Endocrine, *vol. 29, no. 2, 357–361, April 2006* 0969–711X/06/29:357–361/$30.00
ENDO (Online) ISSN 1559-0100

Role of Hypoadiponectinemia in the Metabolic Syndrome and its Association with Post-Glucose Challenge Hyper-Free Fatty Acidemia

A Study in Prediabetic Japanese Males

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

We investigated the role of hypoadiponectinemia in the metabolic syndrome (MS), as well as its association with post-glucose challenge hyper-free fatty acidemia in the clinical setting. The study subjects comprised 177 corporate employees shown to have a fasting plasma glucose (FPG) level of 125 mg/dL or less in a 75 g OGTT in the corporation's healthcare center. When divided into those who met the Japanese criteria for the metabolic syndrome (MS group; $n = 45$) and those who did not (Non-MS group; $n = 132$), the MS group was shown to have significantly lower adiponectin levels than the Non-MS group, and tended to show higher high-sensitivity C-reactive protein (CRP) values than the Non-MS group, while not achieving statistical significance. The MS group showed higher baseline glucose levels; higher baseline, 30-, 60-, and 120-min post-challenge insulin levels; higher 30-, 60-, and 120-min post-challenge free fatty acid levels than the Non-MS group. Additionally, there was a significant, negative correlation between adiponectin levels, area under the free fatty acid curve, and area under the insulin curve at OGTT ($r = -0.24$, $p < 0.01$; $r = -0.21$, $p < 0.01$, respectively). When the patients were divided by adiponectin level into four groups to examine the number of risk factors for MS detected per patient and the incidence of MS, the lower the adiponectin level, the more risk factors were found per patient, with 68% of patients with an adiponectin level of less than 4 µg/mL found to have MS. In those with an adiponectin level of less than 4 µg/mL, BMI values, uric acid levels, HOMA-R values, and the number of risk factors for MS involved per patient were shown to be higher than in those with an adiponectin level of 4 µg/mL or greater. Furthermore, the following risk factors for MS were more frequently found in those with an adiponectin level of less than 4 µg/mL than in those with an adiponectin level of 4 µg/mL or greater: VFA ≥ 100 cm^2 (OR 12.8, $p < 0.001$); TG ≥ 150 mg/dL (OR 3.2, $p < 0.05$); HDL-C < 40 mg/dL (OR 1.9, $p = 0.29$); BP ≥ 130/85 mmHg (OR 2.2, $p = 0.15$); and FPG ≥ 110 mg/dL (OR 1.9, $p = 0.29$). Again, the incidence of MS (OR 7.6, $p < 0.001$) by the ATPIII criteria, as well as that by the Japanese criteria (OR 8.6, $p < 0.001$), was found to be higher in those with an adiponectin level of less than 4 µg/mL than in those with an adiponectin level of 4 µg/mL or greater. Our study results suggest that adiponectin is closely associated with the multiple risk factors that go to make up the MS, suggesting a role for hypoadiponectinemia as a surrogate marker for the MS and further appear to suggest that post-challenge hyper-free fatty acidemia may account in part for hypoadiponectinemia in the MS.

Key Words: Metabolic syndrome; free fatty acid; adiponectin; high-sensitivity CRP; insulin resistance.

Introduction

The metabolic syndrome (MS) is defined as a condition that makes affected individuals highly susceptible to cardiovascular disease characterized by a clustering of multiple risk factors for atherosclerosis, such as abdominal obesity, atherogenic dyslipoproteinemia, high blood pressure, and high fasting plasma glucose levels, in the presence of insulin resistance. In Japan, the Examination Committee for the Metabolic Syndrome drew up and set forth the diagnostic criteria tailored to the needs of the Japanese population in April 2005 *(1)*, in which, as in the IDF diagnostic criteria *(2)*, most characteristically, visceral fat accumulation is included as an essential determinant that makes up the diagnosis of the MS when it is associated with a clustering of any two

Received October 5, 2005; Revised December 16, 2005; Accepted December 27, 2005.

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

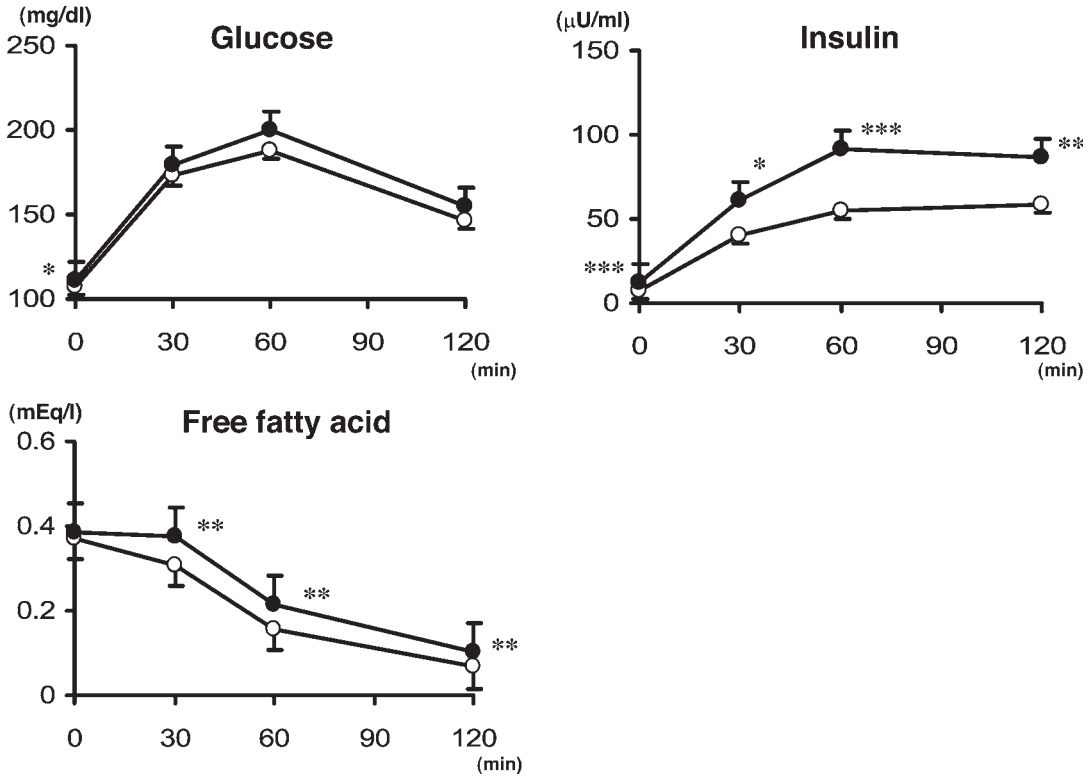

Fig. 1. Plasma glucose, insulin and free fatty acid levels during OGTT in the subjects with or without the metabolic syndrome as defined by the Japanese criteria. -●-, those with the metabolic syndrome ($n = 45$); -○-, those without the metabolic syndrome ($n = 132$). *$p <$ 0.05, **$p < 0.01$,***$p < 0.001$, vs those without the metabolic syndrome. Vertical bars represent SEM.

or more risk factors for the metabolic syndrome from among dyslipoproteinemia (TG ≥ 150 mg/dL and/or HDL-C < 40 mg/dL), high blood pressure (≥ 130/85 mmHg), and FPG ≥ 110 mg/dL, thus positioning visceral fat accumulation as a key player in the metabolic syndrome, in line with the reports of Wajchenberg et al. *(3)* or Carr et al. *(4)*. In these diagnostic criteria, visceral fat accumulation was defined as a waist circumference of 85 cm or greater in men and 90 cm or greater in women, which is deemed equal to a visceral fat area (VFA) of 100 cm^2 as assessed by CT scan at the umbilical level *(5)*. Of note, recent reports have suggested the usefulness of adiponectin as a biomarker for the MS *(6)*, as well as the potential role of increased reactive oxygen species (ROS) as an early instigator of MS *(7)*. In our study, we investigated the role of hypoadiponectinemia in the metabolic syndrome, as well as its potential association with post-glucose challenge changes in free fatty acid levels.

Results

In terms of glucose tolerance, 59 subjects were found with normal glucose tolerance (NGT), 31 with isolated impaired fasting glycemia (IFG), 23 with isolated impaired glucose tolerance (IGT), 39 with combined IFG and IGT, and 25 with diabetes mellitus (DM). The adiponectin levels in those who met the ATPIII diagnostic criteria for the metabolic syndrome (MS group; $n = 48$) and in those who did not (Non-MS group; $n = 129$) were 5.18 ± 0.26 μg/mL and 6.92 ± 0.25 μg/mL, respectively, while the adiponectin levels in those who met the Japanese criteria for the metabolic syndrome (MS group; $n = 45$) and in those who did not (Non-MS group; $n = 132$) were 5.12 ± 0.26 μg/mL and 6.90 ± 0.24 μg/mL, respectively, with the adiponectin levels in those who met either the ATP III or Japanese criteria for the metabolic syndrome found to be significantly ($p < 0.001$) lower than in those who did not. The high-sensitivity C-reactive protein (CRP) values in the MS group who met the ATPIII diagnostic criteria and in the Non-MS group who did not were 851 ± 164 ng/mL and 576 ± 67 ng/mL, respectively, while the high-sensitivity CRP levels in the MS group who met the Japanese criteria and in the Non-MS group who did not were 879 ± 174 ng/mL and 573 ± 65 ng/mL, respectively, with the high-sensitivity CRP levels tending to be higher in both MS groups, while short of reaching statistical significance. Prechallenge glucose levels, prechallenge insulin levels, postchallenge 30-, 60-, and 120-min insulin levels, as well as postchallenge 30-, 60-, and 120-min free fatty acid levels were found to be significantly higher in the MS group than in the Non-MS group (Fig. 1). Furthermore, there was a significant, negative correlation between the area under the insulin curve, area under the free fatty acid curve, and adiponectin levels, with the simple correlation coefficient between the area under the glucose, area under the insulin curve, area under the free fatty acid curve, and

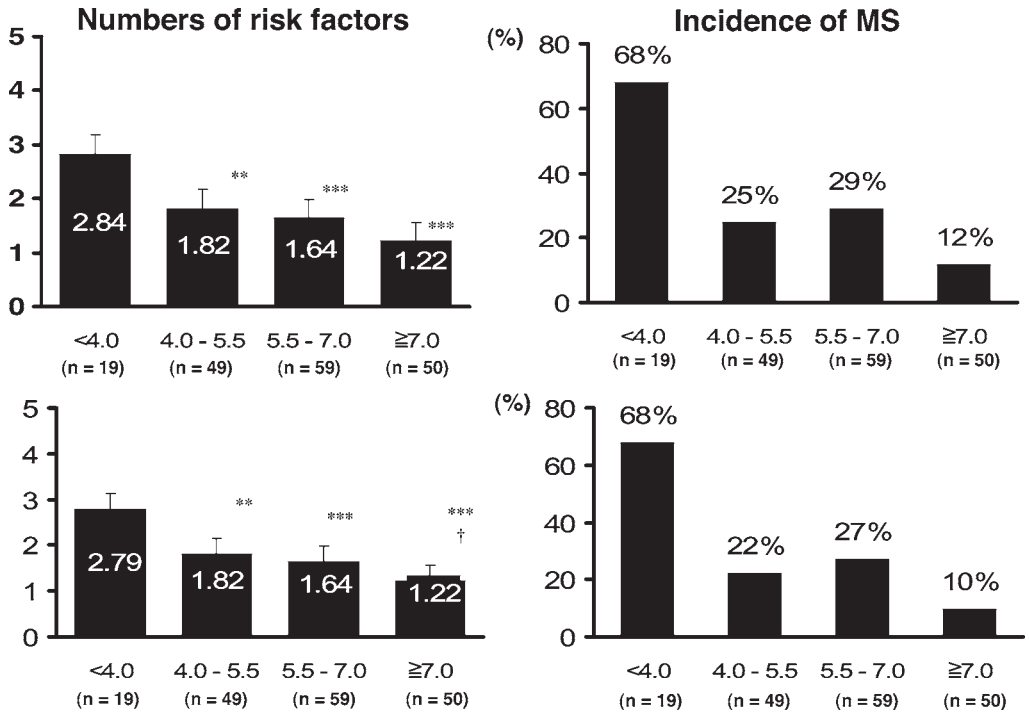

Fig. 2. Number of risk factors involved and incidence of MS as defined by the ATPIII (upper graphs) and Japanese (lower graphs) diagnostic criteria in the subjects stratified by adiponectin level. $^{**}p < 0.01$, $^{***}p < 0.001$ vs those with an adiponectin level of < 4.0 μg/mL, $^{\dagger}p < 0.05$ vs those with an adiponectin level of 4.0–5.5 μg/mL. Vertical bars represent SEM.

adiponectin levels being $r = -0.062$ ($p = 0.41$), $r = -0.21$ ($p < 0.01$), and $r = -0.24$ ($p < 0.01$), respectively.

When the subjects were further stratified by adiponectin level into four groups to compare the number of risk factors per subject, and the incidence of MS between these groups, the lower the adiponectin levels, the more risk factors were detected, and 68% of subjects exhibiting an adiponectin level of less than 4 μg/mL met the diagnosis of MS by either the ATPIII or Japanese diagnostic criteria (Fig. 2).

In subjects with an adiponectin level of less than 4 μg/mL, BMI values, uric acid levels, HOMA-R values, as well as the number of risk factors detected per subject were significantly higher than in those with an adiponectin level of 4 μg/mL or greater. Again, those with an adiponectin level of less than 4 μg/mL were significantly more frequently associated with the risk factors for MS including VFA ≥ 100 cm^2 (OR 12.8, $p < 0.001$), TG ≥ 150 mg/dL (OR 3.2, $p < 0.05$), HDL-C < 40 mg/dL (OR 14.6, $p < 0.01$), BP ≥ 130 / ≥ 85 mmHg (OR 2.2, $p = 0.15$), FPG ≥ 110 mg/dL (OR 1.9, $p = 0.29$), as well as the diagnosis of MS by the ATPIII criteria (OR 7.6, $p < 0.001$) and by the Japanese criteria (OR 8.6, $p < 0.001$) (Table 1).

Discussion

The level of adiponectin, an adipocytockine specific to adipocytes *(8)*, is reported to lower in conjunction with visceral fat accumulation, suggesting an association between hypoadiponectinemia and insulin resistance *(9)* or atherosclerosis *(10,11)*. In our study as well, hypoadiponectinemia was shown to be closely associated with risk factors for the metabolic syndrome including visceral fat accumulation, with the adiponectin levels found to be lower in the MS group who met both the ATPIII and Japanese criteria for the metabolic syndrome than in the Non-MS group who did not. Our study results also suggested that the decrease in adiponectin levels in the MS group might have affected pre- and postchallenge insulin levels in this group.

Meanwhile, recent research using cultured adipocytes *(7)* pointed to the role of ROS as an early "instigator" of MS, which, in agreement with an earlier study using cultured vascular cells *(12)*, led to the hypothesis that an increase in free fatty acid levels resulted in an increase in ROS via an increase in NADPH oxidase production and a decrease in oxidative stress exicionase production, where ROS was assumed to have caused an abnormal production of adipocytokines such as adiponectin in adipose tissues, while at the same time causing a systemic production of oxidative stress. In our study, the MS group showed significantly higher postchallenge free fatty acid levels than the Non-MS group, and a significant, negative correlation between the area under the free fatty acid curve and the adiponectin levels was also found. These findings combine to suggest that postchallenge hyper-free fatty acidemia may have contributed to the hypoadiponectinemia in the MS group. They also suggest that

Table 1
Clinical and Laboratory Test Findings, and Incidence of MS in Japanese Subjects Stratified by Adiponectin Level

	Adiponectin ≧ 4 μg/mL	Adiponectin < 4 μg/mL
Number of subjects	158	19
Age	52.3 ± 1.02	54.1 ± 2.1
BMI	25.7 ± 0.5	29.7 ± 1.8*
Systolic BP (mmHg)	125.8 ± 1.0	131.3 ± 3.0
Diastolic BP (mmHg)	77.1 ± 0.8	80.5 ± 2.1
TC (mg/dL)	208.7 ± 2.7	217.2 ± 5.8
LDL-C (mg/dL)	126.2 ± 25.0	134.0 ± 7.0
HDL-C (mg/dL)	56.8 ± 1.1	57.6 ± 8.2
TG (mg/dL)	131.6 ± 6.4	162.6 ± 24.3
Uric acid (mg/dL)	6.3 ± 0.1	7.1 ± 0.2*
HbA1c (%)	5.5 ± 0.03	5.7 ± 0.05
HOMA-R	2.2 ± 0.2	3.1 ± 0.4*
HOMA-β cell	69.2 ± 5.2	87.9 ± 8.4
ΔINS/ΔPG	0.78 ± 0.2	0.96 ± 0.4
AUC for glucose (mg·h/dL)	331.6 ± 4.7	332.2 ± 13.4
AUC for insulin (μU·h/mL)	102.6 ± 5.9	126.3 ± 15.2
AUC for FFA (mEq·h/mL)	0.41 ± 0.05	0.48 ± 0.02
Adiponectin (μg/mL)	6.84 ± 0.2	3.17 ± 0.1***
Hs-CRP (ng/mL)	647.1 ± 72.5	672.7 ± 116.9
Numbers of risk factors[1] (/subject)	1.56 ± 0.08	2.84 ± 0.23***
Incidence of VFA ≥ 100 cm^2	63/158 (39.9%)	17/19 (89.5%)***
Incidence of TG ≥ 150mg/dL	47/158 (29.7%)	11/19 (57.9%)*
Incidence of HDL < 40mg/dL	2/158 (1.3%)	3/19 (15.8%)**
Incidence of BP ≥ 135/80 mmHg	60/158 (38.0%)	11/19 (57.9%)
Incidence of FPG ≥ 110mg/dL	75/158 (47.5%)	12/19 (63.2%)
Incidence of MS[2] (%)	35/158 (22.2%)	13/19 (68.4%)***
Incidence of MS[3] (%)	32/158 (20.2%)	13/19 (68.4%)***

Mean ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

VFA, visceral fat area; SFA, subcutaneous fat area; AUC, area under the curve; Number of risk factors,[1] risk factors by the ATP III criteria; MS,[2] metabolic syndrome by the ATPIII criteria; MS,[3] metabolic syndrome by the Japanese criteria.

increased ROS in adipocytes may have caused an overproduction of IL-6 in local adipocytes, which likely led to the observed overproduction of CRP in the liver. There is, however, a report to the contrary, demonstrating that drug-induced sustained reduction in the plasma free fatty acid concentration does not alter the plasma adiponectin, resistin, IL-6, and TNF-α levels *(13)*. While this observation directly contradicts ours and the reason for this discrepancy is unclear, it also leads us to further hypothesize that a reduction in adiponectin production may be a primary cause of the metabolic syndrome, as it induces insulin resistance, independently of free fatty acid levels or ROS production.

Our study results demonstrate that adiponectin is closely associated with multiple risk factors that combine to make up the metabolic syndrome, and that hypoadiponectinemia may likely represent a viable surrogate marker for the metabolic syndrome. It is also suggested that postchallenge hyperfree fatty acidemia may likely contribute through ROS to the pathogenesis of hypoadiponectinemia and hyper-high-sensitivity C-reactive proteinemia as part of the metabolic syndrome.

Subjects and Methods

Of the male Matsushima Electric Industrial Corporation employees who underwent 75 g OGTT at the Corporation's Tokyo Healthcare Center, 177 males with FPG ≤ 125 mg/dL who decided to participate in the study voluntarily and who gave written informed consent were included in the study. 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.

The diagnostic criteria for MS as defined by the National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATPIII) *(14)*, as well as visceral fat accumulation [defined as a VFA of 100 cm^2 as measured by CT scans at the umbilical level *(15)*, which is equivalent to a waist circumference of 85 cm or greater], were primarily used for the

diagnosis of MS, while the Japanese criteria for the metabolic syndrome *(1)* were also used, where the subjects with visceral fat accumulation who met any two of the three criteria (TG ≥ 150 mg/dL and/or HDL-C < 40 mg/dL, blood pressure ≥ 130/≥ 85 mmHg, and FPG ≥ 110 mg/dL) were diagnosed as having the metabolic syndrome.

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. All subjects were classified by glycemic status according to the criteria of the ADA *(15)* and WHO *(16)*. Plasma glucose levels were determined by the glucose oxidase 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). Early-phase insulin secretion was calculated as a ratio of the increment of serum insulin (ΔINS) 30 min after the glucose load to plasma glucose (PG) concentration (ΔPG) 30 min after the glucose load (ΔINS/ΔPG). Insulin secretion was also estimated by HOMA-β cell *(17)*. The incremental areas under the insulin (AUC insulin) and glucose (AUC glucose) curve were calculated by the trapezoidal method for 0-, 30-, 60-, 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. *(17)*. Serum lipids (triglycerides, free fatty acid, total cholesterol, HDL cholesterol) were measured enzymatically using enzyme reagents (L-Type TG H, Wako Pure Chemicals, Osaka, Japan; NEFA-SS, Eiken Chemical, Tokyo, Japan; L-Type CHO H, Wako Pure Chemicals; Cholestest N HDL, Daiichi Pure Chemicals, Tokyo, Japan). An estimate of the LDL-cholesterol (LDL-C) concentration was then made from three measurements (TC, HDL-C, TG) using the Friedewald formula when TG levels are below 400 mg/dL. Serum uric acid levels were measured by uricase POD assay using enzyme reagents (L-Type UA F, Wako Pure Chemicals). 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., Tokyo, 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 *(5,18)*.

Statistical Analysis

All data were presented as means ± SEM. An estimation of the reliability of inter-group differences was made using unpaired *t*-test. A p value < 0.05 was considered significant. χ^2 test was used to test for significance all differences detected in the frequency of VFA ≥ 100 cm^2, TG ≥ 150 mg/dL, HDL-C < 40 mg/dL, BP ≥ 130/85 mmHg, FPG ≥ 110 mg/dL, as well as in the incidence of the metabolic syndrome between those with an adiponectin level of less than 4 μg/mL and those with an adiponectin level of 4 μg/mL and greater.

References

1. The Examination Committee on Diagnostic Criteria for Metabolic Syndrome. (2002). *J. Jpn. Soc. Intern. Med.* **94,** 188–203 (in Japanese).
2. International Diabetes Federation. (2000). www.idf.orgIVAT BE433.674.528.
3. Wajchenberg, B. L. (2000). *Endocr. Rev.* **21,** 697–738.
4. Carr, D. B., Utzschneider, K. M., Hull, R. L., et al. (2004). *Diabetes* **53,** 2087–2094.
5. The Examination Committee of Criteria for "Obesity Disease" in Japan, Japan Society for the Study of Obesity. (2002). *Circ. J.* **66,** 987–992.
6. Ryo, M., Nakamura, T., Kihara, S., et al. (2004). *Circ. J.* **68,** 975–981.
7. Furukawa, S., Fujita, T., Shimabukuro, M., et al. (2004). *J. Clin. Invest.* **114,** 1752–1761.
8. Maeda, K., Okubo, K., Shimomura, I., et al. (1996). *Biochem. Biophys. Res. Commun.* **221,** 286–289.
9. Hotta, K., Funahashi, T., Bodkin, N. L., et al. (2001). *Diabetes* **50,** 1126–1133.
10. Ouchi, N., Kihara, S., Arita, Y., et al. (1999). *Circulation* **100,** 2473–2476.
11. Kumada, M., Kihara, S., Sumitsuji, S., et al. (2003). *Arterioscler. Throm. Vasc. Biol.* **23,** 85–89.
12. Inoguchi, T., Li, P., Umeda, F., et al. (2000). *Diabetes* **49,** 1939–1945.
13. Bajaj, M., Suraamornkul, S., Kashyap, S., Cusi, K., Mandarino, L., and DeFronzo, R. A. (2004). *J. Clin. Endocrinol. Metab.* **89,** 4649–4655.
14. National Cholesterol Education Program (NCEP) Expert Panel. (2002). *Circulation* **106,** 3143–3421.
15. The Expert Committee. (1999). *Diabetes Care* **22,** S5–S19.
16. Alberti, K. G. and Zimmet, P. Z. (1998). *Diabet. Med.* **15,** 539–553.
17. Matthews, D. R., Hosker, J. P., Rudenski, A. S., et al. (1985). *Diabetologia* **28,** 412–419.
18. Yoshizumi, T., Nakamura, T., Yamane, M., et al. (1999). *Radiology* **211,** 283–286.