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Differences in the Pathology of the Metabolic Syndrome With or Without Visceral Fat Accumulation

A Study in Pre-Diabetic Japanese Middle-Aged Men

Yutaka Mori,[1] Kyouko Hoshino,[2] Kuninobu Yokota,[3] Yohta Itoh,[1] and Naoko Tajima[3]

[1]Department of Internal Medicine, National Hospital Organization, Utsunomiya National Hospital, Kawachi, Tochigi, Japan; [2]Matsushita Tokyo Health Care Center, Matsushita Electric Industrial Corporation, Tokyo, Japan; and [3]Division of Diabetes and Endocrinology, Department of Internal Medicine, The Jikei University School of Medicine, Tokyo, Japan

To elucidate the role of visceral fat accumulation in the metabolic syndrome, differences in the pathology of the metabolic syndrome with or without visceral fat accumulation were investigated. A total of 472 pre-diabetic Japanese men (mean age, 47.5 ± 7.2 yr) with impaired fasting glycemia (IFG) levels of 110–125 mg/dL were eligible for participation in the study. The study subjects were divided into the following four groups, and intergroup comparisons were made: group I without visceral fat area [VFA] ≥ 100 cm^2 but presenting with fewer than two other risk factors (i.e., TG ≥ 150 mg/dL, HDL-C < 40 mg/dL, BP ≥ 130/ ≥ 85 mmHg, or FPG ≥ 110 mg/dL) (n = 231); group II without VFA of ≥ 100 cm^2 but presenting with three or more other risk factors (n = 57); group III with VFA of ≥ 100 cm^2 accompanied by FPG ≥ 110 mg/dL alone (n = 27); and group IV with VFA ≥ 100 cm^2 and two or more other risk factors (n = 157). The prevalence of patients who had three or more risk factors with or without VFA ≥ 100 cm^2 was 45.3% (214 out of 472 patients), while that of those with VFA ≥ 100 cm^2 who had two or more other risk factors was 33% (157 out of 472 patients). Group II had significantly higher VFA values than group I ($p < 0.05$), and group IV had significantly higher VFA values than group II ($p < 0.001$). While no significant differences in HOMA-R values were seen between groups I and II, these values were significantly higher in group IV compared to groups I and II ($p < 0.001$ and $p < 0.05$, respectively). Furthermore, group IV showed significantly higher 2-h insulin levels after glucose loading compared to group I ($p < 0.001$). While no significant differences were seen between groups II and IV, insulin levels tended to be higher in group IV. Adiponectin levels showed an incremental fall in VFA from group I through groups II and III to group IV. Groups III and IV showed significantly lower adiponectin levels compared to group I ($p < 0.05$, $p < 0.001$, respectively); and group IV showed significantly lower adiponectin levels than group II ($p < 0.05$). A logistic regression analysis using VFA, TG and HDL-C, and BP as explanatory variables showed that the relative risk for high HOMA-R values were 2.65 ($p < 0.001$) for patients with VFA ≥ 100 cm^2; 1.64 ($p < 0.05$) for those with TG ≥ 150 mg/dL and HDL < 40 mg/dL; and 1.79 ($p < 0.01$) for those with BP ≥ 130/ ≥ 85 mmHg. These findings demonstrate that the degree of insulin resistance and the risk of arteriosclerosis vary depending on whether or not the metabolic syndrome accompanied by a clustering of risk factors has visceral fat accumulation as an underlying pathology, strongly suggesting a crucial role for visceral fat accumulation in the metabolic syndrome.

Key Words: Metabolic syndrome; ATPIII; visceral fat accumulation; HOMA-R; adiponectin; high-sensitivity CRP.

Introduction

The metabolic syndrome is defined as a metabolic disorder that predisposes affected individuals to cardiovascular disease by causing insulin resistance, atherogenic lipoprotein abnormalities, and hypertension. A variety of diagnostic criteria for the metabolic syndrome have become available overseas, including the criteria proposed by the National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATPIII) *(1)* and by the World Health Organization (WHO) *(2)*, a cause for some confusion among practicing physicians. In Japan, the Examination Committee on Diagnostic Criteria for Metabolic Syndrome drew up and published diagnostic criteria tailored to the Japanese population in April 2005 *(3)*. One major feature of these diagnostic criteria, in line with those of IDF *(4)*, is their inclusion of visceral fat accumulation, defined as a waist circumference of over 85 cm in men and over 90 cm in women corresponding to a visceral fat area (VFA) of 100 cm^2 in an

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Author to whom all correspondence and reprint requests should be addressed: Yutaka Mori, MD, Department of Internal Medicine, National Hospital Organization, Utsunomiya National Hospital, 2160 Shimookamoto, Kawachi-machi, Kawachi-gun, Tochigi 329-1193, Japan. E-mail: moriyutakajp@yahoo.co.jp

Table 1
Characteristics of Metabolic Syndrome With or Without Visceral Fat Accumulation

	Group			
	I (n = 231)	II (n = 57)	III (n = 27)	IV (n = 157)
VFA ≥ 100 cm^2	—	—	+	+
Other risk factor (incl. FPG ≥ 110)	2↓	3↑	1	2↑
MS (ATP III)	—	+	—	+
MS (new diagnostic criteria)	—	—	—	+
Age*	47 ± 7.4	47 ± 7.2	49 ± 6.6	49 ± 6.8†
Height (cm)	169 ± 5.6	169 ± 6.4	170 ± 6.1	169 ± 5.6
Body weight (kg)***	66.1 ± 7.6	66.8 ± 9.6	75.1 ± 9.9††,‡	75.8 ± 9.4†††,‡‡‡
BMI***	24.1 ± 3.0	24.3 ± 3.0	26.4 ± 2.5†	27.4 ± 4.5†††,‡‡‡
VFA (cm^2)***	60 ± 25	73 ± 23†	118 ± 25†††,‡‡‡	139 ± 37†††,‡‡‡,§§
SFA (cm^2)***	121 ± 58	123 ± 46	162 ± 64††,‡	155 ± 61†††,‡‡
V/S ratio***	0.56 ± 0.3	0.64 ± 0.2	0.81 ± 0.3†	0.96 ± 0.6†††,‡‡‡
TG (mg/dL)***	113 ± 64	219 ± 86†††	119 ± 53‡‡‡	186 ± 107†††,§§
HDL-C (mg/dL)***	58 ± 13	46 ± 1†††	58 ± 16‡‡	51 ± 12†††
SBP (mmHg)***	127 ± 16	139 ± 13	120 ± 10‡‡‡	139 ± 16†††,§§§
DBP (mmHg)***	78 ± 1	85 ± 10†††	75 ± 9	85 ± 11†††,§§§
LDL-C (mg/dL)*	128 ± 31	130 ± 35	131 ± 24	139 ± 34†
Uric acid (mg/dL)**	6.2 ± 1.3	6.6 ± 1.2	6.4 ± 1.4	6.7 ± 1.3††
HbA1c (%)	5.2 ± 0.6	5.3 ± 0.6	5.4 ± 0.5	5.4 ± 0.9
AUC for glucose (mg·h/dL)**	317 ± 52	31 ± 60	24 ± 47	336 ± 52†
ACU for insulin (μU·h/mL)***	94 ± 52	114 ± 54	119 ± 60	135 ± 78†††

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, for trend; $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$, $^{\dagger\dagger\dagger}p < 0.001$ vs I; $^{\ddagger}p < 0.05$, $^{\ddagger\ddagger}p < 0.01$, $^{\ddagger\ddagger\ddagger}p < 0.001$ vs II; $^{\S\S}p < 0.01$, $^{\S\S\S}p < 0.001$ vs III.

VFA, visceral fat area; SFA, subcutaneous fat area; MS, metabolic syndrome; AUC, area under the curve.

abdominal CT scan at the umbilical level *(5)*, as an essential criterion for the metabolic syndrome, which, with two or more risk factors from among lipoprotein abnormalities (TG ≥ 150 mg/dL and/or HDL-C < 40 mg/dL), hypertension (≥ 130/ ≥ 85 mmHg), and FPG ≥ 110 mg/dL, combine to establish the diagnosis of the metabolic syndrome, thus positioning visceral fat accumulation as a key player in the metabolic syndrome *(3)*. Our current study investigated what pathology might be in place in patients who do not meet these new criteria for the metabolic syndrome, despite the observed clustering of multiple risk factors, other than visceral fat accumulation.

Results

The prevalence of the metabolic syndrome based on the ATPIII diagnostic criteria and the new diagnostic criteria was 45.3% (214 out of 472 subjects) and 33.3% (157 out of 472 subjects), respectively. Groups III and IV had significantly higher BMI values than group I ($p < 0.05$ and $p < 0.001$, respectively), and group IV showed significantly higher BMI values than group II ($p < 0.001$) (Table 1). Group II showed significantly higher VFA values than group I ($p < 0.05$), and group IV showed significantly higher values than group II ($p < 0.001$) (Table 1).

As for laboratory variables other than those included in the diagnostic criteria for the metabolic syndrome, group IV had significantly higher LDL-C and uric acid levels than group I ($p < 0.05$ and $p < 0.01$, respectively) (Table 1). While no intergroup differences were seen in HbA1c, the areas under the plasma glucose and insulin curve (in an oral glucose tolerance test [OGTT] using 75 g glucose) were significantly higher in group IV compared to group I ($p < 0.05$ and $p < 0.001$, respectively) (Table 1).

No significant differences were seen in HOMA-R between groups I and II, while HOMA-R values were significantly higher in group IV than in groups I or II ($p < 0.001$ and $p < 0.05$, respectively) (Fig. 1). Additionally, group IV showed significantly higher 2-h insulin levels after glucose loading compared to group I ($p < 0.001$). Furthermore, insulin levels tended to be higher in group IV than in group II (Fig. 1), while not being significantly different. Adiponectin levels showed a declining tendency with increasing VFA from group I to groups II, III, and IV. Groups III and IV had significantly lower adiponectin levels than group I ($p < 0.05$ and $p < 0.001$), and these levels were significantly lower in group IV than in group II ($p < 0.05$) (Fig. 2). Likewise, high-sensitivity CRP values showed an increasing tendency with increasing VFA from group I to groups II, III, and IV.

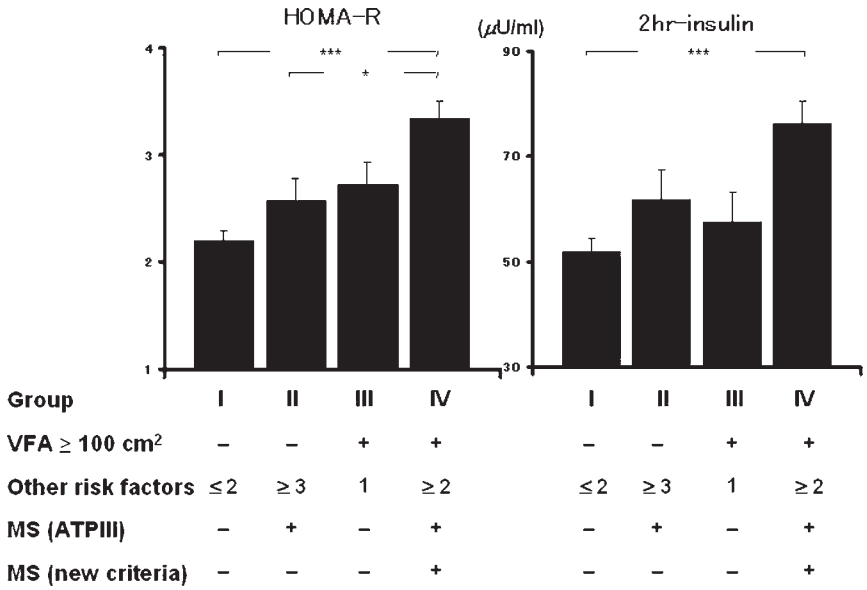

Fig. 1. HOMA-R values and 2-h insulin levels after glucose loading in the metabolic syndrome accompanied by visceral fat accumulation, versus that not accompanied by visceral fat accumulation. Other risk factors for all groups studied include FPG ≥ 110 mg/dL. VFA, visceral fat area; MS, metabolic syndrome.

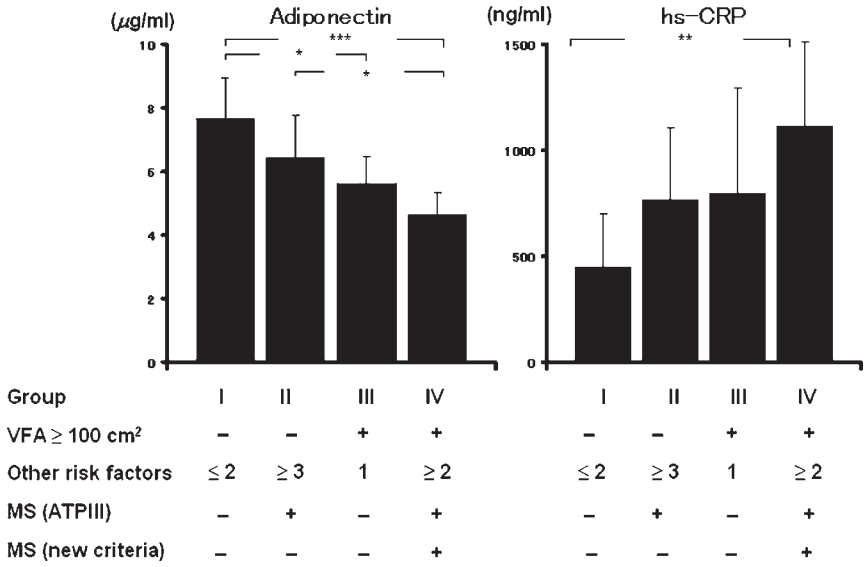

Fig. 2. Adiponectin levels and high-sensitivity CRP values in the metabolic syndrome accompanied by visceral fat accumulation, versus that without visceral fat accumulation. Other risk factors for all groups studied include FPG ≥ 110 mg/dL. VFA, visceral fat area; MS, metabolic syndrome.

Group IV showed significantly higher values than group I ($p < 0.01$) (Fig. 2).

In a logistic regression analysis using high HOMA-R values as the target variable, the relative risk for high HOMA-R values was shown to be 2.65 ($p < 0.001$) for VFA ≥ 100 cm^2, 1.64 ($p < 0.05$) for TG > 150 mg/dL and HDL < 40 mg/dL, and 1.79 ($p < 0.01$) for BP ≥ 130/ ≥ 85 mmHg.

Discussion

In this study, group II who had three or more risk factors, other than VFA ≥ 100 cm^2 (those who met the diagnosis of the metabolic syndrome under the ATPIII diagnostic criteria, but failed to meet the new criteria) showed significantly higher VFA values than group I without VFA ≥ 100 cm^2 who had two or fewer other risk factors (thus failing to meet the ATPIII or new diagnostic criteria for the metabolic syndrome) while there were no significant differences in HOMA-R or in 2-h insulin levels after glucose loading between the two groups. These findings suggest that visceral fat accumulation is implicated in the clustering of risk factors, even in those with VFA < 100 cm^2.

HOMA-R and 2-h insulin levels after glucose loading were more or less similar in group II (those with three or

more other risk factors but not VFA ≥ 100 cm^2) and group III (those with FPG ≥ 110 mg/dL only, in addition to VFA ≥ 100 cm^2). Furthermore, group IV who had two or more other risk factors in addition to VFA ≥ 100 cm^2 (those who met the ATPIII and new diagnostic criteria for the metabolic syndrome) showed significantly higher HOMA-R values as well as high 2-h insulin levels after glucose loading as compared to group II who had three or more other risk factors but not VFA ≥ 100 cm^2.

Published reports have shown that the level of adiponectin, an adipocytokine specific to adipose cells *(6)*, decreases with the accumulation of visceral fat *(7)*, and that hypoadiponectinemia is closely associated with insulin resistance *(8)* and arteriosclerosis *(9,10)*. In this study also, adiponectin levels were shown to decrease with the accumulation of VFA, from group I to groups II, III, and IV, with the adiponectin levels being significantly lower in group IV compared to group II ($p < 0.05$). Furthermore, of the laboratory variables examined other than those included in the diagnostic criteria for the metabolic syndrome, LDL-C and uric acid as well as areas under the glucose and insulin curves at OGTT with 75 g glucose were shown to be significantly higher in group IV than group I, while no significant differences were seen in these variables between groups I and II.

These findings demonstrate that the degree of insulin resistance and the risk of arteriosclerosis vary depending on whether or not the metabolic syndrome accompanied by a clustering of risk factors has visceral fat accumulation as an underlying pathology. Likewise, a logistics regression analysis showed that the relative risk for high HOMA-R values is 2.65 for VFA ≥ 100 cm^2, being higher than for TG > 150 mg/dL and HDL < 40 mg/dL (1.64), and of BP ≥ 130/ ≥ 85 mm Hg (1.79), suggesting that VFA ≥ 100 cm^2 contributed to a greater extent to the development of insulin resistance than the other risk factors. These results strongly support the view that visceral fat accumulation plays a key role in the metabolic syndrome *(11,12)*.

While the mechanism(s) through which insulin resistance may be related to the development of atherosclerosis remains to be clarified, insulin resistance is assumed to be an important factor likely contributing to the development of atherosclerosis by inducing multiple atherosclerotic risk factors as shown in this study. It is also of note that insulin *per se* is thought to act as a growth factor on vascular smooth muscles thus giving rise to the view that hyperinsulinemia is also implicated in atherosclerosis *(13)*. Furthermore, in recent years, research findings have been accumulated suggesting that, in insulin-resistant states, well in advance of the onset of abnormalities such as proliferation of vascular smooth muscle cells, vascular dysfunction as represented by vasodilatory response is found to be present *(14)*. In our study, insulin resistance as part of the metabolic syndrome accompanied by visceral fat accumulation has been shown to contribute more to atherosclerosis than that without visceral fat accumulation. However, whether or not the presence of visceral fat accumulation in insulin resistance may account for any long-term differences in the development of atherosclerosis remains to be clarified in a future, long-term study.

Subjects and Methods

The study subjects were recruited from a pool of employees who received routine care at the outpatient clinic in the Matsushita Electric Industrial Corporation. Only subjects who decided to participate in the study voluntarily and who gave written informed consent were included in the study. A total of 472 pre-diabetic Japanese men (mean age, 47.5 ± 7.2 yr) with impaired fasting glycemia (IFG) (glucose levels of 110–125 mg/dL) based on clinical data provided by the employer were eligible for participation in the study. The subjects were excluded from the study if they were known to have diabetes mellitus (DM) or were receiving drug therapy that affected glucose tolerance, or for hyperlipidemia or hypertension. Glycemic status in this cohort of subjects had been reassessed by repeated oral glucose tolerance test (OGTT). The diagnostic criteria for the metabolic syndrome set forth by the NCEP ATPIII guidelines *(1)*, Japanese new diagnostic criteria for the metabolic syndrome *(3)*, and the Criteria for Obesity Disease in Japan *(5)* were used as references to divide the study subjects into four groups, and intergroup comparisons were made: group I without VFA ≥ 100 cm^2 but accompanied by two or fewer other risk factors (TG ≥ 150 mg/dL, HDL-C < 40 mg/dL, BP ≥ 130/ ≥ 85 mmHg, or FPG ≥ 110 mg/dL) ($n = 231$); group II without VFA ≥ 100 cm^2 but accompanied by three or more other risk factors ($n = 57$); group III with VFA ≥ 100 cm^2 accompanied by FPG ≥ 110 mg/dL only ($n = 27$); and group IV with VFA ≥ 100 cm^2 and two or more other risk factors ($n = 157$).

After fasting overnight, the subjects were subjected to an OGTT with 75-g glucose early in the morning. Blood samples were drawn from the median cubital vein before the test and every 30 min for a period of 2 h. Plasma glucose levels were determined by the glucose dehydrogenase methods. Insulin and adiponectin levels were determined using commercial enzyme immunoassay kits (LS Eiken Insulin Kit, Eiken Chemical, Tokyo, Japan and adiponectin ELISA kit, Otsuka, Tokushima, Japan). High sensitivity C-reactive protein (hs CRP) was measured by latex nephelometry assay (N High Sensitivity CRP, Dade Behring, Marburg GmbH, Marburg, Germany). The incremental areas under the insulin (AUC insulin) and glucose (AUC glucose) curves were calculated by the trapezoidal method for 0-, 30-, 60-, 90-, and 120-min time points.

The estimate of insulin resistance was based on a homeostasis model assessment (HOMA-R) as described by Matthews et al. *(15)* and 2-h insulin levels after glucose loading. Serum

lipids (triglycerides, total cholesterol, HDL cholesterol) were measured enzymatically using enzyme reagents (L-Type TG H, Wako Pure Chemicals, Osaka, Japan; L-Type CHO H, Wako Pure Chemicals, Osaka, Japan; Cholestest N HDL, Daiichi Pure Chemicals, Tokyo, Japan). An estimate of the LDL-cholesterol (LDL-C) concentration was then made from these three measurements using the Friedewald formula when TG levels were below 400 mg/dL. The hemoglobin A1c (HbA1c) was measured by cation exchange high-performance liquid chromatography (Bio-Rad Laboratories, Hercules, CA, USA). Blood pressure was measured at least twice, with the subjects in a seated position after at least 5 min of rest. The average of blood pressure measurements was used for the analysis. Body mass index (BMI [kg/m^2]) was computed from current body weight and height. Abdominal computed tomography (CT; Hitachi model, CTW550, Hitachi Medical Co., Yokyo, Japan) scans at the umbilical level were also performed on all subjects during this same time period. Abdominal VFA and subcutaneous fat area were measured, as described elsewhere *(5,16)*.

Statistical Analysis

All data are presented as means ± SD. An estimation of the reliability of intergroup differences was made using one-way analysis of covariance. The Scheffe method was used to adjust for multiple comparisons in a post hoc analysis. A p value < 0.05 was considered significant. Regression analysis was used to evaluate the relative risk for insulin resistance associated with the number of risk factors involved in the metabolic syndrome, where VFA, TG and HDL-C and BP were used as explanatory variables to evaluate the relative risk for high HOMA-R values associated with the number of risk factors involved in terms of odds ratio. The median value for all subjects (2.59) was used as the cutoff for HOMA-R.

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