/158)	29.3 (166/566)
Model 1	1.0	0.45 (0.27–0.76)	0.44 (0.26–0.74)	0.28 (0.16–0.48)	0.68 (0.54–0.84)
Model 2	1.0	0.49 (0.29–0.83)	0.50 (0.30–0.86)	0.36 (0.20–0.65)	0.76 (0.60–0.96)
Model 3	1.0	0.57 (0.32–1.01)	0.64 (0.36–1.14)	0.48 (0.26–0.90)	0.82 (0.65–1.03)
Model 4	1.0	0.63 (0.36–1.13)	0.79 (0.43–1.44)	0.62 (0.32–1.18)	0.91 (0.73–1.15)

Data are OR (95% CI) unless otherwise indicated. Model 1: adjusted for age and lifestyle (smoking and sports). Model 2: model 1 plus adjusted for leptin (log normal transformed) and WHR. Model 3: model 2 plus adjusted for fasting and postload glucose. Model 4: model 3 plus adjusted for triglycerides (log normal transformed).

women with type 2 diabetes were excluded. An association of ALT with incident type 2 diabetes was previously found in the Hoorn Study (which also disappeared after additional adjustment for baseline glucose levels); however, a possible difference between men and women was not evaluated (22). Our results suggest that the association between adiponectin and incident type 2 diabetes was not explained by ALT.

Adjustment for glucose (particularly postload glucose) and triglycerides substantially reduced the associations between adiponectin and incident type 2 diabetes. This could suggest that the associations are (partly) mediated by these factors, confirming the hypothesis of adiponectin contributing to type 2 diabetes risk through effects on (hepatic) insulin resistance. Previous prospective studies found associations between adiponectin and type 2 diabetes, even independent of fasting glucose levels. It is likely, however, that this is caused by residual effects of postload glucose and triglycerides, which were usually not measured.

There are still unexplained phenomena concerning adiponectin metabolism. The association between adiponectin and type 2 diabetes was stronger in women than in men. Because of differences in design among studies, it is difficult to compare the absolute strength of the association, but a sex difference has previously been shown in younger adults (18), with stronger associations also being shown in women. Other prospective studies on adiponectin and type 2 diabetes did not report on possible differences between men and women (14–17). It is remarkable that, despite their higher body fat percentage, women appear to have higher adiponectin levels than men. Previously, this difference could not be explained by differences in fat distribution (6). Another counterintuitive observation is that adiponectin is positively associated with age. This has also been shown in many other studies in which the association between adiponectin and age was reported (e.g., the study by Cnop et al. [6] and the Funagata Study [16]) but so far has received little attention. So, despite the increase in body fat with aging and the accompanying increased cardiovascular risk, adiponectin levels also increase. These observations emphasize that the mechanisms that underlie the association between adiponectin and disturbed glucose metabolism are poorly understood. Recent work has shown that adiponectin exists in different isoforms, low–molecular weight and high–molecular weight (HMW) complexes (2). Diabetic patients had a significantly decreased HMW-to-total adiponectin ratio (23), and HMW adiponectin correlated better with glucose tolerance than total adiponectin in Indo-Asian males (24). Also, a relatively larger increase in HMW adiponectin after weight reduction has been shown (25). These findings indicate that the HMW adiponectin complex is possibly the active form of this protein. Women have more HMW adiponectin than men (23), which may partly explain the differences that we have found between men and women.

A limitation of the present study may be that we had no repeated measurement of adiponectin at the follow-up examination. Adiponectin levels may have changed considerably after >6 years. Despite the long follow-up duration, however, we were still able to find an association indicating that the relation is very strong. In addition, participants in the follow-up examination were healthier at baseline than the nonparticipants (26). This may have led to an underestimation of the true incidence of type 2 diabetes and consequently to an underestimation of the associations with adiponectin.

In summary, our findings strongly support the hypothesis that adiponectin may play an important role in the pathogenesis of abnormal glucose metabolism. However, further investigation of the underlying mechanisms, focusing on adiponectin isomer distribution, is needed to elucidate the associations of adiponectin with sex and age.

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Brief report

A novel index of insulin resistance determined from the homeostasis model assessment index and adiponectin levels in Japanese subjects

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Abstract

Insulin resistance is the principal cause of glucose intolerance and type 2 diabetes and induces progression of severe atherosclerosis in these patients. Adiponectin, the adipose-specific proteins, is known to correlate negatively with insulin resistance in patients with obesity and type 2 diabetes. The purpose of this study was to evaluate the potential of using serum adiponectin levels as a marker of insulin resistance in various states of insulin resistance. Furthermore, we attempted to establish a modified index of the homeostasis model assessment index (HOMA-IR), calculated from the product of serum insulin and plasma glucose levels divided by serum adiponectin levels (HOMA-AD).

We recruited 117 Japanese subjects with various degrees of glucose tolerance and determined serum adiponectin levels and insulin sensitivity (*M*-value) by using the euglycemic hyperinsulinemic clamp technique. *M*-value, the gold standard index of insulin resistance, correlates significantly and independently with fasting insulin ($r = -0.313$, $P < 0.001$), glucose ($r = -0.319$, $P < 0.001$), and adiponectin ($r = 0.241$, $P < 0.002$) levels. *M*-values were more significantly correlated with HOMA-AD ($r = -0.643$, $P < 0.001$) than HOMA-IR values ($r = -0.591$, $P < 0.001$). In subjects with moderate hyperglycemia (fasting glucose levels > 8.0 mmol/L, $n = 30$), HOMA-AD showed a more significant correlation with the *M*-value than HOMA-IR ($r = -0.535$, $P = 0.005$ versus $r = -0.461$, $P = 0.010$).

We would therefore like to propose a novel index, HOMA-AD, as a simple and adequate index for determining insulin resistance even in diabetic patients with overt hyperglycemia.

Keywords: Insulin resistance; HOMA-IR; Adiponectin

Abbreviations: HOMA-IR, homeostasis model assessment of insulin resistance; BMI, body mass index; NGT, normal glucose tolerance; IGT, impaired glucose tolerance; T2DM, type 2 diabetes mellitus; FPG, fasting plasma glucose levels

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1. Introduction

Insulin resistance, as well as impaired pancreatic β-cell function, is the principal cause of glucose intolerance and type 2 diabetes. Insulin resistance is also well-correlated with central obesity, lipid abnormality, and hypertension, and accumulation of these metabolic abnormalities, so called metabolic syndrome, results in severe atherosclerosis. Treatment of insulin resistance with thiazolidine diones or metformin improves mortality and decreases cardiovascular events. It is therefore important to evaluate the extent of insulin resistance in obese and type 2 diabetic patients, and to treat them with improvement of life styles and/or medication.

Several techniques are used to evaluate insulin resistance in humans, and among them, the *M*-value determined by the euglycemic hyperinsulinemic clamp technique is considered the gold standard [1]. Since this method is complicated and expensive to be utilized in epidemiological studies, the homeostasis model assessment index (HOMA-IR) [2], calculated from fasting glucose and insulin levels, has been mainly applied in clinical studies. However, several studies demonstrated that HOMA-IR failed to detect insulin resistance in individuals with normal and impaired glucose tolerance [3,4]. In addition, this index may be inadequate in diabetic subjects with moderate hyperglycemia, where insulin secretory ability fails to compensate for the impaired homeostasis of glucose [5]. Therefore, a more accurate index is necessary for determining insulin resistance especially in diabetic patients.

Adiponectin, the most abundant of adipose-specific proteins, is known to modulate the action of insulin via activation of AMP-activated protein kinase in the muscle and the liver [6]. Serum adiponectin levels were found to correlate directly with whole-body insulin sensitivity in patients with obesity and type 2 diabetes [7]. Therefore, in this study, we examined the correlation between adiponectin levels and HOMA-IR and *M*-values, and established a novel index of insulin resistance, which can be used even for individuals with hyperglycemia by modifying HOMA-IR by taking into account adiponectin levels.

2. Materials and methods

A total of 117 Japanese subjects with various degrees of glucose tolerance, including individuals with type 2 diabetes (T2DM; $n = 89$, 54.7 ± 10.8 years, BMI 26.2 ± 18.5), impaired glucose tolerance (IGT; $n = 5$, 43.2 ± 19.8 years, BMI 25.3 ± 3.2), and normal glucose tolerance (NGT; $n = 23$, 49.7 ± 10.2 years, BMI 25.5 ± 4.3) were enrolled in this study carried out in Osaka University Hospital, Osaka City University Hospital, and Ryukyu University Hospital. The study was approved by the Ethical Committee for Human Studies at each hospital. After giving a detailed explanation of the study using a document, written informed consent was obtained from each subject.

During the euglycemic hyperinsulinemic clamp study, the target levels of plasma glucose and insulin were 5.5 mmol/L and 600 pmol/L, respectively. When the rate of exogenous glucose infusion reached a steady-state level, we evaluated insulin sensitivity as the average rate of exogenous glucose infusion for 30 min (*M*-value). We also determined plasma glucose, insulin, and adiponectin concentrations at the beginning of the glucose clamp test to simultaneously assess insulin sensitivity. Adiponectin levels were determined using a validated latex kit (LTX) employing an adiponectin-specific antibody (Otsuka Pharmaceutical and Mitsubishi Kagaku Iatron, Tokyo, Japan) [8]. We calculated the HOMA-IR values, and divided it by adiponectin levels to establish a more accurate index (HOMA-AD) for determining insulin resistance using the following formula:

$$\text{HOMA-AD} = \frac{\text{fasting insulin (mU/L)} \times \text{fasting glucose (mg/dL)}}{\text{adiponectin (}\mu\text{g/mL)}}$$

Data are represented as means ± S.D. Laboratory data were compared using unpaired *t*-test. Pearson's correlation co-efficient and stepwise multivariate regression analysis was performed to evaluate the relationship between the *M*-value and other parameters. The indexes of insulin resistance were log-transformed to yield a normal distribution before analysis.

3. Results

Fasting glucose levels were significantly higher in T2DM (7.4 ± 1.7) than NGT and IGT (5.1 ± 0.6 and 5.9 ± 1.2 mmol/L, $P < 0.001$). Fasting insulin and adiponectin levels were similar among three groups (NGT, 48.0 ± 20.4, 5.8 ± 2.2; IGT, 46.8 ± 18.0, 6.8 ± 3.3; T2DM, 56.7 ± 51.6 pmol/L, 6.1 ± 2.8 μg/mL). HOMA-IR was significantly higher in T2DM (3.1 ± 2.6) than NGT and IGT (1.8 ± 0.8, 2.1 ± 0.9, $P < 0.05$). The *M*-values were significantly higher in NGT (6.0 ± 2.5 mg/kg/min) than T2DM and IGT (4.6 ± 2.3, 4.9 ± 1.9 mg/kg/min, $P < 0.05$).

The *M*-value significantly correlated with glucose ($r = -0.345$, $P < 0.001$), insulin ($r = -0.518$, $P < 0.001$), adiponectin ($r = 0.369$, $P < 0.001$), and HOMA-IR ($r = -0.591$, $P < 0.001$). Multivariate regression analysis revealed that the *M*-value correlates significantly and independently with fasting insulin ($r = -0.313$, $P < 0.001$), glucose ($r = -0.319$, $P <$

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0.001), adiponectin ($r = 0.241$, $P < 0.002$) levels and BMI ($r = -0.185$, $P = 0.032$).

HOMA-AD was significantly higher in T2DM (257 ± 250, $P < 0.05$) than NGT and IGT (166 ± 115, 100 ± 35.5). HOMA-AD values showed the highest correlation with M-values ($r = -0.643$, $P < 0.001$, Fig. 1) among the evaluated parameters, including HOMA-IR ($r = -0.591$, $P < 0.001$). Multivariate regression analysis also revealed that the M-value correlates most significantly and independently with HOMA-AD ($r = -0.284$, $P < 0.001$). We also validated the relation of HOMA-IR and HOMA-AD with M-value in patients with type 2 diabetes ($n = 87$). We found the similar results that HOMA-AD showed higher correlation with M-value ($r = -0.667$) than HOMA-IR ($r = -0.617$). Moreover, HOMA-IR is known to become inaccurate under hyperglycemic condition, where the homeostasis between glucose and insulin is disrupted. In subjects with moderate hyperglycemia (fasting glucose levels > 7.8 mmol/L, $n = 30$), HOMA-AD showed a more significant correlation with the M-value than HOMA-IR ($r = -0.538$, $P = 0.002$ versus $r = -0.461$, $P = 0.010$).

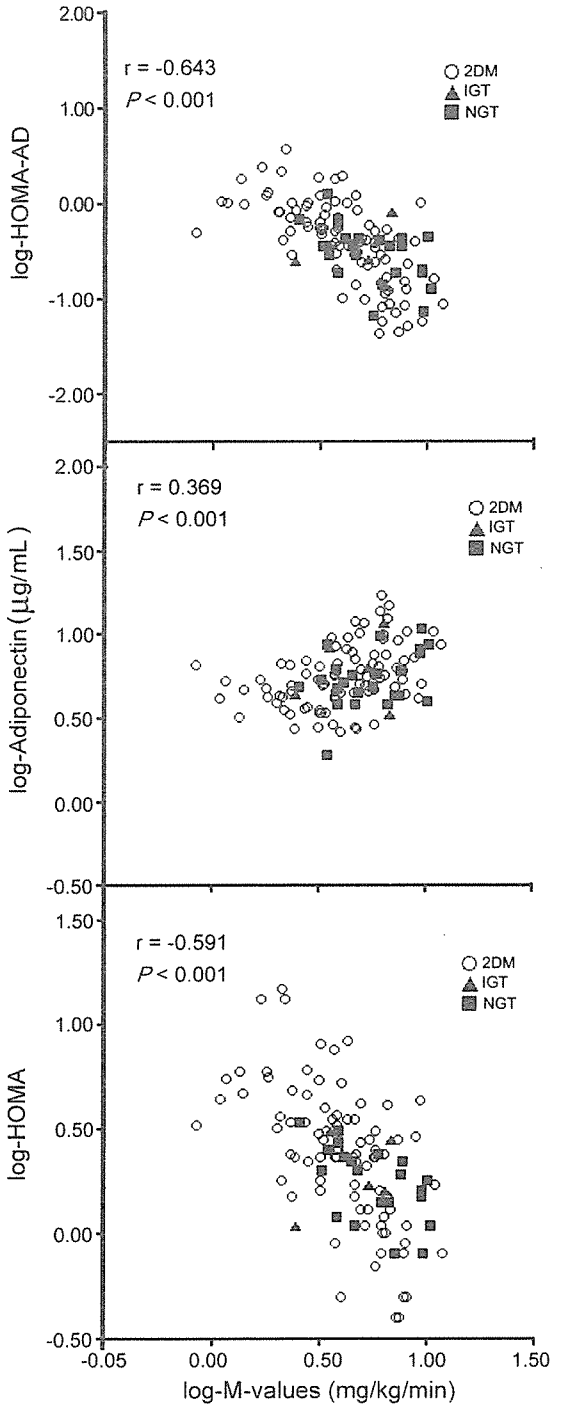

Fig. 1. Correlation between adiponectin levels, HOMA-IR, and HOMA-AD, and M-values determined by the euglycemic hyperinsulinemic clamp test in patients with various states of glucose tolerance.

4. Discussion

In the present study, the M-value significantly correlated with fasting plasma glucose and fasting serum insulin and adiponectin levels in Japanese subjects. Therefore, we generated a modified index of HOMA-IR (HOMA-AD) by taking account into adiponectin levels; found that this index is a more accurate indicator for assessing insulin resistance than HOMA-IR. This index is simply based on glucose, insulin, and adiponectin levels in fasting blood sample.

Although HOMA-IR is easy to calculate, there are several limitations in its use, especially in subjects with fasting hyperglycemia. In this study, HOMA-IR showed lower correlation with M-value in type 2 diabetic patients with moderate hyperglycemia (FPG $\geqq$ 8 mmol/L). Since hyperglycemia is induced by the inadequate secretion of insulin, the homeostasis between fasting glucose and insulin levels may be disrupted in these subjects. Moreover, circulating glucose is excreted into the urine under moderate hyperglycemic condition. Therefore, we speculate that the accuracy of these surrogate insulin resistance indexes could be lowered by elevation of plasma glucose in type 2 diabetic patients.

Modification of HOMA-IR with adiponectin levels resulted in an index exhibiting a good correlation with M-values even in subjects with moderate hyperglycemia. Therefore, we would like to propose HOMA-AD as a novel and beneficial index for determining insulin resistance in individuals with various levels of insulin resistance.

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HOMA-IR is known to correlate with insulin resistance in various races, but the distribution and average of HOMA-IR were different among these races [9,10]. The adiponectin level is also known to correlate with the level of insulin resistance, as well as HOMA-IR, in the various races [5,7]. Therefore, the findings in this study could be limited for Japanese subjects. The further study is needed to evaluate the usefulness of HOMA-AD in the various races.

In summary, the present study demonstrated that HOMA-AD is a more adequate predictor of insulin resistance in non-diabetic and diabetic individuals compared with the established surrogate index, HOMA-IR. HOMA-AD is therefore the most suitable index for use in epidemiological studies, even for diabetic patients with moderate hyperglycemia.

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Pravastatin improved glucose metabolism associated with increasing plasma adiponectin in patients with impaired glucose tolerance and coronary artery disease

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Abstract

Reduced incidence of type-2 diabetes has been shown in patients treated with pravastatin. Adiponectin can exhibit beneficial effects on glucose metabolism. We investigated whether pravastatin could improve glucose tolerance associated with increasing adiponectin levels in patients with impaired glucose tolerance (IGT). This study consisted of 40 coronary artery disease (CAD) patients with IGT assessed by oral glucose tolerance test (OGTT). Patients were randomized to receive pravastatin ($n = 20$) or no lipid-lowering medications (control group, $n = 20$) for 6 months, after which OGTT was repeated and adiponectin levels were measured. Pravastatin treatment significantly decreased levels of total cholesterol (16%), low-density lipoprotein cholesterol (23%) and high-sensitivity C-reactive protein (37%) ($p < 0.01$, respectively). At 2 h in OGTT, pravastatin significantly improved hyperglycemia (−14%) and hyperinsulinemia (−23%). Pravastatin treatment significantly elevated plasma adiponectin levels (35%; $p < 0.001$) but not in the control group. The glucose reduction at 2 h post-OGTT was significantly associated with increased levels of adiponectin ($r = -0.462$; $p = 0.003$). Pravastatin treatment is an independent predictor for improvement of post-loaded hyperglycemia (odds ratio; 5.7; 95% confidence interval 1.7–19.3; $p = 0.003$) and achieved beneficial conversion from IGT to normal glucose tolerance (40%; $p = 0.03$). Pravastatin exhibits beneficial effects on glucose metabolism especially in the postprandial state associated with increasing plasma adiponectin levels in CAD patients with IGT.

Keywords: Coronary artery disease; Diabetes mellitus; Statin; Glucose tolerance; Adiponectin; Impaired glucose tolerance

1. Introduction

Pravastatin is a competitive 3-hydroxy-3-methylglutaryl coenzyme-A reductase inhibitor (statin) that inhibits hepatic de novo cholesterol synthesis, improving both fasting and postprandial hyperlipidemia. The efficacy of statins for prevention of cardiovascular events has been established in a number of landmark randomized clinical trials [1–3] however, whether statin has beneficial effects on glucose metabolism still remains controversial. The West of Scotland Coronary Prevention Study (WOSCOPS) demonstrated a significant risk reduction in the development of type-2 diabetes

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in hypercholesterolemic patients treated with pravastatin [4] while several studies with other statins have not shown this beneficial effect on glucose metabolism [2,3]. Furthermore, it has recently been reported that high-dose atorvastatin significantly associated with worse glycemic control after acute coronary syndrome [5] suggesting the protective effect unique to pravastatin. Many coronary artery disease (CAD) patients with abnormal lipid metabolism also have impaired glucose metabolism; together these are key components of metabolic syndrome. Because low-density lipoprotein (LDL)-cholesterol is definitely an important target to prevent cardiovascular complications in patients with CAD and the patients are allowed to take cholesterol-lowering medicines all along their life, treatment of ischemic heart disease with consideration to the patient's glucose metabolism and cholesterol levels is a clinically valuable approach.

Postprandial metabolic disorders, including hyperglycemia and hyperinsulinemia, are involved in the process of atherogenesis, which can lead to major clinical cardiovascular events. Postprandial hyperglycemia is defined as impaired glucose tolerance (IGT) following an oral glucose tolerance test (OGTT). IGT itself has been shown to be a significant risk factor for cardiovascular disease, and is also considered to presage the onset of type-2 diabetes mellitus, which is associated with a significantly worse prognosis in patients with CAD [6]. Now it is recognized that IGT may be a practical treatment target to prevent the development of overt diabetes [7] and a modifiable risk factor for cardiovascular disease.

Adiponectin is an adipocyte-derived secreted protein that has several important metabolic and endocrinologic functions which are of particular relevance to glucose metabolism and the development of type-2 diabetes [8]. Patients with CAD have decreased adiponectin levels [9] and hypoadiponectinemia is associated with an increased risk of myocardial infarction [10]. In humans, increased serum concentrations of adiponectin are associated with increased insulin sensitivity and glucose tolerance [11]. We hypothesized that treatment with pravastatin would result in improvements in glucose tolerance associated with elevation of plasma levels of adiponectin in patients with CAD. To test this hypothesis, we evaluated the change in plasma levels of adiponectin and glucose tolerance assessed by OGTT before and after 6 months of treatment with pravastatin in CAD patients with IGT.

2. Methods

2.1. Patients

This was a single-center, open-label, prospective randomized study carried out at Kumamoto University Hospital, Kumamoto, Japan, between 2001 and 2004. Patients were eligible if they were non-hypercholesterolemic (total cholesterol <220 mg/dL; Japan Atherosclerosis Society) and non-diabetic (fasting glucose <126 mg/dL, 2 h post-loaded glucose <200 mg/dL and glycosylated hemoglobin A1c (HbA1c) <6.4%; Japan Diabetes Society) and without taking any lipid-lowering medications and underwent elective diagnostic coronary angiography at Kumamoto University Hospital because of an abnormality in the electrocardiogram or angina-like chest symptoms upon effort. All patients underwent a 75 g OGTT to identify those with IGT, as defined by the criteria of the World Health Organization (fasting glucose level <126 mg/dL, and 140 mg/dL ≤2 h post-load glucose level <200 mg/dL). Patients were randomly divided into two treatment groups using the permuted block method, a control group without any lipid-lowering therapy and a pravastatin group. The follow-up time was 6 months. At enrollment, all patients received dietary counseling to ensure a total daily calorie intake of 25–30 kcal/kg in order to prevent weight gain, and were asked to maintain their current level of physical activity during the study. Intensive lifestyle modification (structured diet education and an increase in physical exercise) was not initiated. Advise for smoking cessation was provided in each group. Patients in the pravastatin group initially received 10 mg/day, which was increased to a final dose of 20 mg/day as required to achieve target LDL-cholesterol levels of <100 mg/dL. Patients in the control group received additional dietary counseling according to the Adult Treatment Panel III [12] to reach the target LDL levels (<100 mg/dL). Excluded were patients with valvular disease, trauma within 1 month prior to study entry, cardiomyopathy, malignant disease, infectious disease, chronic inflammatory disease, end-stage renal failure, autoimmune diseases or acute coronary syndromes. Informed consent was obtained from all patients. This study was performed in accordance with the ethics principles in the Declaration of Helsinki and the Ethics Committee at Kumamoto University Hospital approved the study protocol.

2.2. Oral glucose tolerance test

A 75 g OGTT was performed after an overnight fast. Venous blood samples were obtained in the fasting state and at 30, 60 and 120 min after ingestion of 75 g glucose to evaluate blood glucose and insulin concentrations. The glucose and insulin area under the concentration–time curve values ($AUC_{glucose}$ and $AUC_{insulin}$) were calculated. An insulinogenic index was defined as the ratio of plasma insulin to glucose at 30 min after glucose loading. An insulin sensitivity index was calculated using the following Matsuda and DeFronzo formula: insulin sensitivity index = 10,000/square root of [(fasting glucose × fasting insulin) × (mean glucose × mean insulin during OGTT)] [13]. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using the following formula: HOMA-IR (μU/mL × mg/dL) = (fasting insulin × fasting glucose)/405. The homeostasis model assessment of pancreatic β-cell function (HOMA-Iβ) was calculated using the following formula: HOMA-Iβ [(μU/mL)/(mg/dL)] = (fasting insulin × 360)/(fasting glucose − 63).

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Table 1
Baseline clinical characteristics of IGT patients with CAD

	Control ($n = 20$)	Pravastatin ($n = 20$)	p-Value
Age (years)	65.7 ± 9.2	68.2 ± 8.3	0.28
Gender (male/female)	15/5	13/7	0.49
Smoking	9 (45)	10 (50)	0.75
Hypertension	18 (90)	17 (85)	0.63
Number of diseased vessel			
One	14 (70)	12 (60)	0.51
Two	5 (25)	6 (30)	0.72
Three	1 (5)	2 (10)	0.55
Old myocardial infarction	5 (25)	7 (35)	0.49
Medication			
β-Blocker	5 (25)	7 (35)	0.49
Calcium antagonist	16 (80)	15 (75)	0.71
ACE inhibitor or ARB	15 (75)	13 (65)	0.49
Nitrate	5 (25)	4 (20)	0.71
Aspirin	20 (100)	20 (100)	–

ACE, angiotensin converting enzyme inhibitor; ARB, angiotensin II receptor blocker. Values in parentheses are given in percentage.

2.3. Follow-up

All patients were followed-up monthly at the Kumamoto University Hospital outpatient clinic. Patients were asked to keep a diary of physical activity and diet, which was checked at each visit by the primary physicians and nutritionists. Peripheral blood samples were collected in the morning before breakfast after an overnight fast at the beginning of the study and at the end of the follow-up period. Serum lipid parameters and high-sensitivity C-reactive protein (hsCRP) were measured in the hospital laboratory. Serum LDL-cholesterol concentrations were measured by the direct assay. Plasma levels of adiponectin were measured by enzyme-linked immunosorbent assay as described previously [14]. After the fasting blood sample was taken, a 75 g OGTT was performed with each patient at baseline and after 6 months of treatment. During the follow-up period, the standard medical therapies at baseline in Table 1 were kept unchanged in all patients. We compared the change from baseline to 6 months in the time course of blood glucose levels at 2 h in OGTT and plasma levels of adiponectin. Furthermore, the change from baseline to 6 months in the time course of blood insulin and glucose levels during OGTT ($AUC_{glucose}$ and $AUC_{insulin}$), the incidence of new-onset diabetes and the normalization rate of glucose tolerance were evaluated.

2.4. Sample size calculation

On the bases of our preliminary observation, we proposed that pravastatin would reduce about 15% of glucose levels at 2 h in OGTT after 6 months treatment. Given this effect size, a probability of type-I error of 0.05 (two-tailed), a power of 0.8, expected S.D. of the 2 h glucose values as 20 mg/dL, the required sample size based on a unpaired t-test was 18 patients in each group by power-analysis (GraphPad, StatMate).

2.5. Statistical analysis

Results are expressed as the mean value ± S.D. in tables and mean value ± S.E.M. in graphs. Levels of non-parametric variables were expressed as medians with interquartile range. The frequencies between the two groups were compared using Chi-square analysis. Other comparisons between two groups were carried out using the unpaired t-test and Mann–Whitney U-test. Comparisons in each group from baseline to follow-up were assessed using the paired Student's t-test and Wilcoxon signed rank test. Associations between changes in body mass index (BMI) and continuous serum and plasma parameters and change in 2 h glucose levels in OGTT were examined by simple regression analysis and stepwise regression analysis. Multiple regression analysis was then performed with change in the 2 h glucose levels as the dependent variable and significant parameters in the stepwise regression ($F > 3.0$) as the independent variables. Logistic regression analysis with the covariates listed in Table 1 was performed to determine effective factors for reduction of more than 10 mg/dL glucose values at the 2 h in OGTT. Statistical significance was defined as $p < 0.05$.

3. Results

3.1. Baseline characteristics

Forty IGT patients with CAD were enrolled and randomized to the pravastatin group ($n = 20$) or the control group ($n = 20$). All patients had angiographic documentation of organic stenosis (≥50%) in more than one major coronary artery (single-vessel, $n = 26$; two-vessel, $n = 11$; three-vessel, $n = 3$; Table 1). They were on stable condition for more than 3 months and they did not undergo new coronary intervention procedure at the enrollment. All patients were on the stable concomitant medication before enrollment and there were no change in the concomitant medication including β-blockers during the follow-up.

Baseline clinical characteristics of the patients are shown in Table 1. All patients completed the study protocol and were evaluated as the intent-to-treat population. In the pravastatin group, the final doses were 10 mg/day in 15 patients and 20 mg/day in 5 patients. There were no significant differences in all baseline demographic parameters between the two groups. Levels of physical activity did not significantly change during the study. Pravastatin was well tolerated by all patients, and none had adverse side effects.

3.2. Changes in body mass index, lipoproteins, hsCRP, HOMAs and adiponectin levels

Table 2 shows changes from baseline to the end of follow-up in BMI, body weight, lipoproteins, hsCRP, adiponectin levels and HOMAs. The change in BMI from baseline was not significantly different between the two groups.

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