Increased Visceral Fat and Serum Levels of Triglyceride Are Associated With Insulin Resistance in Japanese Metabolically Obese, Normal Weight Subjects With Normal Glucose Tolerance

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OBJECTIVE — The purpose of this study was to investigate the association between visceral adiposity or triglyceride (TG) metabolism and insulin resistance in metabolically obese, normal weight (MONW) Japanese individuals with normal glucose tolerance.

RESEARCH DESIGN AND METHODS — We evaluated body fat areas, lipid profiles, and the glucose infusion rate (GIR) during a euglycemic-hyperinsulinemic clamp study in 20 MONW subjects (BMI <25 kg/m^{2}and visceral fat areas 100 cm^2) with normal glucose tolerance. Body fat areas were measured by computed tomography scans. Control data were obtained from 20 normal subjects (BMI <25 kg/m^2 and visceral fat areas <100 cm^2).

RESULTS — MONW subjects showed a significant increase in fasting serum levels of TG ($P <$ 0.01) and a decrease in GIR ($P < 0.01$) compared with normal subjects. There were significant correlations between visceral fat areas ($r = -0.563$, $P < 0.01$) or serum levels of TG ($r = -0.474$, $P < 0.05$) and GIR in MONW subjects. Multiple regression analyses showed that visceral fat areas ($F = 7.702$, $P < 0.02$) and serum levels of TG ($F = 7.114$, $P < 0.05$) were significantly associated with GIR in all (MONW and normal) subjects.

CONCLUSIONS — Increased visceral fat and serum levels of TG are associated with insulin resistance in Japanese MONW subjects with normal glucose tolerance. Excess visceral fat and elevated TG levels may play important roles in the development of insulin resistance in Japanese MONW subjects with normal glucose tolerance.

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Recent reports described the existence of individuals with normal body weight but with a cluster of obesity-related characteristics (1,2). They are characterized by excess visceral fat, insulin resistance, and hyperinsulinemia and have been called metabolically obese, normal weight (MONW) subjects. Ethnic differences should be considered for identifying MONW subjects. In the Japanese population, nonobese (BMI <25 kg/m^2) subjects with increased visceral fat areas (100 cm^2) fulfill the criteria for categorizing them in the MONW group (2–6). Regarding the association of the MONW state with diabetes, higher prevalence of hyperglycemia has been observed in MONW subjects than in normal individuals (3,4).

Previous studies have demonstrated that visceral fat areas are associated with insulin resistance in Japanese subjects with normal glucose tolerance and impaired glucose tolerance and in nonobese Japanese patients with type 2 diabetes (7–9). Visceral fat accumulation is also associated with serum triglyceride (TG) levels, and the disturbance of TG metabolism precedes the development of insulin resistance in nonobese Japanese type 2 diabetic patients (9,10). However, the relationships of visceral adiposity and TG metabolism with insulin resistance in Japanese MONW subjects with normal glucose tolerance have not been evaluated. To clarify these points, in the present study, we investigated the relationship between visceral fat areas or serum levels of TG and insulin resistance in Japanese MONW subjects with normal glucose tolerance.

RESEARCH DESIGN AND METHODS

Subjects

This study comprised 20 MONW and 20 age-matched normal subjects (BMI <25 kg/m^2 and visceral fat areas <100 cm^2). BMI was calculated as body weight (in kilograms) divided by the square of height (in meters).

None of the subjects had impaired glucose tolerance or diabetes according to the diagnostic criteria of the American Di-

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Abbreviations: AUC, area under the curve; GIR, glucose infusion rate; MONW, metabolically obese, normal weight; TG, triglyceride.

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

abetes Association based on the 75-g oral glucose tolerance test (Trelan G 75; Shimizu, Shimizu, Japan) (11). Eleven subjects in the MONW group had hyperlipidemia (total cholesterol 5.69 mmol/l or TG 1.69 mmol/l). Arterial hypertension was not observed in any subject. None of the subjects were receiving any medication that could affect insulin levels and insulin sensitivity, and the subjects were not under any regular exercise or dietary therapy before the beginning of this study. Three normal and five MONW subjects had a family history of diabetes among their first-degree relatives. We asked all subjects whether some member of their family had obesity (BMI 25 kg/m^2) or had a past history of obesity. One normal and two MONW individuals had a family history of obesity among their first-degree relatives.

Informed consent was obtained from all subjects before the beginning of the study.

Table 1—*Clinical characteristics of the study subjects*

Parameters	Normal subjects	MONW subjects
n	20	20
Age (years)	33.1 ± 1.6	34.3 ± 1.5
Sex (male/female)	19/1	19/1
BMI (kg/m^2)	21.0 ± 0.3	23.5 ± 0.2†
Body fat weight (kg)	11.8 ± 0.6	17.1 ± 0.5†
Visceral fat area (cm^2)	57.5 ± 5.5	134.4 ± 5.1†
Subcutaneous fat area (cm^2)	97.9 ± 7.4	157.5 ± 15.2†
Fasting plasma glucose (mmol/l)	4.9 ± 0.3	5.0 ± 0.1
Glucose AUC (mmol/l)	749.8 ± 25.0	812.1 ± 27.6
Fasting serum insulin (pmol/l)	26.4 ± 1.6	49.8 ± 6.0†
Insulin AUC (pmol/l)	33,542.7 ± 3,213.1	46,438.2 ± 5,011.2*
$\Delta I_{30}/\Delta G_{30}$	124.3 ± 10.8	162.7 ± 31.3
Total cholesterol (mmol/l)	4.31 ± 0.20	4.37 ± 0.21
TG (mmol/l)	1.05 ± 0.07	1.89 ± 0.23†
HDL cholesterol (mmol/l)	1.31 ± 0.06	1.21 ± 0.04
Serum free fatty acid (mmol/l)	0.46 ± 0.05	0.47 ± 0.04
Systolic blood pressure (mmHg)	116.2 ± 2.3	127.4 ± 2.2†
Diastolic blood pressure (mmHg)	71.7 ± 2.1	80.0 ± 1.3†
GIR (μmol · kg^{-1} · min^{-1})	60.2 ± 2.9	45.4 ± 1.4†

Data are means ± SE. *$P < 0.05$; †$P < 0.01$ vs. normal subjects.

Methods

Several parameters in blood samples, body fat weight, body fat distribution, insulin resistance, the capacity to secrete insulin, and blood pressure were evaluated in all subjects. Venous blood was collected at 8:00 A.M. after an overnight bed rest. After centrifugation, the plasma and serum samples were separated in small aliquots and then frozen at −20°C until use.

The plasma glucose level was measured by an automated enzymatic method. Serum levels of total cholesterol, TG, HDL cholesterol, and free fatty acids were measured by enzymatic methods using an autoanalyzer (TBA60M; Toshiba, Tokyo, Japan). Serum insulin levels were measured using an immunoradiometric assay kit (Insulin Riabead II kit; Dainabot, Tokyo, Japan). This kit included 125I-labeled and unlabeled anti-human insulin mouse monoclonal antibodies. The intra- and interassay coefficients of variation of the assay were 1.9% and 2.0%, respectively. No cross-reactivity or interference was observed between insulin and proinsulin, C-peptide, glucagon, secretin, and gastrin-I.

Body fat weight was measured by bioelectric impedance using a TBF-101 (Tanita, Tokyo, Japan). Body fat area was evaluated by a previously described method (5,6,12). At 8:00 A.M., after an overnight fast of 11 h, all subjects underwent a single abdominal-computed tomography scan at the umbilical level. Any intraperitoneal region having the same density as the subcutaneous fat layer was defined as a visceral fat area; this area was measured by tracing object contours on film using a computerized planimetric method.

Insulin resistance was evaluated by the euglycemic-hyperinsulinemic clamp technique using an artificial pancreas (Nikkiso STG-22; Nikkiso, Tokyo, Japan) (5,6,13). At 8:00 A.M., a priming dose of insulin (Humulin R; Shionogi, Osaka, Japan) was administered during the initial 10 min in a logarithmically decreasing manner to rapidly raise serum insulin to the desired level (1,200 pmol/l); this level was then maintained by continuous infusion of insulin at a rate of 13.44 pmol · kg^{-1} · min^{-1} for 120 min. The mean insulin level, from 90 to 120 min after starting the clamp study, was stable (MONW subjects, 1,235.4 ± 52.2 pmol/l; normal subjects, 1,187.4 ± 46.8 pmol/l). Blood glucose was monitored continuously and maintained at the target clamp level (5.24 mmol/l) by infusing 10% glucose. The mean amount of glucose given during the last 30 min was defined as the glucose infusion rate (GIR) and was used as a measure of peripheral insulin sensitivity.

The 75-g oral glucose tolerance test was started at 8:00 A.M. after an overnight bed rest (fasting for 11 h). Blood was taken at 0, 30, 60, and 120 min, and plasma glucose and serum insulin were evaluated. The capacity to secrete insulin in response to oral glucose stimulation was estimated by the ratio of I_{30} to G_{30} (I_{30}/G_{30} was calculated as follows: increment of serum insulin from 0 to 30 min/increment of plasma glucose from 0 to 30 min) and the insulin area under the curve (AUC). Plasma glucose AUC was also calculated. We also measured blood pressure in supine position after a 5-min rest.

Statistical analysis

Comparisons between MONW and normal subjects were done using the two-tailed Student's *t* test. Correlations were evaluated by univariate regression analysis and multivariate analysis. All statistical analyses were performed using the StatView 4.0 software (Abacus Concepts, Berkeley, CA) for the Macintosh. $P < 0.05$ was considered statistically significant.

RESULTS — BMI ($P < 0.01$), body fat weight ($P < 0.01$), visceral ($P < 0.01$) and subcutaneous fat areas ($P < 0.01$), and systolic ($P < 0.01$) and diastolic blood pressure ($P < 0.01$) were significantly increased in MONW subjects compared with normal subjects (Table 1).

MONW individuals showed significant hyperinsulinemia and hypertriglyc-

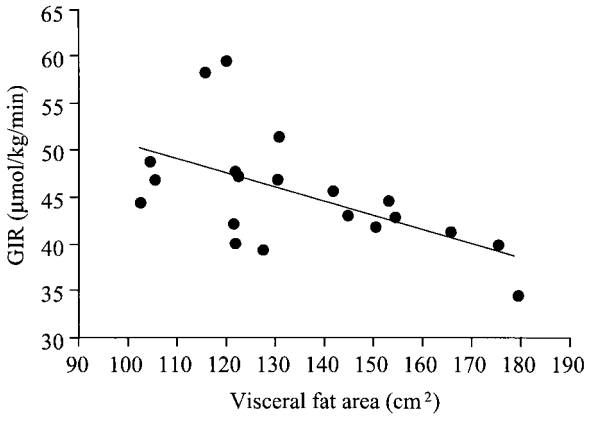

Figure 1—*Correlation between visceral fat areas and GIR in Japanese MONW subjects with normal glucose tolerance. Visceral fat areas were significantly correlated with GIR* (r = *−0.563,* P < *0.01).*

eridemia (fasting insulin levels, $P < 0.01$; insulin AUC, $P < 0.05$; TG, $P < 0.01$) compared with normal subjects. The GIR in MONW subjects were significantly decreased compared with normal subjects ($P < 0.01$).

There were significant correlations between visceral fat areas ($r = -0.563$, $P < 0.01$) (Fig. 1) or serum levels of TG ($r = -0.474$, $P < 0.05$) (Fig. 2) and GIR in MONW subjects. Visceral fat areas were significantly correlated with serum levels of TG ($r = 0.533$, $P < 0.02$) and fasting serum levels of insulin ($r = 0.503$, $P < 0.05$) in MONW subjects.

No significant correlations were observed between subcutaneous ($r = -0.249$, $P = 0.290$) or total (visceral and subcutaneous) fat areas ($r = -0.366$, $P = 0.113$) and GIR in MONW subjects.

Multiple regression analyses showed that visceral fat areas ($F = 7.702$, $P < 0.02$) and serum levels of TG ($F = 7.114$, $P < 0.05$) were significantly associated with GIR in all (MONW and normal) subjects.

CONCLUSIONS — This is the first report that demonstrates a significant relationship of increased visceral fat or elevated serum TG levels with insulin resistance in Japanese MONW subjects with normal glucose tolerance.

Recent studies have demonstrated that visceral rather than subcutaneous adipose tissue secrete adipocytokines and that their circulating levels are correlated with the development of insulin resistance and with the occurrence of cardiovascular diseases (4,14–18). Visceral fat accumulation may be a major contributor for the development of insulin resistance in Japanese MONW subjects with normal glucose tolerance. The MONW state can be diagnosed in subjects at a young age (2); therefore, it is necessary to diagnose this syndrome early to prevent the occurrence of several obesity-related disorders. Scoring methods should be proposed for identifying MONW subjects in the Japanese population as well as in other ethnic groups (2).

The explanation for the significant correlation between serum levels of TG and insulin resistance in Japanese MONW subjects with normal glucose tolerance remains unknown. Normalization of TG metabolism through surgical procedures has been reported to improve insulin resistance in two sisters with extreme hypertriglyceridemia due to familial lipoprotein lipase deficiency (19). Our MONW subjects showed minimally elevated serum levels of TG. Mild but not severe hypertriglyceridemia may lead to insulin resistance in Japanese MONW subjects with normal glucose tolerance.

Diet combined with exercise therapy is useful to reduce visceral fat and improve insulin resistance in nonobese patients with type 2 diabetes (5,6). It has been reported that improvement of TG metabolism by bezafibrate reduces insulin resistance in nonobese Japanese patients with type 2 diabetes (20). Bezafibrate with diet and exercise therapy may be indicated in MONW subjects with normal glucose tolerance to prevent the development of glucose intolerance.

In the present study, a significant relationship between visceral fat areas and serum TG levels was observed in Japanese MONW subjects with normal glucose tolerance. Taniguchi et al. (9) reported the same relationship in nonobese Japanese type 2 diabetic patients. It was reported that free fatty acids released from intraperitoneal fat tissue is drained directly into the liver through the portal system, leading to increased serum TG levels (21). This may also occur in Japanese MONW subjects with normal glucose tolerance. Thus, it is conceivable that visceral fat accumulation is the primary factor for the development of insulin resistance in Japanese MONW subjects with normal glucose tolerance.

In conclusion, the present study showed that increased visceral fat and serum levels of TG are associated with insulin resistance in Japanese MONW subjects with normal glucose tolerance. Excess visceral fat and elevated TG levels may play important roles in the development of insulin resistance in nonobese Japanese subjects with and without type 2 diabetes.

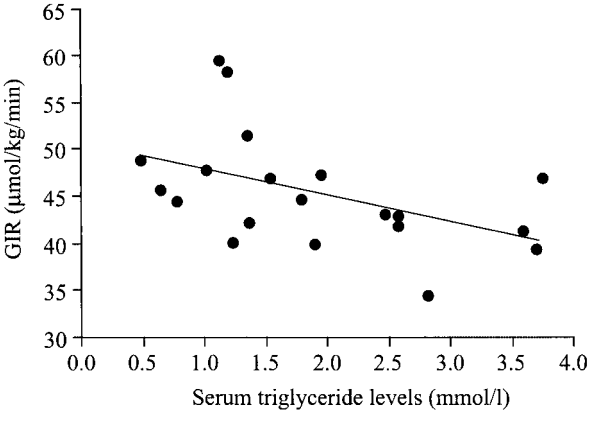

Figure 2—*Correlation between serum levels of TG and GIR in Japanese MONW subjects with normal glucose tolerance. Serum levels of TG were significantly correlated with GIR* (r = *−0.474,* P < *0.05).*

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