

Association Between Adiponectin and Mediators of Inflammation in Obese Women

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Low plasma levels of the anti-inflammatory factor adiponectin characterize obesity and insulin resistance. To elucidate the relationship between plasma levels of adiponectin, adiponectin gene expression in adipose tissue, and markers of inflammation, we obtained blood samples, anthropometric measures, and subcutaneous adipose tissue samples from 65 postmenopausal healthy women. Adiponectin plasma levels and adipose-tissue gene expression were significantly lower in obese subjects and inversely correlated with obesity-associated variables, including high-sensitive C-reactive protein (hs-CRP) and interleukin-6 (IL-6). Despite adjustment for obesity-associated variables, plasma levels of adiponectin were significantly correlated to adiponectin gene expression (partial $r = 0.38$, $P < 0.05$). Furthermore, the inverse correlation between plasma levels of hs-CRP and plasma adiponectin remained significant despite correction for obesity-associated variables (partial $r = -0.32$, $P < 0.05$), whereas the inverse correlation between adiponectin plasma levels or adiponectin gene expression in adipose tissue with plasma IL-6 were largely dependent on the clustering of obesity-associated variables. In conclusion, our data suggest a transcriptional mechanism leading to decreased adiponectin plasma levels in obese women and demonstrate that low levels of adiponectin are associated with higher levels of hs-CRP and IL-6, two inflammatory mediators and markers of increased cardiovascular risk. *Diabetes* 52:942–947, 2003

Adipocytes are a rich source of molecules that modulate cardiovascular and metabolic risk (1,2). The recently identified hydrophilic protein adiponectin, also known as Acrp30, AdipQ, apM1, and GBP28, is exclusively produced by adipocytes and has high homology to complement factor C1q (3). Plasma adiponectin levels are decreased in obesity (4), insulin resistance (5), type 2 diabetes (6,7), and dyslipide-

mia (8) and are particularly low in patients with coronary artery disease (6,9). Conversely, weight loss (10) and pharmacological improvement of insulin sensitivity by thiazolidinediones (11–13) are associated with increased adiponectin levels. Lack of adiponectin resulted in increased susceptibility to diet-induced insulin resistance (14), and treatment of animals with adiponectin improved insulin resistance and other metabolic abnormalities associated with obesity and lipodystrophy (15–18). In vitro, adiponectin suppressed adhesion molecule expression on endothelial cells (19), reduced vascular inflammatory responses by inhibition of endothelial cell nuclear factor (NF)- κ B signaling (20), and suppressed macrophage function (21,22). In vivo, adiponectin adhered to endothelial lesions (23) and prevented neointimal formation (24) and atherosclerosis in mice (25). In contrast to other adipocyte-derived molecules, adiponectin thus seems to have protective metabolic and anti-inflammatory properties. As little is known about adiponectin regulation, we tested the hypothesis that decreased adiponectin levels in obesity are determined by adiponectin gene expression in human adipose tissue. Furthermore, we examined the relationship between decreased adiponectin levels and interleukin-6 (IL-6), tumor necrosis factor- α (TNF- α), and high-sensitive C-reactive protein (hs-CRP) as mediators of inflammation and markers of cardiovascular risk in non-diabetic postmenopausal women.

RESEARCH DESIGN AND METHODS

Subjects and study protocol. The institutional review board approved the study, and all volunteers gave written consent. A total of 65 postmenopausal white women participated. None had diabetes, renal or liver disease, congestive heart failure, or known coronary heart disease. All medication was stopped at least 7 days and hormonal replacement therapy at least 4 weeks before the study. The women had not experience weight changes >1 kg during the last 3 months. After an overnight fast, anthropometric measurements and blood samples were obtained at 9:00 A.M. Body composition was determined by bioelectrical impedance analysis (Akern RJL, BIA 101/S, Data Input, Frankfurt aM, Germany). Abdominal subcutaneous adipose tissue samples (1.5–3 g) were obtained by needle biopsy from the periumbilical region. The specimen were washed three times in 0.9% NaCl and separated from blood cells and blood clots by centrifugation for 5 min at 200g and 20°C in each washing step. One hour after the biopsy, 24-h ambulatory blood pressure measurement was begun with appropriate cuffs (14 cm for 24–32 cm upper arm circumference, 16 cm for >32 cm upper arm circumference, SPACELABS 90207; Spacelabs, Kaarst, Germany).

Adipose-tissue gene expression. RNA was isolated by the Qiagen RNeasy mini kit (including the RNase-free DNase set; Qiagen, Hilden, Germany) as described previously (26), followed by determination of quality and quantity using the RNA 6000 Chip and the 2100 Bioanalyzer (Agilent Technologies, Waldbronn, Germany). Two micrograms of total RNA were reverse transcribed in 20 μ l of final volume for 1 h at 37°C using 100 units of Superscript Reverse Transcriptase, 5.4 μ g of random primer, 0.5 mmol/l dNTPs, 10 mmol/l DTT, and 1 \times RT buffer (all reagents by Invitrogen, Karlsruhe, Germany).

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CRP, C-reactive protein; CV, coefficient of variation; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HOMA, homeostasis model assessment; hs-CRP, high-sensitive CRP; IL-6, interleukin-6; NF, nuclear factor; TNF- α , tumor necrosis factor- α .

Relative quantitation of gene expression was performed with the ABI 5700 sequence detection system for "Real Time PCR" (TaqMan technology: all machines, software, and chemicals by PE Biosystems, Weiterstadt, Germany) using the standard curve method and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as endogenous control (27). PCRs were performed in a total volume of 25 μ l. The two-step PCR conditions were 2 min at 50°C, 10 min at 95°C, and 45 cycles of 15 s at 95°C and 1 min at 62°C.

Sequences of primers and probe for adiponectin cDNA were derived from GenBank accession number NM_004797 using the Primer Express software: 5'-GCTCTGTGCTCCTGCATCTG-3' (forward primer, 300 nmol/l final concentration), 5'-ACGCTCTCCTCCCATACA-3' (reverse primer, 900 nmol/l final concentration), 5' FAM-AGGTGGGCGACCAAGTCTGGCTC-TAMRA 3' (probe, 125 nmol/l final concentration). Ready-to-use TaqMan assays for human IL-6, TNF- α , and GAPDH genes were purchased from PE Biosystems. A standard human adipocyte cDNA was included in every TaqMan run to determine interassay coefficients of variation (CVs) for GAPDH (1.1%), adiponectin (1.5%), IL-6 (0.7%), and TNF- α (0.9%).

Blood and urine variables. Serum lipids were determined by standard procedures. Insulin resistance was individually calculated by homeostasis model assessment (HOMA) from fasting glucose (polarography; Beckmann, München, Germany) and insulin levels (radioimmunoassay; DPC Biermann, Bad Nauheim, Germany) in three blood samples drawn at 5-min intervals (28). Adiponectin plasma levels were measured in duplicate by radioimmunoassay (Linco Research, St. Charles, MO) with murine ¹²⁵I-adiponectin as a competitor to plasma adiponectin and a multispecies adiponectin rabbit antiserum. Plasma adiponectin was calculated using a standard curve with recombinant human adiponectin according to the supplier. A human plasma control sample ($n = 8$) was included to determine intra-assay (5.4%) and interassay (7.2%) CVs. IL-6 and TNF- α were measured by enzyme-linked immunosorbent assay (Quantikine ELISA; R&D Systems, Minneapolis, MN) as described by the manufacturer. Control plasma was analyzed on each plate, demonstrating intra-assay CV of 7.2 and 4.5% and interassay CV of 5.4 and 21.2% for IL-6 and TNF- α , respectively. hs-CRP was determined by nephelometry (goat and mouse anti-human CRP antibodies; Synchron LX Systems, Beckman Coulter, Krefeld, Germany) with an interassay CV of 7.5%.

Statistical analysis. Data were analyzed with the SPSS 10.0.7 statistical software package. All variables were normally distributed as tested with the Kolmogorov-Smirnov test. Group comparison was performed by ANOVA followed by Bonferroni's multiple t test. Stepwise multiple linear regression models for plasma levels and gene expression of adiponectin, IL-6, TNF- α , and hs-CRP were calculated including BMI, waist circumference, percentage of body fat, HOMA index of insulin resistance, daily mean values of systolic and diastolic blood pressure, plasma triglycerides, and HDL cholesterol as independent variables. Results were considered statistically significant at $P < 0.05$.

RESULTS

Adiponectin. Demographic data from the study population are presented in Table 1. Adiponectin plasma levels and adipose-tissue gene expression were decreased in overweight and obese women by approximately one-third compared with lean women (Fig. 1). Additional analysis of adiponectin plasma levels and gene expression revealed the best linear curve fitting by logarithmic transformation. Stepwise multiple linear regression analysis of log₁₀-transformed adiponectin plasma levels demonstrated that plasma triglycerides and percentage of body fat explain most of the variability of the complete model ($r = -0.58$, $r^2 = 0.33$, $P < 0.001$ vs. $r = -0.62$, $r^2 = 0.39$, $P = 0.001$ for the complete model). Stepwise multiple linear regression analysis of log-transformed adiponectin gene expression revealed the HOMA index and plasma triglycerides as the strongest predictors ($r = -0.52$, $r^2 = 0.27$, $P < 0.001$) compared with the complete model ($r = -0.56$, $r^2 = 0.32$, $P = 0.007$). The significant relationship between adiponectin gene expression and plasma levels (Fig. 2) was independent of BMI, HOMA index, waist circumference, percentage of body fat, blood lipids, and blood pressure (partial $r = 0.38$, $P = 0.012$).

IL-6, TNF- α , and hs-CRP. Plasma levels of IL-6 and hs-CRP, as well as adipose-tissue gene expression of IL-6

TABLE 1
Demographic characteristics of the study population

Variable	Lean	Overweight	Obese
<i>n</i>	20	25	20
Age (years)	56 \pm 3	57 \pm 4	58 \pm 4
BMI (kg/m ²)	23.1 \pm 1.4	27.3 \pm 1.4*	35.2 \pm 3.9*†
Waist circumference (cm)	72 \pm 18	88 \pm 10*	102 \pm 10*†
Body fat (%)	29 \pm 3	34 \pm 3*	41 \pm 4*†
ABPM _{systolic} daytime (mmHg)	118 \pm 9	128 \pm 14	136 \pm 15*
ABPM _{diastolic} daytime (mmHg)	74 \pm 6	80 \pm 8	82 \pm 8*
Mean daily heart rate (min ⁻¹)	74 \pm 5	81 \pm 8*	82 \pm 8*
Total cholesterol (mmol/l)	5.4 \pm 1.1	5.5 \pm 0.7	5.5 \pm 0.7
HDL cholesterol (mmol/l)	1.5 \pm 0.4	1.4 \pm 0.3	1.3 \pm 0.3
LDL cholesterol (mmol/l)	3.5 \pm 1.0	3.7 \pm 0.8	3.7 \pm 0.8
Triglycerides (mmol/l)	1.0 \pm 0.4	1.0 \pm 0.4	1.3 \pm 0.6
Glucose (mmol/l)	4.9 \pm 0.6	5.1 \pm 0.5	5.4 \pm 0.5*
Insulin (mU/l)	3.0 \pm 1.8	3.6 \pm 1.7	6.6 \pm 3.3*†
HOMA index	0.7 \pm 0.4	0.9 \pm 0.4	1.6 \pm 0.9*†

All data were normally distributed and are presented as mean \pm SD. ABPM, 24-h ambulatory blood pressure measurement. Group comparison by ANOVA followed by Bonferroni's multiple t test. * $P < 0.05$ vs. lean group; † $P < 0.05$ vs. overweight group.

and TNF- α increased significantly with BMI (Fig. 1). HOMA and BMI were the strongest predictors of plasma hs-CRP ($r = 0.70$, $r^2 = 0.49$, $P < 0.001$ vs. $r = 0.73$, $r^2 = 0.53$, $P < 0.001$ for the complete model) and of plasma IL-6 ($r = 0.68$, $r^2 = 0.46$, $P < 0.001$ vs. $r = 0.70$, $r^2 = 0.49$, $P < 0.001$ for the complete model). BMI and plasma triglycerides predicted expression of the adipose-tissue IL-6 gene nearly as well as the complete regression model ($r = 0.56$, $r^2 = 0.31$, $P < 0.001$ vs. $r = 0.57$, $r^2 = 0.32$, $P < 0.05$). Using the same multiple linear regression model, neither plasma levels nor gene expression of TNF- α were significantly related to obesity-associated variables in this study. In contrast to adiponectin, plasma levels and adipose-tissue gene expression of IL-6 and TNF- α were not correlated ($r = 0.17$, $P = 0.28$ for IL-6, and $r = 0.10$, $P = 0.43$ for TNF- α).

Relationship between adiponectin, IL-6, TNF- α , and hs-CRP. Whereas the significant association between plasma levels of IL-6 and hs-CRP ($r = -0.57$, $r^2 = 0.32$, $P < 0.001$) was independent of BMI, HOMA index, waist circumference, percentage of body fat, blood lipids, and blood pressure (partial $r = 0.47$, $P = 0.005$), the association between adipose-tissue (IL-6) gene expression and hs-CRP was dependent on these confounding obesity-associated variables (partial $r = 0.28$, $P = 0.1$). No significant correlation between TNF- α and IL-6 or hs-CRP was found. Increased plasma levels of IL-6 and hs-CRP, however, were strongly associated with decreased plasma levels of log-transformed adiponectin (Fig. 3). The correlation between adiponectin and hs-CRP remained significant despite adjustment for the above-mentioned obesity-associated variables (partial $r = -0.32$, $P < 0.05$), whereas the correlation between adiponectin and IL-6 was largely dependent on these variables. At the tissue level, log-transformed adiponectin gene expression was significantly and independently related to IL-6 plasma levels ($r = -0.51$, $r^2 = 0.27$, $P < 0.001$; partial $r = -0.35$, $P = 0.031$

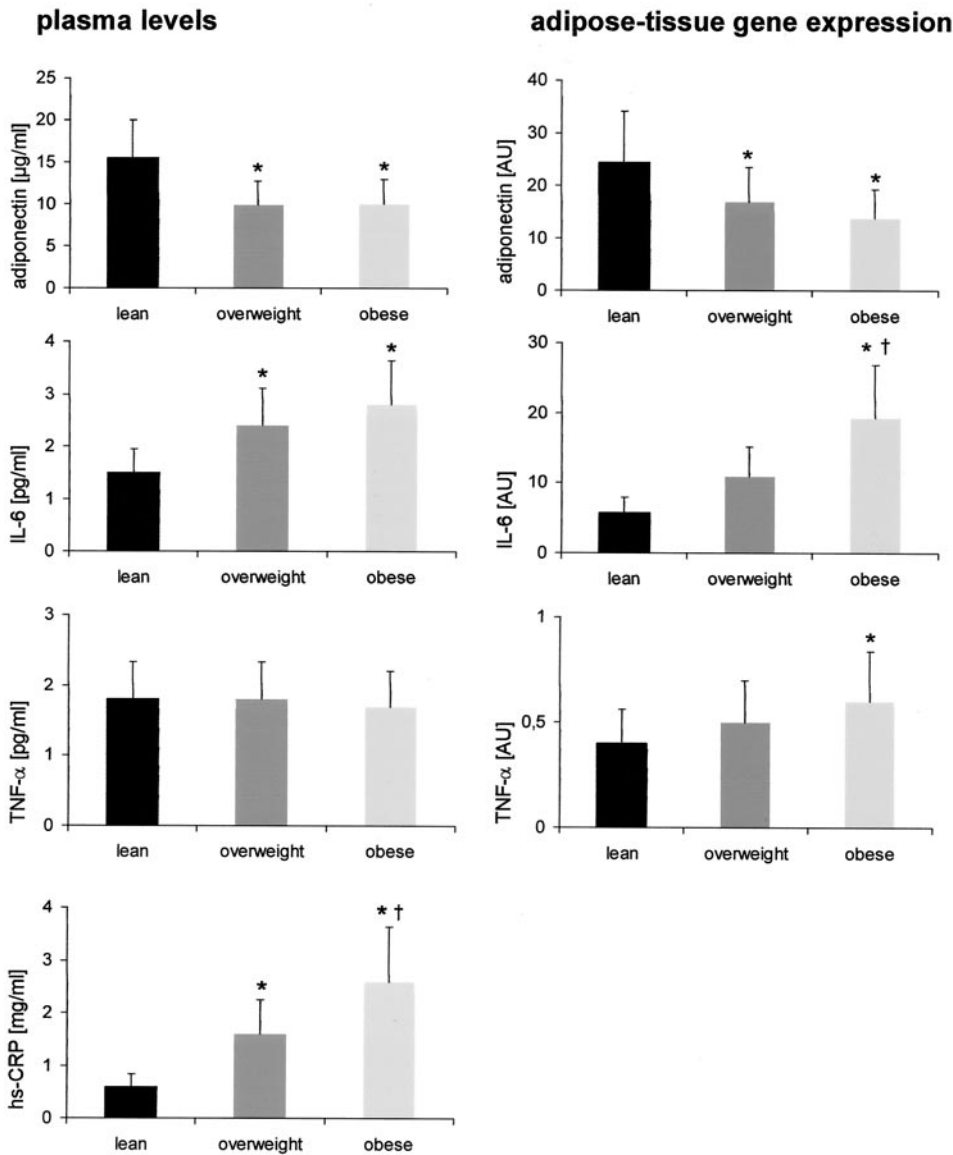


FIG. 1. Plasma levels (left) and adipose-tissue gene expression (right) of adiponectin and the proinflammatory molecules IL-6, TNF- α , and hs-CRP in the study population (mean BMIs for the groups are lean, 23.1 ± 1.4 kg/m²; overweight, 27.3 ± 1.4 kg/m²; and obese, 35.2 ± 3.9 kg/m²). Gene expression data of adiponectin, IL-6, and TNF- α are normalized by the expression of the internal control gene GAPDH, resulting in the calculation of arbitrary units (AU). Data are expressed as mean \pm SD. Group comparison was performed by ANOVA followed by Bonferroni's multiple *t* test. **P* < 0.05 vs. lean group; †*P* < 0.05 vs. overweight group.

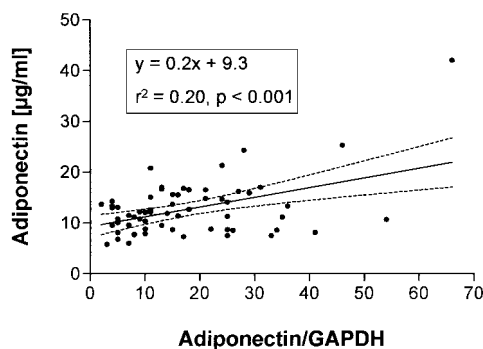


FIG. 2. Prediction of adiponectin plasma levels by subcutaneous abdominal adipose-tissue gene expression as calculated by linear regression ($r = 0.45$, $r^2 = 0.20$, $P < 0.001$; $y = 0.2x + 9.3$; regression line and 95% CI for the regression model are displayed). Gene expression data of adiponectin are normalized by the expression of the internal control gene GAPDH and expressed in arbitrary units. The significant relationship between adiponectin gene expression and plasma levels is independent of the influence of BMI, HOMA index, waist circumference, percentage of body fat, blood lipids, and blood pressure on both variables (partial $r = 0.38$, $P = 0.012$).

after adjustment for obesity-associated variables) but not to the expression of IL-6 or TNF- α genes.

DISCUSSION

Our study confirms the previously reported inverse correlation between obesity-associated changes and plasma adiponectin levels (4–8) and extends these findings to healthy postmenopausal white women. More important, decreased adiponectin plasma levels were associated with reduced adiponectin gene expression in subcutaneous abdominal adipose tissue in our study. This finding is in contrast to a previous report in Rhesus monkeys (7), in which subcutaneous adiponectin expression was not correlated with adiponectin plasma levels. For thiazolidinedione-treated mice, however, a parallel increase in adipose-tissue adiponectin gene expression and plasma levels was reported (11). In one of the first descriptions of adiponectin, Hu et al. (29) reported lower adiponectin mRNA levels in four severely obese humans. Statnick et al. (30) recently reported downregulation of adiponectin gene expression

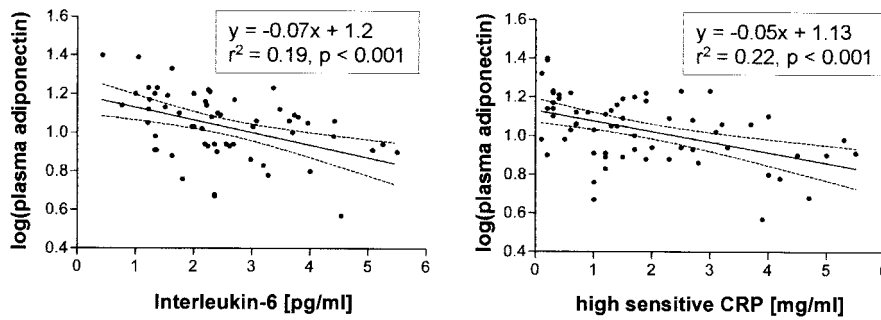


FIG. 3. Relationship between plasma levels of inflammatory markers and log-transformed adiponectin plasma levels as determined by linear regression analysis (IL-6: $r = -0.40$, $r^2 = 0.19$, $P < 0.001$; $y = -0.07x + 1.2$; hs-CRP: $r = -0.45$, $r^2 = 0.22$, $P < 0.001$; $y = -0.05x + 1.13$). The relationship between adiponectin and hs-CRP remained significant despite correction for BMI, HOMA index, waist circumference, percentage of body fat, blood lipids, and blood pressure (partial $r = -0.32$, $P < 0.05$), whereas the relationship between adiponectin and IL-6 is dependent on these variables.

in visceral adipose tissue of obese patients with diabetes compared with nondiabetic lean and obese subjects. However, these data were obtained in fewer severely obese patients ($n = 24$; BMI >45 kg/m²). Our data, although restricted to subcutaneous abdominal adipose tissue, clearly demonstrate that adiponectin gene expression is significantly reduced in adipose tissue of obese nondiabetic subjects. The strongest effect of adiposity on downregulation of adiponectin gene expression was clearly seen at a BMI <35 kg/m². The effect of small differences in adiposity at lower BMI levels is also reflected by the fact that adiponectin expression and plasma levels both decreased in a logarithmic manner with obesity-associated variables such as BMI or HOMA index of insulin resistance.

Insulin resistance (as indicated by the HOMA index) was a strong negative predictor of adiponectin gene expression. This correlation was independent of other confounding variables such as BMI, waist circumference, or percentage of body fat. Insulin possibly plays a role in the downregulation of adiponectin expression, or perhaps decreased adiponectin levels lead to insulin resistance and hyperinsulinemia. Although in vitro data are not conclusive on the role of insulin on adiponectin regulation (31–33), in vivo data clearly showed that hyperinsulinemia downregulates adiponectin plasma levels (34). However, administration of adiponectin reversed insulin resistance as a result of obesity or lipodystrophy (15), positively influenced hepatic glucose metabolism (17,18), and stimulated muscular fatty acid oxidation (16,35), whereas adiponectin knockout mice developed insulin resistance (14). Together, these observations suggest that insulin resistance is a consequence rather than a determinant of decreased adiponectin expression in adipose tissue. This view is supported by recent reports that low adiponectin plasma levels significantly predict the risk to develop insulin resistance or type 2 diabetes in small Pima Indian cohorts (36,37) and in a large genetically heterogeneous white cohort (38). As the insulin-sensitizing thiazolidinediones increase adipose-tissue adiponectin gene expression and plasma levels (11–13), their beneficial effects may be partly due to the stimulation of adiponectin.

Our data suggest that the clustering of increased metabolic and adiposity measures is associated with lower adiponectin levels, rather than any single variable. Our study also shows that lower adiponectin levels are attributable at least in part to transcriptional alterations in adipose tissue. Recent studies have shown that decreased generation of adiponectin observed in cultured adipose-tissue explants can be abolished by unspecific inhibition of transcription and protein synthesis, suggesting the formation of an autocrine or paracrine factor that acts to

destabilize adiponectin protein or mRNA (32). This factor remains to be identified, but it may well be involved in the downregulation of adiponectin secretion from adipocytes of obese subjects. Other hormonal mechanisms implicated in the downregulation of adiponectin expression include glucocorticoids (31) and β -adrenergic activation (39), both of which may be increased in obese individuals.

Increased adiposity is associated with increased plasma levels of inflammatory markers such as IL-6 (40,41), hs-CRP (42,43), and, less consistently, TNF- α (44–46). Especially IL-6 and hs-CRP are established risk markers for cardiovascular events (47,48). As several cytokines are also produced by adipose tissue (2), it was postulated that an “adipo-vascular” axis (24) may contribute to the increased risk of cardiovascular events in obese subjects. Recent studies suggest that adiponectin may play a role in the modulation of inflammatory vascular response by inhibiting the expression of adhesion molecules on endothelial cells (19), inhibiting endothelial cell NF- κ B signaling (20), and suppressing macrophage function (21,22). Furthermore, adiponectin knockout mice are prone to increased neointimal formation after vascular injury, and this susceptibility was reversed by adenoviral transfer of adiponectin to these mice (24).

Given the anti-inflammatory and vasculoprotective actions of adiponectin and the presentation of obesity as a chronic inflammatory state, the inverse association between decreased plasma adiponectin levels and increased plasma levels of hs-CRP and IL-6 in our study is not surprising. However, the inverse correlation of adiponectin and hs-CRP remained statistically significant even after adjustment for obesity-associated confounders such as anthropometric variables, ambulatory blood pressure, HOMA index of insulin resistance, and blood lipids. hs-CRP is an established early marker of vascular damage and is strongly predictive for future cardiovascular events (42,43,48). Thus, our finding of an independent inverse correlation between plasma adiponectin levels and hs-CRP may suggest that decreased production of adiponectin contributes to the systemic and vascular inflammation commonly found in obesity.

Decreased adiponectin plasma levels with increasing obesity and insulin resistance were best described by logarithmic functions, suggesting that adiponectin plasma levels deteriorate early in the development of obesity and rapidly achieve trough levels with further weight gain. Thus, the significance of low adiponectin levels for the metabolic and cardiovascular risk may not be apparent in patients beyond a certain BMI cutoff or in patients with already visible end-organ damage. This idea is supported by the observation that although low adiponectin plasma

levels were predictive for new cardiovascular events in hemodialysis patients (9), they were not correlated with existing retinopathy in patients with diabetes (6).

Our data suggest that decreased plasma and expression levels of adiponectin may serve as a marker of increased metabolic and inflammatory risk. As for IL-6 (49), depot-specific differences may exist for the regulation of adiponectin in adipose tissue. However, the subcutaneous abdominal adipose tissue examined in our study may be regarded as a suitable model, as we demonstrated the association between adiponectin gene expression and plasma levels. That expression levels of IL-6 or TNF- α , in contrast to adiponectin, were not related to the expression of these genes in adipose tissue is consistent with the finding that these molecules are secreted by a number of other cell types, whereas adiponectin is secreted exclusively by adipocytes (49).

Future studies should address the regulation of adiponectin gene expression in adipose tissue and determine whether inflammatory mediators influence this regulation. TNF- α suppressed adiponectin expression in mouse 3T3-L1 clonal preadipocytes (31,33). However, 3T3-L1 cells have several different characteristics compared with human adipocytes, and we were not able to detect a close relationship between adiponectin gene expression and the expression of TNF- α and IL-6 within the same adipose tissue samples. These data suggest that TNF- α and IL-6 do not directly downregulate adiponectin gene expression in humans. In contrast, we observed a close relationship between plasma IL-6 and adiponectin gene expression, although not independent of obesity-associated variables. This finding again supports the notion that "obesity" is a syndrome composed of metabolic disturbances, increased anthropometric variables, increased blood pressure, increased inflammatory, and possibly decreased anti-inflammatory mediators.

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