

CLINICAL STUDY

Inverse relationship between plasma adiponectin and leptin concentrations in normal-weight and obese women

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Abstract

Background: Adiponectin, a novel adipocyte-derived collagen-like protein, is the gene product of the adipose most-abundant gene transcript 1 (apM1), which has been considered to have anti-inflammatory and anti-atherogenic effects.

Objective: To characterize the relationship between adiponectin and leptin, the *ob* gene product, in normal-weight and obese women.

Design and methods: In this cross-sectional study, we measured fasting plasma adiponectin by ELISA, leptin concentrations by RIA, and related parameters such as blood pressure, body mass index (BMI), body fat mass, lipids, fasting blood glucose and insulin in 353 non-diabetic adult women with a wide range of BMI values.

Results: Plasma adiponectin concentrations in women with the highest tertile of BMI (at least 25.0 kg/m²) were decreased compared with those in the middle (22.0–25.0 kg/m²) or lowest (≤ 22.0 kg/m²) tertile of BMI (means \pm S.E.M.: 6.7 \pm 0.3 μ g/ml compared with 8.6 \pm 0.4 μ g/ml and 9.2 \pm 0.3 μ g/ml; both $P < 0.0001$). Serum leptin concentrations in women with the highest tertile of BMI were increased compared with those in women in the middle or lowest tertile of BMI (13.2 \pm 0.4 ng/ml compared with 8.1 \pm 0.2 ng/ml and 5.2 \pm 0.2 ng/ml; both $P < 0.0001$). These relationships were similar after adjustment for BMI or body fat mass. Adiponectin was negatively correlated with serum leptin concentration, fasting immunoreactive insulin, calculated insulin resistance (homeostasis model assessment), BMI and body fat mass. These negative relationships became stronger after adjustment for BMI or body fat mass. In stepwise regression analyses, leptin was the significant independent variable for adiponectin, and adiponectin was also the significant independent variable for leptin before and after adjustment for BMI or body fat mass.

Conclusions: In this study, we found an inverse correlation between adiponectin and leptin *in vivo*.

European Journal of Endocrinology 147 173–180

Introduction

Adipose tissue was once considered to be an inert depot for storing fuel as lipids, to be released only during times of hardship such as starvation. Now adipose tissue is known to operate as an endocrinologically active tissue that releases peptides, such as plasminogen activator inhibitor 1 (1), leptin (2), resistin (3) and adiponectin (4), in response to specific extracellular stimuli or changes in metabolic status. Because these secreted peptides seem to share some structural properties of cytokines, they are called 'adipocytokines' (5).

Leptin, the *obese* (*ob*) gene product, is believed to be a lipostatic hormone that contributes to body weight regulation through modulating feeding behavior and

energy expenditure (2, 6, 7). Serum leptin concentration was shown to be increased in humans with obesity, insulin resistance and dyslipidemia, after adjustment for body composition (7, 8). The increased serum leptin concentration in obesity was proposed to be secondary to 'leptin resistance'. However, as leptin causes an oxidative stress in endothelial cells, and has a vascular calcifying effect, it has been suggested that leptin promotes atherogenesis (9–11). Adiponectin, the gene product of the adipose most-abundant gene transcript 1 (*apM1*) gene that is exclusively and abundantly expressed in white adipose tissue, is a 244-amino acid protein with high structural homology to collagen VIII, X and complement C1q (4, 12). This protein was also identified independently by three other groups using different approaches, and was named by them respectively as gelatin-binding protein

(GBP28) (13), adipocyte complement-related protein of 30 kDa (Acrp30) in mouse (14) or AdipoQ in mouse (15). As adiponectin accumulates in injured vessel walls and dose-dependently inhibits the tumor necrosis factor (TNF)- α signaling pathway in human aortic endothelial cells and reduces TNF- α production in macrophages, adiponectin was suggested to have anti-atherogenic and anti-inflammatory properties (12, 16–19). Plasma adiponectin concentrations were found to be decreased in patients with obesity (16), non-insulin-dependent diabetes mellitus (20), insulin resistance (21), dyslipidemia (22) and cardiovascular disease (12).

In the present cross-sectional study, we examined the relationship between fasting plasma adiponectin and leptin concentrations in a large group of non-diabetic Japanese individuals. Because sex differences have been reported in plasma adiponectin (16), leptin (6, 23), triglyceride, high-density lipoprotein-cholesterol (HDL-C), uric acid and percent body fat mass, we chose to study women only.

Subjects and methods

Subjects

Three hundred and fifty-three Japanese women residing in Hokkaido, Japan, aged 16–86 years (mean \pm S.E.M.: 52.6 ± 0.6 years) were included in this cross-sectional study. Women taking the birth control pill, and any with diabetes mellitus (fasting blood glucose >7.0 mmol/l or blood glucose >11.1 mmol/l 2 h after 75 g oral glucose loading), renal failure (serum creatinine >159 μ mol/l or blood urea nitrogen (BUN) >10.7 mmol/l) or untreated endocrine diseases were excluded. Approximately 34% and 12% of the women had hypercholesterolemia (total cholesterol >5.69 mmol/l) and hypertriglyceridemia (>1.69 mmol/l) respectively. Approximately 30% and 21% of them had systolic (>160 mmHg) and diastolic hypertension (>90 mmHg), and 48 were receiving calcium channel blockers, angiotensin converting enzyme inhibitors, or both. Body mass index (BMI) was calculated as weight in kilograms divided by the square of the height in meters. Body fat mass was determined by bioelectrical impedance analysis; this value was the average determined using both a Tanita Body Fat Analyzer (TBF-541, Tanita, Tokyo) and an Omron Body Fat Analyzer (HBF-301, Omron, Tokyo) (23). All the women provided informed consent.

Biochemical analyses

Blood glucose was measured by the glucose oxidase method; serum lipids, total protein, albumin, uric acid and BUN were measured using commercially available kits. Immunoreactive insulin (IRI) was determined by a specific EIA with reagents from Dainabot Co. Ltd, Japan (8). Insulin resistance was calculated by the

homeostasis model assessment (HOMA) method, using fasting blood glucose and insulin concentrations (8, 24). Assuming that normal-weight normal individuals <35 years of age have an insulin resistance of 1, the value for insulin resistance can be assessed by the formula: fasting blood glucose (mmol/l) \times fasting IRI (μ U/ml)/22.5. Serum leptin concentration was measured with an RIA (Linco Research Inc., St Charles, MO, USA), which uses a polyclonal antibody against recombinant human leptin, raised in rabbits (6, 8). Blood samples for measurement of fasting plasma adiponectin concentrations were drawn into 1/10 volume EDTA–aprotinin tubes, and immediately placed on ice. All tubes were centrifuged at 4 °C for collection of plasma and stored at -80 °C until required for analysis at Otsuka Assay Institute, Tokushima, Japan. Adiponectin was determined with a validated sandwich ELISA using an adiponectin-specific monoclonal and polyclonal antibody (16). Cross reaction with leptin, insulin and several cytokines, such as TNF- α and interleukin (IL) 1- β and IL-8, was not observed in this ELISA system. The recovery rate was almost 100%, and the intra- and interassay coefficients of variation for adiponectin were 3.3% and 7.4% respectively (22).

Statistical analyses

All the women were stratified into tertiles of BMI values (≤ 22.0 kg/m², 22.0–25.0 kg/m², ≥ 25.0 kg/m²), because in Japan a BMI value >25.0 kg/m² is considered an increased value, according to Japan Obesity Society criteria. The differences across tertiles of various continuous parameters, leptin and adiponectin before and after adjustment for BMI or body fat mass were tested with analysis of variance (ANOVA). Because preliminary analyses indicated that the distributions of plasma adiponectin, leptin, triglycerides, IRI and calculated insulin resistance were skewed, log transformation was used, which yielded more normally distributed data. Linear regression was performed to determine which factor among serum total protein, albumin, fasting blood glucose, IRI, calculated insulin resistance (homeostasis model assessment) and leptin correlated with log-transformed adiponectin before and after adjustment for BMI or body fat mass. We had previously reported significant positive correlations between adiponectin and age, BUN and HDL-C concentrations, and the negative correlation of adiponectin with serum triglyceride concentrations (22). Stepwise multiple regression analyses were used to identify independent determinants for adiponectin before and after adjustment for BMI or body fat mass, and the percentage of variance in adiponectin that they explained (r^2). The same analyses were also used to identify independent determinants for leptin. Two-way ANOVA was performed to determine possible relations for plasma adiponectin concentration, adjusting for

body fat mass (kg), between tertiles of leptin and several stratified parameters, such as age, diastolic blood pressure (DBP), BMI, serum triglycerides, HDL-C or calculated insulin resistance. Results are expressed as means \pm s.e.m. A *P* value less than 0.05 was considered to be statistically significant.

Results

The mean age of the women studied was 52.6 years and their mean BMI was 22.9 ± 0.2 kg/m² (range 14.8–36.3 kg/m²). The fasting serum leptin concentration ranged from 1.2 to 44.5 ng/ml, with an arithmetic mean of 8.1 ng/ml, and the fasting plasma adiponectin concentration ranged from 0.9 to 26.1 μ g/ml, with an arithmetic mean of 8.4 μ g/ml. Age, systolic blood pressure (SBP) and DBP, body fat mass, serum uric acid, total cholesterol, triglycerides, fasting blood glucose, IRI and calculated insulin resistance were increased when the values in the highest tertile of BMI were compared with those in the lowest tertile (all *P* < 0.0001), whereas serum HDL-C decreased (*P* < 0.0001) (Table 1). Serum leptin concentrations increased gradually by BMI tertile (5.2 ± 0.2 ng/ml and 8.1 ± 0.2 ng/ml compared with

13.2 ± 0.4 ng/ml; both *P* < 0.0001), and this significant increase remained after adjustment for BMI or body fat mass. Plasma adiponectin concentrations by BMI tertile decreased progressively (9.2 ± 0.3 μ g/ml and 8.6 ± 0.4 μ g/ml compared with 6.7 ± 0.3 μ g/ml; both *P* < 0.0001), and this significant decrease also remained after adjustment for BMI or body fat mass (Table 1).

BMI (*r* = -0.26, *P* < 0.0001), body fat mass (*r* = -0.25, *P* < 0.0001), fasting IRI (*r* = -0.39, *P* < 0.0001), calculated insulin resistance (*r* = -0.37, *P* < 0.0001) and leptin concentration (*r* = -0.35, *P* < 0.0001) were negatively correlated with plasma adiponectin concentrations (Table 2) and the correlations became stronger after adjustment was made for BMI or body fat mass (Table 2; Fig. 1).

In a stepwise regression analysis model, age, BUN, triglycerides, calculated insulin resistance and leptin concentration were significant independent determinants of adiponectin concentration, explaining a total of 32% of the variance in these measures (*r*² = 0.32) (Table 3). These relationships became stronger after adjustment for BMI or body fat mass, explaining a total of 47–63% of the variance in these measures, adding BMI as the significant independent determinant (*r*² = 0.47–0.63) (Table 3). Moreover,

Table 1 Relationship between stratified body mass index (BMI) and the variables associated with metabolic syndrome, plasma adiponectin and leptin concentrations, before and after adjustment for body composition. BFM, body fat mass; BP, blood pressure; IRI, immunoreactive insulin; HOMA-IR, homeostasis model assessment ratio: fasting blood glucose (FBG) (mmol/l) \times fasting IRI (μ U/ml)/22.5. Data are presented as means \pm s.e.m.

Variable	BMI (kg/m ²)			<i>P</i> (Tertile 1 vs 3)
	Tertile 1 (≤ 22.0)	Tertile 2 (22.0–25.0)	Tertile 3 (≥ 25.0)	
Number (%)	158 (44.7)	102 (28.9)	93 (26.4)	
Age (years)	49.0 \pm 0.9	54.8 \pm 1.0	55.9 \pm 1.0	<0.0001
Systolic BP (mmHg)	137.3 \pm 1.1	145.4 \pm 1.3	147.3 \pm 1.3	<0.0001
Diastolic BP (mmHg)	76.8 \pm 0.5	81.3 \pm 0.7	85.1 \pm 0.8	<0.0001
Serum total protein (g/l)	73 \pm 0.3	73 \pm 0.4	74 \pm 0.4	0.0788
Albumin (g/l)	46 \pm 0.2	46 \pm 0.2	46 \pm 0.2	0.0969
Uric acid (μ mol/l)	262 \pm 4	274 \pm 5	291 \pm 5	<0.0001
BUN (mmol/l)	4.9 \pm 0.2	5.2 \pm 0.2	5.2 \pm 0.2	0.563
BMI (kg/m ²)	19.9 \pm 0.1	23.5 \pm 0.1	27.8 \pm 0.2	<0.0001
BFM (kg)	12.4 \pm 0.1	17.3 \pm 0.2	24.0 \pm 0.3	<0.0001
BFM (%)	25.4 \pm 0.2	31.3 \pm 0.2	36.9 \pm 0.3	<0.0001
Total cholesterol (mmol/l)	5.10 \pm 0.05	5.37 \pm 0.06	5.66 \pm 0.06	<0.0001
Triglycerides* (mmol/l)	0.98 \pm 0.03	1.18 \pm 0.05	1.45 \pm 0.05	<0.0001
HDL-C (mmol/l)	1.74 \pm 0.02	1.67 \pm 0.03	1.51 \pm 0.02	<0.0001
FBG (mmol/l)	5.1 \pm 0.1	5.2 \pm 0.1	5.5 \pm 0.1	0.0001
Fasting IRI* (μ U/ml)	6.0 \pm 0.3	6.8 \pm 0.4	9.8 \pm 0.5	<0.0001
HOMA-ratio*	1.4 \pm 0.1	1.6 \pm 0.1	2.4 \pm 0.1	<0.0001
Serum leptin* (ng/ml)	5.2 \pm 0.2	8.1 \pm 0.2	13.2 \pm 0.4	<0.0001
Leptin/BMI*	0.26 \pm 0.01	0.34 \pm 0.01	0.47 \pm 0.01	<0.0001
Leptin/BFM (kg)*	0.42 \pm 0.01	0.46 \pm 0.01	0.54 \pm 0.02	<0.0001
Leptin/BFM (%)*	0.20 \pm 0.01	0.25 \pm 0.01	0.35 \pm 0.01	<0.0001
Plasma adiponectin* (μ g/ml)	9.2 \pm 0.3	8.6 \pm 0.4	6.7 \pm 0.3	<0.0001
Adiponectin/BMI*	0.47 \pm 0.02	0.37 \pm 0.02	0.25 \pm 0.01	<0.0001
Adiponectin/BFM (kg)*	0.79 \pm 0.04	0.51 \pm 0.03	0.29 \pm 0.01	<0.0001
Adiponectin/BFM (%)*	0.37 \pm 0.01	0.28 \pm 0.01	0.18 \pm 0.01	<0.0001

* Log-transformed statistics.

Table 2 Correlation between plasma adiponectin and leptin concentrations, before and after adjustment for body composition, and the variables associated with metabolic syndrome.

Factor	Plasma adiponectin*		Adiponectin/BMI*		Adiponectin/BFM (kg)*		Adiponectin/BFM (%)*	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
Serum total protein	-0.155	0.0036	-0.175	0.0010	-0.193	0.0003	-0.202	0.0002
Albumin	-0.124	0.0205	-0.095	0.0761	-0.085	0.1158	-0.107	0.0475
FBG	-0.113	0.0348	-0.162	0.0025	-0.167	0.0019	-0.160	0.0030
Fasting IRI*	-0.375	<0.0001	-0.458	<0.0001	-0.479	<0.0001	-0.456	<0.0001
HOMA-ratio*	-0.359	<0.0001	-0.447	<0.0001	-0.466	<0.0001	-0.443	<0.0001
BMI	-0.264	<0.0001	-0.526	<0.0001	-0.683	<0.0001	-0.561	<0.0001
BFM (kg)	-0.254	<0.0001	-0.498	<0.0001	-0.705	<0.0001	-0.561	<0.0001
BFM (%)	-0.215	<0.0001	-0.458	<0.0001	-0.660	<0.0001	-0.563	<0.0001
Serum leptin*	-0.353	<0.0001	-0.525	<0.0001	-0.638	<0.0001	-0.567	<0.0001
Leptin/BMI*	-0.341	<0.0001	-0.468	<0.0001	-0.556	<0.0001	-0.506	<0.0001
Leptin/BFM (kg)*	-0.278	<0.0001	-0.333	<0.0001	-0.330	<0.0001	-0.341	<0.0001
Leptin/BFM (%)*	-0.340	<0.0001	-0.458	<0.0001	-0.524	<0.0001	-0.473	<0.0001

*Log-transformed statistics. BFM, body fat mass; FBG, fasting blood glucose; IRI, immunoreactive insulin; HOMA, homeostasis model assessment.

adiponectin was independently associated with leptin concentration before and after adjustment for BMI or body fat mass in women in these stepwise regression analysis models (Table 4). In contrast, age, DBP, serum triglycerides and BUN were not independently related to the leptin concentrations. The results presented in Tables 3 and 4 were essentially unchanged when fasting IRI was substituted for calculated insulin resistance, serum HDL-C was substituted for triglycerides, or SBP was substituted for DBP (data not shown).

In two-way ANOVA, despite adjustment for stratified age, DBP, BMI, serum triglycerides, HDL-C or calculated insulin resistance, the plasma adiponectin/body fat mass (kg) value was lower in the highest tertiles of serum leptin concentrations than in the lowest tertiles (Fig. 2).

Discussion

The present study demonstrated that plasma adiponectin concentrations were inversely correlated with

leptin concentrations in non-diabetic normal-weight and obese women. We also confirmed that the mean plasma adiponectin concentration before and after adjustment for body composition was decreased, and that leptin increased in obesity. Plasma adiponectin/body fat mass was lower in the high-leptin group after adjustment was made for age, blood pressure, BMI, lipids and calculated insulin resistance.

Increased serum leptin concentrations in obesity have been suggested to be the result of 'leptin resistance'. However, they have also been observed in patients with insulin resistance, dyslipidemia and hyperuricemia, after adjustment for body composition (8, 25, 26). Bouloumie *et al.* (9, 10) reported that leptin exerts angiogenic and atherogenic effects through the generation of oxidative stress in endothelial cells. Parhami *et al.* (11) recently demonstrated a vascular calcifying effect of leptin. It has also been reported that leptin promotes human platelet aggregation (27). Increased adipose tissue in obesity requires an increased vascular bed to maintain its baseline circulation (10, 28). Thus this adaptation may, conversely, promote arteriosclerosis over long periods of

Table 3 Stepwise regression analyses of selected variables (age, BMI, diastolic blood pressure (BP), blood urea nitrogen (BUN), log-transformed triglyceride, homeostasis model assessment ratio (HOMA-R) and leptin) on log-transformed plasma adiponectin, before and after adjustment for body composition. The *F* value was set at 4.0 at each step.

Independent variable	Plasma adiponectin			Adiponectin/BMI			Adiponectin/BFM (kg)			Adiponectin/BFM (%)		
	<i>r</i> ²	β	<i>F</i>	<i>r</i> ²	β	<i>F</i>	<i>r</i> ²	β	<i>F</i>	<i>r</i> ²	β	<i>F</i>
	0.323			0.465			0.627			0.497		
Age		0.279	22.204		0.249	21.201		0.245	29.500		0.205	15.286
BMI			0.016		-0.293	16.940		-0.450	57.423		-0.280	16.542
Diastolic BP			0.012			0.011			0.649			0.018
BUN		0.122	4.566		0.110	4.650		0.093	4.780		0.109	4.840
Log(triglyceride)		-0.297	27.326		-0.267	27.667		-0.205	23.501		-0.261	28.147
Log(HOMA-R)		-0.210	11.606		-0.180	10.532		-0.138	8.871		-0.159	8.816
Log(leptin)		-0.161	6.659		-0.146	4.005		-0.204	11.301		-0.223	9.962

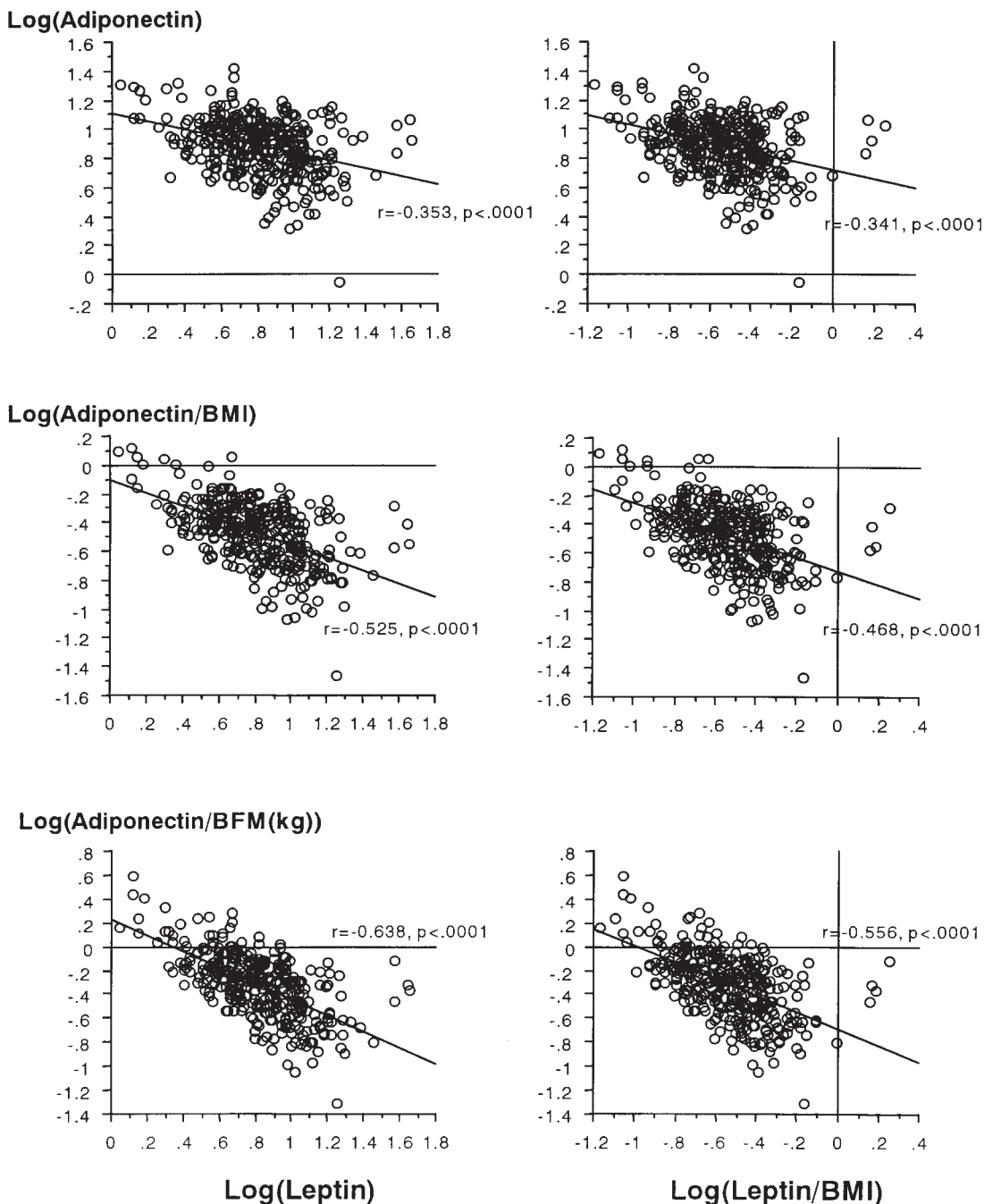


Figure 1 Correlation between log-transformed plasma adiponectin and leptin concentrations before and after adjustment for body composition. BFM, body fat mass.

time. It has been indicated that adiponectin has potential anti-atherogenic and anti-inflammatory properties (4, 12, 16–19). In the early stages of atherosclerosis, endothelial cell activation by various inflammatory stimuli, including TNF- α , results in the synthesis of adhesion molecules and increases the adherence of monocytes. This adhesion of monocytes to the arterial endothelium is considered crucial for the development

of vascular diseases. Adiponectin has been shown to inhibit both the production and action of TNF- α , a cytokine which has direct effects on the adhesion molecules (12, 16–19). Hotta *et al.* (29) reported that plasma adiponectin (determined as an arbitrary value) decreased, and leptin concentrations increased, in parallel with the progression of non-insulin-dependent diabetes mellitus in male rhesus monkeys. A recent genomic

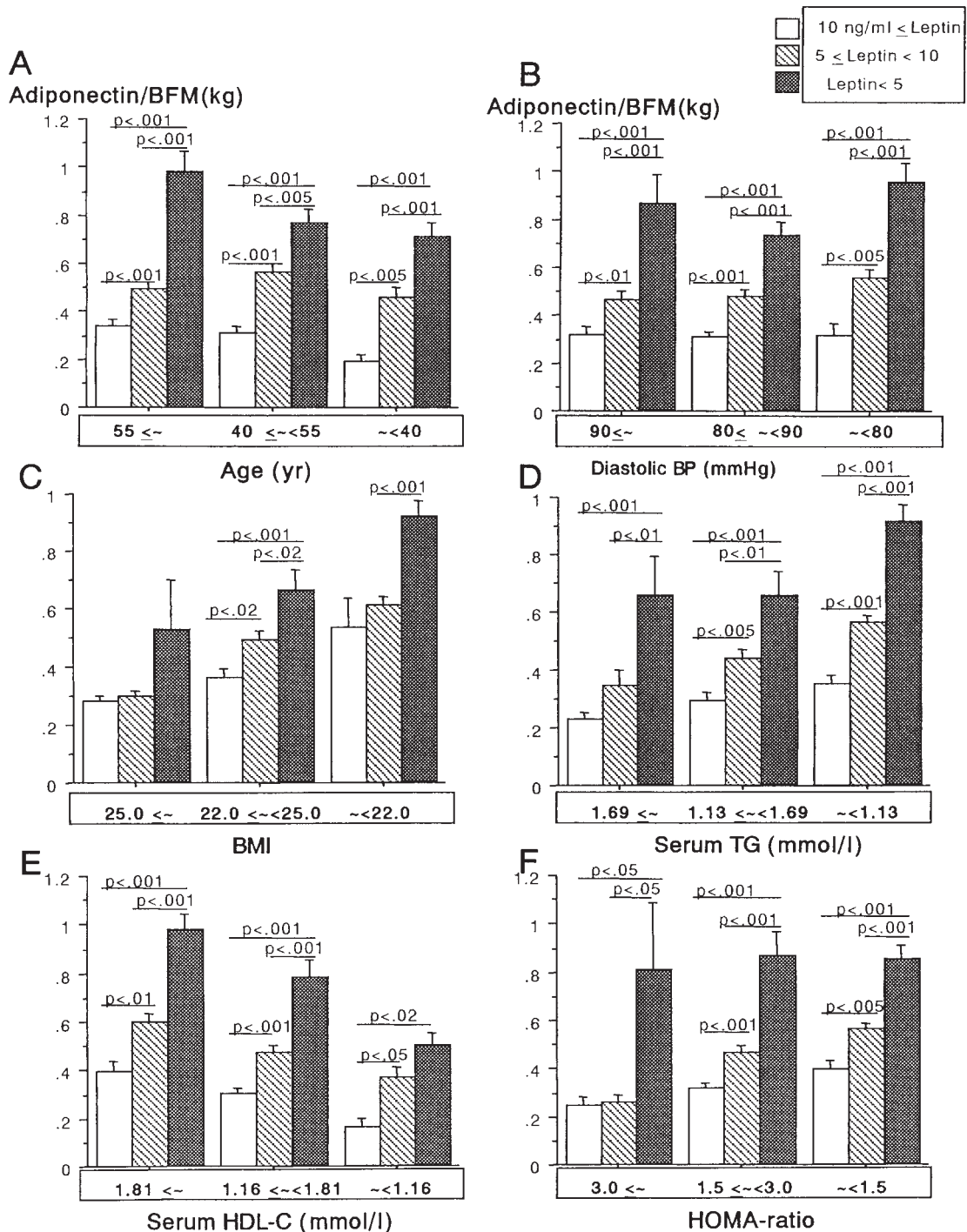


Figure 2 (A) Age, (B) diastolic blood pressure (BP), (C) body mass index (BMI), (D) serum triglyceride (TG) concentration, (E) high-density lipoprotein-cholesterol (HDL-C) concentration, and (F) calculated insulin resistance (homeostasis model assessment (HOMA)-ratio)-adjusted plasma adiponectin/body fat mass (BFM; kg) by tertiles of serum leptin concentration. Data are means ± s.e. Statistical analyses were performed after log-transformation.

scan study (30) has revealed linkage of the metabolic syndrome both to regions on chromosome 3q27 where the gene encoding adiponectin is located (31), and to regions on chromosome 17p12 that are strongly linked to plasma leptin concentrations.

The mechanism underlying the decreased adiponectin production in obesity remains unknown, but a decreased mRNA expression of apM1 in adipose tissue has been reported in obesity (32). Hyperleptinemia or 'leptin resistance' might contribute to the decrease in

Table 4 Stepwise regression analyses of selected variables (age, BMI, diastolic blood pressure (BP), blood urea nitrogen (BUN), log-transformed triglyceride, homeostasis model assessment ratio (HOMA-R) and adiponectin) on log-transformed plasma serum leptin, before and after adjustment for body composition. The *F* value was set at 4.0 at each step.

Independent variable	Serum leptin			Leptin/BMI			Leptin/BMI (kg)			Leptin/BMI (%)		
	<i>r</i> ²	β	<i>F</i>	<i>r</i> ²	β	<i>F</i>	<i>r</i> ²	β	<i>F</i>	<i>r</i> ²	β	<i>F</i>
	0.583			0.403			0.165			0.378		
Age			1.370			1.397			0.492			3.442
BMI		0.615	179.189		0.431	61.418			3.489		0.387	47.674
Diastolic BP			0.154			0.174			0.111			0.386
BUN			0.618			0.664			2.328			0.417
Log(triglyceride)			0.295			0.232			1.919			0.004
Log(HOMA-R)		0.192	16.010		0.236	16.866		0.313	24.917		0.248	17.891
Log(adiponectin)		-0.117	6.851		-0.138	6.686		-0.167	7.123		-0.156	8.205

adiponectin production in adipose tissue. Alternatively, the excess of adipose tissue and calories in obesity might cause 'leptin resistance' and the decline in adiponectin production, separately. Further study is needed to clarify this mechanism. Yamauchi *et al.* (33) reported that the concomitant replenishment of adiponectin and leptin completely resolved the insulin resistance in lipoatrophic and obese diabetic mice. Because adiponectin increases the gene expression of fat-combustion-related substances such as CD36, acyl CoA oxidase and uncoupling protein-2 in muscle, this peptide causes both the decrease in triglyceride and free fatty acid content, blood glucose and body weight, and the improvement in insulin resistance (33, 34). Supplementation of adiponectin in insulin resistance and obesity may possibly become the standard treatment for these diseases.

Conclusion

We observed hypoadiponectinemia and hyperleptinemia in non-diabetic obese women, and a significant inverse relationship between plasma adiponectin and leptin concentrations that was independent of age, BUN, blood pressure, body composition, lipid and insulin resistance. Whether hypoadiponectinemia and hyperleptinemia may work together to accelerate atherosclerosis in obese individuals merits further investigation.

References

- Shimomura I, Funahashi T, Takahashi M, Maeda K, Kotani K, Nakamura T *et al.* Enhanced expression of PAI-I in visceral fat: possible contributor to vascular disease in obesity. *Nature Medicine* 1996 **2** 800–803.
- Zhang Y, Proenca R, Maffei M, Barone M, Leopold L & Friedman JM. Positional cloning of the mouse obese gene and its human homologue. *Nature* 1994 **372** 425–432.
- Steppan CM. The hormone resistin links obesity to diabetes. *Nature* 2001 **409** 307–312.
- Maeda K, Okubo K, Shimomura I, Funahashi T, Matsuzawa Y & Matsubara K. cDNA cloning and expression of a novel adipose specific collagen-like factor, apMI (adipose most abundant gene transcript 1). *Biochemical and Biophysical Research Communications* 1996 **221** 286–289.
- Matsuzawa Y, Funahashi T & Nakamura T. Molecular mechanism of metabolic syndrome X: contribution of adipocytokines adipocyte-derived bioactive substances. *Annals of the New York Academy of Sciences* 1999 **892** 146–154.
- Auwerx J & Staels B. Leptin. *Lancet* 1998 **351** 737–742.
- Mantzoros CS. The role of leptin in human obesity and disease: a review of current evidence. *Annals of Internal Medicine* 1999 **130** 671–680.
- Matsubara M, Chiba H, Maruoka S & Katayose S. Elevated serum leptin concentrations in women with components of multiple risk factor clustering syndrome. *Journal of Atherosclerosis and Thrombosis* 2000 **7** 231–237.
- Bouloumie A, Marumo T, Lafontan M & Busse R. Leptin induces oxidative stress in human endothelial cells. *FASEB Journal* 1999 **13** 1231–1238.
- Bouloumie A, Drexler HCA, Lafontan M & Busse R. Leptin, the product of *ob* gene, promotes angiogenesis. *Circulation Research* 1998 **83** 1059–1066.
- Parhami F, Tintut Y, Ballard A, Fogelman AM & Demer LL. Leptin enhances the calcification of vascular cells: artery wall as a target of leptin. *Circulation Research* 2001 **88** 954–960.
- Ouchi N, Kihara S, Arita Y, Maeda K, Kuriyama H, Okamoto Y *et al.* Novel modulator for endothelial adhesion molecules: adipocyte-derived plasma protein adiponectin. *Circulation* 1999 **100** 2473–2476.
- Nakano Y, Tobe T, Choi-Miura NH, Mazda T & Tomita M. Isolation and characterization of GBP28, a novel gelatin-binding protein purified from human plasma. *Journal of Biochemistry* 1996 **120** 803–812.
- Scherer PE, Williams S, Fogliano M, Baldini G & Lodish HF. A novel serum protein similar to C1q, produced exclusively in adipocytes. *Journal of Biological Chemistry* 1995 **270** 26746–26749.
- Hu E, Liang P & Spiegelman BM. AdipoQ is a novel adipose-specific gene dysregulated in obesity. *Journal of Biological Chemistry* 1996 **271** 10697–10703.
- Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J *et al.* Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochemical and Biophysical Research Communications* 1999 **257** 79–83.
- Okamoto Y, Arita Y, Nishida M, Muraguchi M, Ouchi N, Takahashi M *et al.* An adipocyte-derived plasma protein, adiponectin, adheres to injured vascular walls. *Hormone and Metabolic Research* 2000 **32** 47–50.
- Ouchi N, Kihara S, Arita Y, Okamoto Y, Maeda K, Kuriyama H *et al.* Adiponectin, an adipocyte-derived plasma protein, inhibits

- endothelial NF-kappa B signaling through a c-AMP-dependent pathway. *Circulation* 2000 **102** 1296–1301.
- 19 Yokota T, Oritani K, Takahashi I, Ishikawa J, Matsuyama A, Ouchi N *et al.* Adiponectin, a new member of the family of soluble defense collagens, negatively regulates the growth of myelomonocytic progenitors and the functions of macrophages. *Blood* 2000 **96** 1723–1732.
- 20 Hotta K, Funahashi T, Arita Y, Takahashi M, Matsuda M, Okamoto Y *et al.* Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. *Arteriosclerosis, Thrombosis and Vascular Biology* 2000 **20** 1595–1599.
- 21 Weyer C, Funahashi T, Tanaka S, Hotta K, Matsuzawa Y, Platley RE *et al.* Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. *Journal of Clinical Endocrinology and Metabolism* 2001 **86** 1930–1935.
- 22 Matsubara M, Maruoka S & Katayose S. Decreased plasma adiponectin concentrations in women with dyslipidemia. *Journal of Clinical Endocrinology and Metabolism* 2002 **87** 2764–2769.
- 23 Matsubara M, Yoshizawa T, Morioka T & Katayose S. Serum leptin and lipids in patients with thyroid dysfunction. *Journal of Atherosclerosis and Thrombosis* 2000 **7** 50–54.
- 24 Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF & Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985 **28** 412–419.
- 25 Donahue RP, DeCourten M, Prineas RJ, Collier G, Donahue RDC, Goldberg RB *et al.* Is fasting leptin associated with insulin resistance among nondiabetic individuals? *Diabetes Care* 1999 **22** 1092–1096.
- 26 Matsubara M, Chiba H, Maruoka S & Katayose S. Elevated serum leptin concentrations in women with hyperuricemia. *Journal of Atherosclerosis and Thrombosis* 2002 **9** 28–34.
- 27 Nakata M, Yada T, Soejima N & Maruyama I. Leptin promotes aggregation of human platelets via the long form of its receptor. *Diabetes* 1999 **48** 426–429.
- 28 Sierra-Honigmann HR, Nath AK, Murakami C, Garcia-Cardena G, Papapetropoulos A, Sessa WC *et al.* Biological action of leptin as an angiogenic factor. *Science* 1998 **281** 1683–1686.
- 29 Hotta K, Funahashi T, Bodkin NL, Ortmeier HK, Arita Y, Hansen BC *et al.* Circulating concentrations of the adipocyte protein adiponectin are decreased in parallel with reduced insulin sensitivity during the progression to type 2 diabetes in rhesus monkeys. *Diabetes* 2001 **50** 1126–1133.
- 30 Kissebah AH, Sonnenberg GE, Myklebust J, Goldstein M, Broman K, James RG *et al.* Quantitative trait loci on chromosomes 3 and 17 influence phenotypes of the metabolic syndrome. *PNAS* 2000 **97** 14478–14483.
- 31 Takahashi M, Arita Y, Yamagata K, Matsukawa Y, Okutomi K, Horie M *et al.* Genomic structure and mutations in the adipose-specific gene, adiponectin. *International Journal of Obesity* 2000 **24** 861–868.
- 32 Statnick MA, Beavers LS, Conner LJ, Corominola H, Johnson D, Hammond CD *et al.* Decreased expression of apM1 in omental and subcutaneous adipose tissue of humans with type 2 diabetes. *International Journal of Experimental Diabetes Research* 2000 **1** 81–88.
- 33 Yamauchi T, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K *et al.* The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nature Medicine* 2001 **7** 941–946.
- 34 Fruebis J, Tsao TS, Javorschi S, Ebbets-Reed D, Erickson MR, Yen FT *et al.* Proteolytic cleavage product of 30-kDa adipocyte complement-related protein increases fatty acid oxidation in muscle and causes weight loss in mice. *PNAS* 2001 **98** 2005–2010.

Received 7 February 2002

Accepted 10 April 2002