Hypoadiponectinemia Is Associated With Visceral Fat Accumulation and Insulin Resistance in Japanese Men With Type 2 Diabetes Mellitus

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The aim of the present study was to investigate the association of serum adiponectin concentration with regional adiposity and insulin resistance in subjects with type 2 diabetes mellitus. A total of 73 Japanese men with type 2 diabetes (aged 59 ± 11 years and body mass index [BMI] 23.8 ± 3.0 kg/m^2, mean ± SD) were studied. Fasting serum adiponectin and leptin concentrations were determined by radioimmunoassay. Regional adiposity was measured by abdominal computed tomography (CT) at the umbilical level, and insulin resistance was estimated by homeostasis model assessment (HOMA-R). Univariate regression analysis showed that serum adiponectin levels were negatively correlated with subcutaneous and visceral fat areas. With multivariate regression analysis, visceral fat area was a predominant determinant of serum adiponectin levels. In contrast, subcutaneous fat area was strongly associated with serum leptin concentrations. Among subcutaneous and visceral fat areas, BMI, and serum leptin levels, both subcutaneous and visceral fat areas were independently associated with HOMA-R. In another model incorporating serum adiponectin levels, serum adiponectin levels were selected as an independent determinant of HOMA-R instead of visceral fat area. In conclusion, hypoadiponectinemia was associated with visceral fat accumulation rather than subcutaneous fat depot in Japanese men with type 2 diabetes mellitus. Both subcutaneous and visceral fat accumulation contribute to insulin resistance in these subjects, and the contribution of visceral fat may be mediated, in part, by hypoadiponectinemia.

CIRCULATING CONCENTRATIONS of adiponectin, the product of the adipose most abundant gene transcript 1 (*apM1*) gene, are decreased in subjects with obesity, type 2 diabetes mellitus, and coronary heart disease.[1,2] In a cross-sectional study involving varying degrees of glucose tolerance, hypoadionectinemia was strongly related to insulin resistance.[3] In addition, the decrease in serum adiponectin is proposed to be predictive of the future decrease in insulin sensitivity[4] and the development of type 2 diabetes in Pima Indians.[5] These results suggest that hypoadiponectinemia may play an important role in the pathogenesis of type 2 diabetes.

Despite the close association of hypoadiponectinemia with obesity,[1,2] the relative contribution of regional adiposity to serum adiponectin level is not well understood. In addition, the contribution of adiponectinemia to insulin resistance has not been fully investigated in subjects with type 2 diabetes. Therefore, in the present study, we analyzed the association of serum adiponectin concentration with regional adiposity and insulin resistance in Japanese men with type 2 diabetes mellitus.

MATERIALS AND METHODS

Patients

A total of 73 Japanese type 2 diabetic male patients (aged 59 ± 11 years and body mass index [BMI] 23.8 ± 3.0 kg/m^2, mean ± SD) were investigated in the present study. A portion of the subjects were analyzed in our previous study.[6] Female subjects were not included in this study, because there are sexual differences in serum levels of the adipocyte-derived proteins, adiponectin and leptin.[1,7] Type 2 diabetes was diagnosed based on the criteria of the World Health Organization (WHO).[8] Estimated duration of diabetes was 9 ± 8 years. Forty-four of the 73 patients were treated with diet alone, and the rest were treated with diet in combination of sulfonylureas (gliclazide). None of the subjects had significant renal, hepatic, or cardiovascular disease. All of the subjects provided informed consent.

Measurements

Blood was drawn in the morning after an overnight fast. Before blood sampling, all subjects had ingested at least 150 g of carbohydrate for 3 days, and did not consume alcohol or perform heavy exercise for at least 1 week. Fasting plasma glucose, hemoglobin A_{1c} (HbA_{1c}), and levels of serum total and high-density lipoprotein (HDL) cholesterol, remnant-like particle cholesterol, and triglyceride were measured as described previously.[9] Serum insulin and C-peptide levels were measured using commercial radioimmunoassay kits (Shionogi, Osaka, Japan). Serum adiponectin and leptin concentrations were also determined by radioimmunoassay kits (Linco Research, St Charles, MO). The intra-assay and interassay coefficients of variation were less than 5% for adiponectin and leptin.

Insulin resistance was estimated by homeostasis model assessment (HOMA).[10] HOMA insulin resistance index (HOMA-R) was calculated with the formula: fasting insulin (μU/mL) × fasting glucose (mg/dL)/405. HOMA-R was validated in diabetic subjects treated with diet therapy alone and in those treated with sulfonylureas.[11,12]

All subjects underwent computed tomography (CT) at umbilical level to measure cross-sectional abdominal subcutaneous and visceral fat areas. Details of the procedures have been described previously.[6] In this method, abdominal and visceral fat areas represent a different proportion of their respective total areas. Therefore, there may be some uncertainty about how these different areas correlate with other physiological parameters, although this method is validated by other determinations of fat accumulation[13,14] and widely adopted as a practical method to evaluate regional adiposity.

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Statistical Analysis

All data were presented as means ± SD. Simple linear regression analysis was performed to calculate a correlation. Multiple stepwise regression analysis was used to determine the contribution of subcutaneous and visceral fat areas to serum adiponectin and leptin levels, and to analyze independent determinants of HOMA-R. The statistical package StatView (Abacus Concepts, Berkeley, CA) for Macintosh version 5.0 was employed for these analyses. A P value less than 5% was considered significant. In a multiple regression analysis, an F value greater than 4 was considered significant.

RESULTS

Characteristics of the Subjects

Clinical characteristics of the subjects are summarized in Table 1. The ranges of BMI, and visceral and subcutaneous fat areas were from 16.2 to 31.3 kg/m^2, from 12 to 206 cm^2, and from 28 to 296 cm^2, respectively. There was a positive correlation between subcutaneous and visceral fat area ($r = 0.644$, $P < .0001$). Subcutaneous and visceral areas were also correlated with BMI ($r = 0.832$, $P < .0001$ and $r = 0.600$, $P < .0001$, respectively). Overall, glycemia was fairly controlled with a mean HbA_{1c} level less than 7%. There was a wide variation in insulin resistance calculated from HOMA-R (range, 0.8 to 9.7). Thirty (41%) of the 73 subjects had HOMA-R greater than 2.5, indicating that they were insulin-resistant.[9] Serum levels of lipids, adiponectin, and leptin are as shown in Table 1.

Relation of Serum Adiponectin and Leptin Levels to Regional Adiposity

Serum concentrations of adiponectin were negatively associated with visceral fat area, subcutaneous fat area, and BMI, respectively (Table 2). In a multivariate analysis, visceral fat area, but not subcutaneous fat area, was independently contributed to adiponectinemia (Table 2). In contrast, serum leptin levels were positively related to visceral fat area ($r = 0.426$, $P = .0002$), subcutaneous fat area ($r = 0.560$, $P < .0001$), and BMI ($r = 0.566$, $P < .0001$), respectively. In a multivariate analysis, subcutaneous fat area ($F = 32.5$), but not visceral fat area ($F = 0.741$), was an independent determinant of serum leptin concentrations.

Table 1. Characteristics of Diabetic Patients

Characteristic	Mean ± SD
BMI (kg/m^2)	23.8 ± 3.0
Visceral fat area (cm^2)	103 ± 46
Subcutaneous fat area (cm^2)	124 ± 55
Fasting glucose (mg/dL)	144 ± 28
HbA_{1c} (%)	6.9 ± 1.1
Serum insulin (μU/mL)	7.7 ± 4.6
Serum C-peptide (ng/mL)	2.3 ± 0.9
HOMA-R	2.8 ± 0.9
Serum total cholesterol (mg/dL)	197 ± 36
Serum HDL cholesterol (mg/dL)	50 ± 14
Serum RLP cholesterol (mg/dL)	5.6 ± 3.1
Serum triglyceride (mg/dL)	125 ± 66
Serum adiponectin (μg/mL)	12.8 ± 7.4
Serum leptin (ng/mL)	4.0 ± 2.6

Abbreviation: RLP, remnant-like particle.

Table 2. Correlation of Serum Adiponectin Concentration to Measures of Variables in Diabetic Patients

Variables	Univariate		Multivariate
	r	P	F
Visceral fat area	−0.398	.0005	13.336
Subcutaneous fat area	−0.327	.0048	0.717
BMI	−0.446	<.0001	
Fasting glucose	−0.053	NS	
HbA_{1c}	−0.136	NS	
Insulin	−0.406	.0004	
C-peptide	−0.400	.0005	
HOMA-R	−0.375	.0011	
Total cholesterol	−0.092	NS	
HDL cholesterol	0.355	.0021	
RLP cholesterol	−0.301	.0097	
Triglyceride	−0.341	.0031	
Age	0.175	NS	
Duration of diabetes	0.176	NS	

Abbreviation: NS, not significant.

Relation of Serum Adiponectin Levels to Dyslipidemia

Serum adiponectin concentrations were not related to fasting glucose and HbA_{1c} levels, but those were negatively associated with fasting insulin, C-peptide, and HOMA-R in a univariate analysis (Table 2). Although serum adiponectin levels were not correlated with total cholesterol concentrations, the levels were correlated positively with serum HDL cholesterol concentrations, and negatively with the levels of serum remnant-like particle cholesterol and triglyceride. There was no association between serum adiponectin concentrations and blood pressure (data not shown).

Determinants of HOMA-R

There were significant associations of HOMA-R with serum levels of adiponectin and leptin, visceral and subcutaneous fat areas, and BMI in a univariate analysis (Fig 1). The associations of HOMA-R with fat areas and BMI seemed less tight with increasing fat areas and BMI.

To explore independent determinants of HOMA-R, multiple stepwise regression analysis was applied (Table 3). Among 4 variables (model 1), subcutaneous fat area was the strongest determinant of HOMA-R, followed by visceral fat area. In a model incorporating serum adiponectin concentrations (model 2), subcutaneous fat area and serum adiponectin concentrations were independently associated with HOMA-R.

DISCUSSION

In the present study, we first investigated the contribution of regional adiposity to adionectinemia, and demonstrated that serum adiponectin levels were independently associated with abdominal visceral fat area, but not with subcutaneous fat area, in Japanese men with type 2 diabetes. As for mRNA expression of adiponectin in regional adipose tissues, Statnick et al[15] revealed that *apM1* (adiponectin) mRNA expression was significantly reduced in omental adipose tissue of obese patients

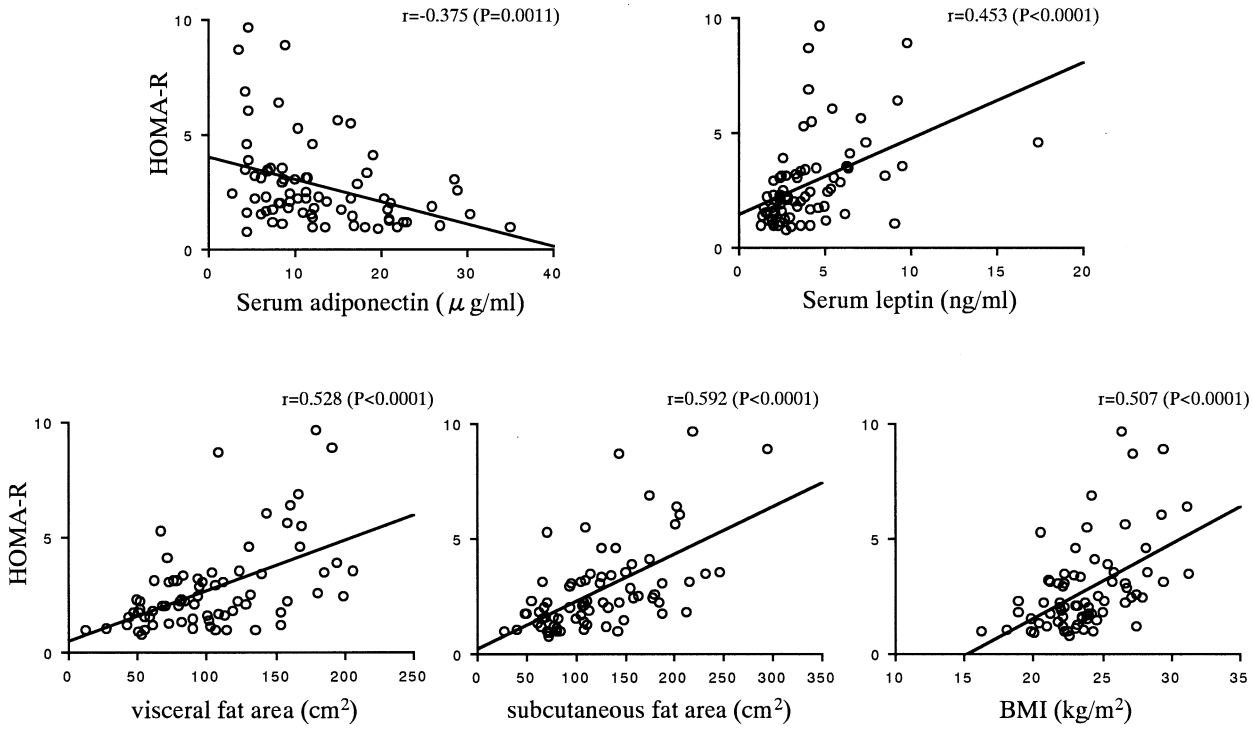

Fig 1. Associations of HOMA-R with measures of variables.

with type 2 diabetes, while the decrease in *apM1* mRNA expression was less pronounced in subcutaneous fat tissue. This observation is in accordance with the present results. The association of serum adiponectin levels to visceral fat area is in striking contrast to the dependence of leptinemia on subcutaneous fat area.

Of interest was the relationship between insulin resistance and serum adiponectin levels. In a univariate regression analysis, insulin resistance was correlated negatively with serum adiponectin levels and positively with serum leptin concentrations, visceral and subcutaneous fat areas, and BMI. To reveal independent determinants of insulin resistance, 2 models of the multivariate analysis were applied. In both models, subcutaneous fat area was selected as a first determinant of insulin resistance in the diabetic male patients of the present study. In the model incorporating serum adiponectin levels (model 2), hypoadiponectinemia was a second independent determinant of insulin resistance, whereas visceral fat area was selected as a second determinant in the model excluding adiponectinemia (model 1). Considering the close relationship between adiponectinemia and visceral fat area, hypoadiponectinemia might be responsible, at least in part, for the association of insulin resistance and visceral fat accumulation in Japanese men with type 2 diabetes. Very recently, it is shown that adiponectin replacement therapy dramatically reverses insulin resistance and glucose intolerance in obese diabetic mice[16,17] and in adiponectin-knockout mice.[18] It is therefore plausible that hypoadiponectinemia associated with visceral fat accumulation could also increase insulin resistance in human subjects. The precise mechanisms remain to be elucidated by which the deficiency of adiponectin leads to insulin resistance, although Fruebis et al[19] demonstrated that the cleavage product of adiponectin increases fatty acid oxidation in skeletal muscle in mice.

Table 3. Determinants of HOMA-R by Multivariate Analyses

	Model 1 *F*	Model 2 *F*
Adiponectin	—	4.204
Leptin	1.862	2.513
Visceral fat area	4.167	2.500
Subcutaneous fat area	12.455	28.264
BMI	0.001	0.183

NOTE. R^2 = 0.387 (model 1) and 0.388 (model 2), respectively.

The contribution of regional adiposity to insulin resistance is a point of controversy in subjects with type 2 diabetes. In the present study, although both subcutaneous and visceral fat areas were independently associated with insulin resistance, the contribution of subcutaneous fat area was much greater than visceral fat area. This observation is substantially in accordance with our previous study, in which both subcutaneous and visceral fat areas independently contributed to insulin resistance in nonobese Japanese subjects with type 2 diabetes.[6] Consistently, Abate et al[20] revealed that subcutaneous fat, but not intraperitoneal or retroperitoneal fat, was associated with insulin resistance in non-Hispanic whites with type 2 diabetes. In contrast, visceral fat accumulation was predominantly related to insulin resistance in type 2 diabetic patients from black, white and Asian Indian populations.[21-24] In the present study, the association of subcutaneous and visceral fat areas with insulin resis-

tance was less tight with increasing degree of obesity. It seems likely that the discrepancies among several studies may result from the different degree of obesity of the participants, as well as the difference in ethnicity, gender, degree of hyperglycemia, and duration of diabetes.

Serum adiponectin concentrations were closely associated with dyslipidemia in the diabetic male patients, consistent with observations in diabetic and nondiabetic subjects.[2,25] Hypoadiponectinemia was associated with the decreased levels of serum HDL-cholesterol and the increased concentrations of remnant-like particle cholesterol and triglyceride. This phenotype of dyslipidemia is known to be associated with visceral fat accumulation and/or insulin resistance.[6,9,26,27] It seems possible that hypoadiponectinemia could increase insulin resistance and result in dyslipidemia. Conversely, dyslipidemia may be one of the candidates for increased insulin resistance associated with hypoadiponectinemia, since this phenotype of dyslipidemia is often accompanied with the increase in serum free fatty acids, which can cause insulin resistance in various tissues.[28]

In the present study, we demonstrated that hypoadiponectinemia in Japanese men with type 2 diabetes is associated with visceral fat accumulation, insulin resistance, and dyslipidemia, all of which are known to be important components of the metabolic syndrome. Since adiponectin is proposed to have potent antiatherogenic properties,[29-31] hypoadiponectinemia could be one of the mediators between the metabolic syndrome and atherosclerosis. In fact, serum adiponectin levels were decreased in subjects with established coronary heart disease[2] and in patients carrying the rare mutation I164T of the adiponectin gene, who showed features of the metabolic syndrome and high frequency of the atherosclerotic diseases.[32]

Since nondiabetic subjects or female patients with type 2 diabetes were not included in the present study, it remains unclear whether the relationships among hypoadiponectinemia, visceral fat accumulation, and insulin resistance can be applied to those subjects. However, regarding this point, Weyer et al[3] demonstrated that insulin sensitivity and waist-to-thigh ratio independently associate with adiponectinemia in their study involving both male and female subjects with varying degrees of glucose tolerance. Further analysis is necessary to establish a firm relationship of adiponectinemia with regional adiposity and insulin resistance.

In conclusion, although the present study was performed among the limited number of patients (N = 73), hypoadiponectinemia was associated with visceral fat accumulation rather than subcutaneous fat in Japanese men with type 2 diabetes mellitus. Both subcutaneous and visceral fat accumulation contribute to insulin resistance in these subjects, and the contribution of visceral fat may be mediated, in part, by hypoadiponectinemia.

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