Adiponectin Expression From Human Adipose Tissue

Relation to Obesity, Insulin Resistance, and Tumor Necrosis Factor-α Expression

Philip A. Kern, Gina B. Di Gregorio, Tong Lu, Negah Rassouli, and Gouri Ranganathan

Adiponectin is a 29-kDa adipocyte protein that has been linked to the insulin resistance of obesity and lipodystrophy. To better understand the regulation of adiponectin expression, we measured plasma adiponectin and adipose tissue adiponectin mRNA levels in nondiabetic subjects with varying degrees of obesity and insulin resistance. Plasma adiponectin and adiponectin mRNA levels were highly correlated with each other ($r = 0.80$, $P < 0.001$), and obese subjects expressed significantly lower levels of adiponectin. However, a significant sex difference in adiponectin expression was observed, especially in relatively lean subjects. When men and women with a BMI <30 kg/m^2 were compared, women had a twofold higher percent body fat, yet their plasma adiponectin levels were 65% higher (8.6 ± 1.1 and 14.2 ± 1.6 μg/ml in men and women, respectively; $P < 0.02$). Plasma adiponectin had a strong association with insulin sensitivity index (S_I) ($r = 0.67$, $P < 0.0001$, $n = 51$) that was not affected by sex, but no relation with insulin secretion. To separate the effects of obesity (BMI) from S_I, subjects who were discordant for S_I were matched for BMI, age, and sex. Using this approach, insulin-sensitive subjects demonstrated a twofold higher plasma level of adiponectin (5.6 ± 0.6 and 11.2 ± 1.1 μg/ml in insulin-resistant and insulin-sensitive subjects, respectively; $P < 0.0005$). Adiponectin expression was not related to plasma levels of leptin or interleukin-6. However, there was a significant inverse correlation between plasma adiponectin and tumor necrosis factor (TNF)-α mRNA expression ($r = -0.47$, $P < 0.005$), and subjects with the highest levels of adiponectin mRNA expression secreted the lowest levels of TNF-α from their adipose tissue in vitro. Thus, adiponectin expression from adipose tissue is higher in lean subjects and women, and is associated with higher degrees of insulin sensitivity and lower TNF-α expression. *Diabetes* 52:1779–1785, 2003

From the Central Arkansas Veterans Healthcare System and the Department of Medicine, Division of Endocrinology, University of Arkansas for Medical Sciences, Little Rock, Arkansas.

Address correspondence and reprint requests to Philip A. Kern, Associate Chief of Staff, Research, Central Arkansas Veterans Healthcare System, 598/151 LR, 4300 West 7th St., Little Rock, AR 72205. E-mail: kernphilipa@uams.edu.

Received for publication 6 December 2002 and accepted in revised form 4 April 2003.

AIR_{glu}, acute insulin response to glucose; DI, disposition index; DMEM, Dulbecco's modified Eagle's medium; IL-6, interleukin-6; FSIVGT, frequently sampled intravenous glucose tolerance test; RIA, radioimmunoassay; SI, insulin sensitivity index; TNF, tumor necrosis factor.

Obesity has become widespread in developed countries along with a corresponding increase in the prevalence of type 2 diabetes (1–3). Although the precise underlying mechanisms in the development of diabetes are as yet unknown, the initial pathophysiological event is usually insulin resistance, which involves a genetic component that is exacerbated by obesity and a sedentary lifestyle. There is a significant correlation between obesity and insulin resistance in nondiabetic subjects, and obesity exacerbates insulin resistance in diabetic subjects (4–7). However, the degree of insulin resistance that accompanies obesity varies considerably, and the relation among obesity, insulin resistance, and type 2 diabetes is not well understood.

Obesity represents an increase in adipose tissue mass. It is not known how this leads to insulin resistance, which is a phenomenon that primarily involves the uptake and metabolism of glucose by skeletal muscle (8). Knowledge about this metabolic syndrome has moved forward with the description of numerous adipocyte secretory products. The term "adipokines" has been used to describe the numerous adipocyte secretory products, which include tumor necrosis factor (TNF)-α, interleukin-6 (IL-6), leptin, resistin, and adiponectin (9–12). There is growing evidence that adipocyte secretory products are important determinants of insulin resistance, through either a traditional (circulating) hormonal effect or local effects on the adipocyte.

The expression of adiponectin (also called ACRP 30) is low in rodent models of insulin resistance and is accompanied by low muscle and liver lipid accumulation (13–15). When adiponectin was given along with leptin to a mouse model of lipodystrophy, the insulin resistance was reversed. Adiponectin knockout mice develop severe insulin resistance in response to a high-fat diet, in association with higher levels of TNF-α and increased tissue lipid accumulation (16).

Several studies have examined plasma adiponectin levels in humans and have found decreased levels in obese and diabetic subjects and significant inverse associations with some measure of insulin resistance (17). However, few human studies have been able to determine whether adiponectin is associated specifically with insulin resistance, independent of obesity. In addition, human studies have not thoroughly examined adiponectin gene expres-

TABLE 1
Baseline characteristics of subjects

	Women	Men
n	50	12
Age (years)	39 ± 1.4	42 ± 2.9
Triglycerides (mg/dl)	111 ± 10	147 ± 25
LDL (mg/dl)	122 ± 5.0	127 ± 5.5
HDL (mg/dl)	53 ± 1.1	45 ± 0.51
Fasting blood glucose (mg/dl)	90 ± 1.0	98 ± 1.3
HbA_{1c} (%)	5.1 ± 0.09	5.2 ± 0.22
BMI (kg/m^2)	36 ± 1.3	32 ± 3.5
Fat (%)	42 ± 1.3	26 ± 2.4

Data are means ± SE.

sion and have not determined the relation between adiponectin and other cytokines.

This study examined adiponectin expression in plasma and adipose tissue in nondiabetic subjects with varying degrees of obesity and insulin resistance. We found a significant inverse association between plasma adiponectin and insulin resistance, an effect that was independent of obesity. In addition, there was an inverse association between adipose tissue adiponectin and TNF-α expression, but there was no association between adiponectin and other cytokines. These data suggest that adiponectin, like TNF-α and IL-6, plays an important role in obesity-associated insulin resistance.

RESEARCH DESIGN AND METHODS

Subjects. This study involved 62 weight-stable subjects ages 23–61 years. All subjects gave informed consent, and the research was approved by the institutional review board. Subjects initially underwent a 75-g oral glucose tolerance test, and subjects with diabetes (fasting glucose >126 mg/dl, 2-h glucose >200 mg/dl) were excluded. Of the 62 subjects, 15 had impaired glucose tolerance based on a 2-h glucose test of 140–200 mg/dl, and 4 of these subjects had impaired fasting glucose based on a fasting glucose of 110–126 mg/dl. Subjects then underwent a frequently sampled intravenous glucose tolerance test (FSIVGT) and an adipose tissue biopsy, which were performed on separate days.

Characteristics of the study subjects are shown in Table 1. Blood lipids, glucose, and HbA_{1c} were measured using standard clinical assays. Of the 62 subjects studied, 50 were women and 12 were African American. The subjects ranged from lean to very obese (BMI range 19–65 kg/m^2). Some subjects demonstrated moderate dyslipidemia, but no subject demonstrated fasting triglycerides >400 mg/dl. Body composition was determined using bioelectric impedance (18).

Insulin sensitivity measurements. The measurement of in vivo insulin sensitivity was performed in the fasting state using the classic tolbutamide-modified minimal model analysis of the FSIVGT (19,20), which has been validated against the euglycemic clamp (21,22). Four basal blood samples were obtained at time 0. Patients were then given an intravenous glucose bolus (11.4 g/m^2) and, 20 min later, an injection of tolbutamide (125 mg/m^2). Frequent blood sampling was then performed according to the standard protocol. Glucose was measured using the glucose oxidase method in a glucose analyzer and insulin was measured using radioimmunoassay (RIA; Endocrinology Laboratory of the Indiana University School of Medicine, Indianapolis, IN). The insulin sensitivity index (S_I) was calculated using the MINMOD program, along with the acute insulin response to glucose (acute insulin response to glucose [AIR_{glu}]) (20).

Adipose tissue biopsy. Abdominal subcutaneous adipose tissue (~5 g) was removed from each patient by incision. Some of the tissue was immediately frozen in liquid N_2 for later RNA extraction, whereas the rest of the tissue was placed into cold Dulbecco's modified Eagle's medium (DMEM) for other assays. To measure the secretion of adiponectin and TNF-α, ~500 mg of adipose tissue were minced and placed into serum-free DMEM (pH 7.4, 10 mmol/l HEPES) at 37° for varying times, as previously described (23). To compare cytokine secretion among different subjects, we measured cytokine levels in the medium after 2 h at 37°. All data were normalized to either adipose DNA content (24) or cell number to control for differences in fat cell size. Cell number was measured using the method of DiGirolamo et al. (25).

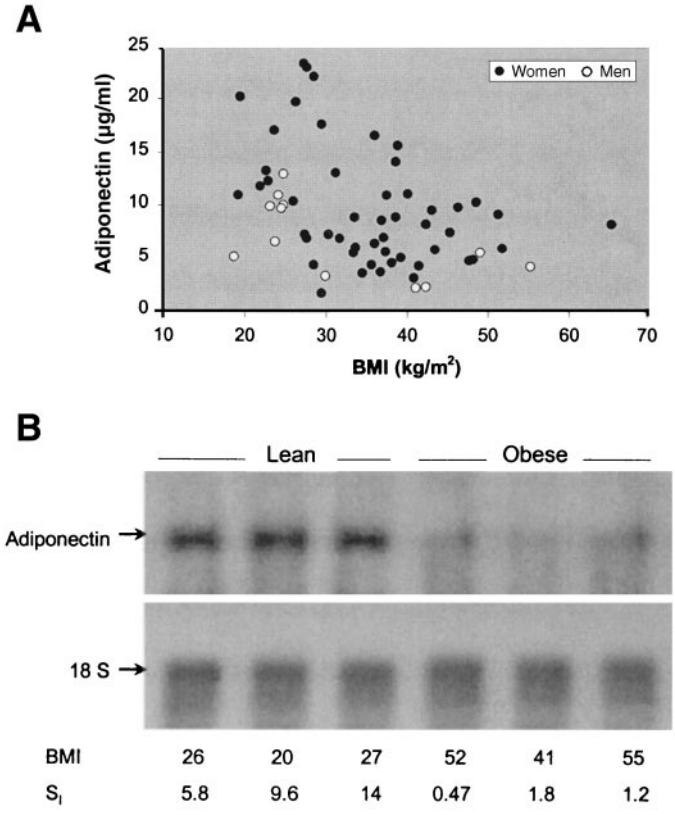

FIG. 1. Adiponectin expression in lean and obese subjects. *A*: Plasma adiponectin levels expressed in relation to subject BMI. *B*: Representative Northern blot from three lean and three obese women (see text for explanation).

Measurement of cytokine expression. The measurement of adiponectin protein used a radioimmunoassay (Linco Research, St. Charles, MO). This assay demonstrates a 4.3% intra-assay variation and a 7.1% interassay variation. This assay method was used to measure adiponectin in fasting plasma as well as secretion by adipose tissue, as described above. Adiponectin mRNA levels were measured by Northern blotting using the cDNA to human adiponectin, and the same blots were reprobed with the cDNA to 18S RNA as a constitutive probe (Fig. 1*B*). To quantitate adiponectin mRNA expression, the blots were analyzed by densitometry, and the adiponectin/18S RNA ratio was given as arbitrary units.

TNF-α expression. TNF-α mRNA levels were measured as previously described using competitive RT-PCR (26). In brief, 0.4 μg of total RNA from adipose tissue were added to increasing quantities of a competing RNA construct containing an internal 49-nt deletion. After the RT and PCR reactions, the products were resolved on a 2% agarose gel and the ethidium bromide–stained gel was quantitated. Data are expressed as the "number of copies" per microgram of total RNA, where "number of copies" refers to the number of copies of cRNA added at the equivalence point between TNF-α mRNA product and cRNA. TNF-α and IL-6 protein were measured using enzyme-linked immunosorbent assays (R&D Systems, Minneapolis, MN), and leptin was measured using an RIA (Linco Research).

Statistics. All data are expressed as means ± SE. Analysis of trends was performed using linear regression. When comparing two groups, a Student's *t* test was used, and to analyze data among groups of three or more, a one-way ANOVA was performed and secondary analysis was performed with the Student's *t* test with Bonferroni correction.

RESULTS

Adiponectin expression and relation to obesity. Adiponectin was measured in plasma, in the medium secreted from adipose tissue, and adiponectin mRNA levels were measured by Northern blotting. To examine the relation between adiponectin and obesity, adiponectin expression

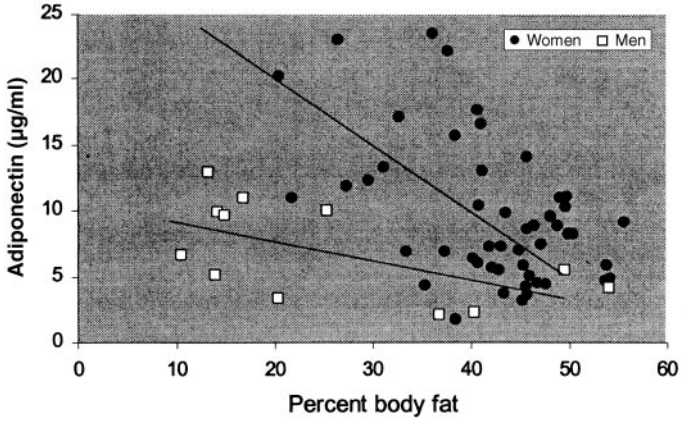

FIG. 2. Plasma adiponectin levels expressed in relation to percent body fat. The regression lines are drawn using a least-squares fit.

was measured in subjects covering a wide range of BMI and percent body fat. When all subjects were considered together, there was a significant inverse association between BMI and plasma adiponectin ($r = -0.39$, $P < 0.002$, $n = 62$) (Fig. 1*A*). In addition, adiponectin mRNA was assessed by Northern blotting, which yielded a single band for adiponectin (Fig. 1*B*). There was a strong correlation between adiponectin mRNA levels and plasma adiponectin levels ($r = 0.80$, $P < 0.001$, $n = 24$; data not shown), and likewise a significant inverse correlation between adiponectin mRNA and BMI ($r = -0.55$, $P < 0.01$, $n = 24$; data not shown).

Gender differences in adiponectin expression. Among subjects with similar BMIs, there was considerable variation in plasma adiponectin level. As can be seen in Fig. 1*A*, some of this variation could be attributed to lower adiponectin levels in men. To more appropriately analyze this association and account for gender differences, adiponectin expression was analyzed in relation to percent body fat. This resulted in a stronger association, along with significant gender differences. With increasing body fat, there was an overall significant decrease in plasma adiponectin when all subjects were considered together ($r = -0.27$, $P < 0.02$, $n = 62$). However, as shown in Fig. 2, this association was stronger when women and men were considered separately (women: $r = -0.50$, $P < 0.001$, $n = 50$; men: $r = -0.60$, $P < 0.05$, $n = 12$). At similar degrees of body fat, women demonstrated considerably higher plasma adiponectin levels than men, although this difference was less apparent with obesity and high body fat levels. To more directly compare men and women, we examined plasma adiponectin levels in normal weight and mildly overweight men and women (BMI <30 kg/m^2). As shown in Table 2, these men and women had the same BMI and the women had higher body fat, as expected. According to the trend described in Fig. 1*A*, involving lower plasma adiponectin levels with increasing adiposity, women would be expected to have lower plasma adiponectin levels because of their higher body fat. Instead, as shown in Table 2, women had significantly higher plasma adiponectin levels (8.6 ± 1.1 and 14.2 ± 1.6 μg/ml in men and women, respectively; $P < 0.02$) and also higher adiponectin mRNA levels.

Adiponectin expression and insulin sensitivity. Insulin sensitivity was expressed as S_I using the MINMOD analysis of the FSIVGT, as described in RESEARCH DESIGN AND METHODS. Figure 3*A* shows the relation between adiponectin and S_I. There was a significant positive relation between S_I and plasma adiponectin ($r = 0.67$, $P < 0.0001$, $n = 51$) and a similar relation between S_I and adiponectin mRNA levels ($r = 0.53$, $P < 0.01$, $n = 24$; data not shown). This relation between adiponectin expression and S_I was unaffected by gender (data not shown). To determine whether adiponectin affected insulin secretion, the AIR_{glu} was assessed in these subjects. No relations between insulin secretion, using the AIR_{glu}, and adiponectin was observed (data not shown). Previous studies have reported the product of $S_I \times AIR_{glu}$, called the disposition index (DI), as a heritable trait that indicates the ability of the β-cell to compensate for insulin resistance (19,27). We observed no significant relation between DI and adiponectin expression (data not shown).

TABLE 2
Effect of gender on adiponectin expression in nonobese subjects

	Women	Men
n	16	8
BMI (kg/m^2)	25 ± 0.9	24 ± 1.1
Fat (%)	33 ± 1.7	16 ± 1.6*
Plasma adiponectin (μg/ml)	14.2 ± 1.6	8.6 ± 1.1†
Adiponectin mRNA	3.1 ± 0.6	1.2 ± 0.3‡

Data are means ± SE. Adiponectin mRNA given in arbitrary units normalized to 18S RNA (see RESEARCH DESIGN AND METHODS). *$P < 0.01$; †$P < 0.02$; ‡$P = 0.06$.

Plasma adiponectin: relation to insulin sensitivity independent of obesity. As described by numerous previous studies, there was significant relations between obesity and insulin sensitivity. Among the subjects in this study, there was a significant correlation between BMI and S_I ($r = -0.60$, $P < 0.001$) and a similar correlation with percent body fat. We wanted to determine whether there was a significant relation between plasma adiponectin levels and insulin resistance that was independent of obesity. To assess this, we examined adiponectin expression in subjects who had similar degrees of adiposity, but were discordant for S_I. Therefore, paired subjects were identified who were matched for BMI (± 3 kg/m^2), age (± 10 years), and gender, but who were discordant for S_I. Because S_I is a continuous variable that varies across the population, we used the median value of our population, which is 2.6 units, to separate insulin-sensitive from insulin-resistant subjects. Using these criteria, we were able to match 11 subjects with S_I <2.6 with 11 subjects with S_I >2.6 (in each subject pair, the difference between S_I values was >2.0 units). The result of this matching is shown in Fig. 3*B*. There were no significant differences between the insulin-resistant and insulin-sensitive group with regard to BMI, age, percent body fat, or plasma leptin, but there were significant differences in S_I, based on the selection. Although these subjects were well matched with regard to adiposity, the insulin-sensitive subjects demonstrated a two-fold higher level of serum adiponectin (5.6 ± 0.6 and 11.2 ± 1.1 μg/ml in insulin-resistant and insulin-sensitive subjects, respectively; $P < 0.0005$).

Adiponectin and TNF-α expression. Adipose tissue expresses a number of cytokines, and previous studies

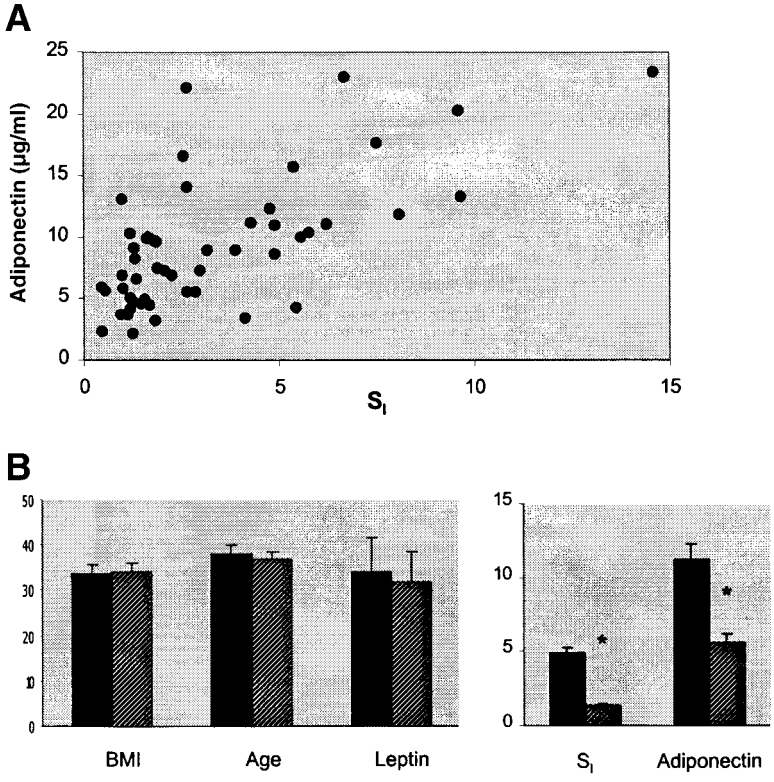

FIG. 3. Relation between plasma adiponectin and insulin sensitivity. *A*: Plasma adiponectin is shown in relation to S_I. *B*: Plasma adiponectin in subjects discordant for insulin resistance. We identified 11 subjects who were discordant for S_I (see text) but matched for BMI, age, and sex. There were no significant differences in BMI, age, or plasma leptin. *$P < 0.05$ vs. insulin-sensitive group.

have implicated adipose tissue TNF-α, IL-6, and leptin in obesity-related insulin resistance (23,28). To determine whether adiponectin expression was related to the expression of other cytokines, plasma IL-6, leptin, TNF, and TNF expression were measured in these subjects. There was no significant relation between adiponectin expression and plasma leptin ($r = -0.12$, NS; data not shown). In previous studies, plasma IL-6 correlated strongly with S_I (23). Even though IL-6 and adiponectin were both strongly associated with S_I, there was only a nonsignificant inverse trend between plasma IL-6 and plasma adiponectin ($r = -0.24$, $P = 0.10$, $n = 48$). However, there was a significant relation between adiponectin and TNF-α expression in adipose tissue. As shown in Fig. 4, subjects with high plasma adiponectin levels demonstrated low levels of TNF mRNA expression ($r = -0.47$, $P < 0.005$, $n = 36$). In a similar fashion, adiponectin mRNA levels were significantly associated with TNF mRNA levels ($r = -0.48$, $P < 0.05$, $n = 20$; data not shown). Previous studies demonstrated that TNF-α secretion from adipose tissue was strongly associated with obesity-related insulin resistance (23), suggesting that TNF-α may function in a paracrine fashion in adipose tissue. To examine the relation between TNF secretion and adiponectin, we examined TNF secretion in tertiles of adiponectin mRNA. As shown in Fig. 5, the low, middle, and high tertiles of adiponectin mRNA levels were associated with a significant inverse relation with adipose TNF-α secretion. Gender did not influence the relation between adiponectin and TNF-α. Therefore, the expression of adiponectin by adipose tissue was significantly associated with TNF-α expression, including TNF-α secretion, but not with the expression or plasma levels of IL-6 or leptin.

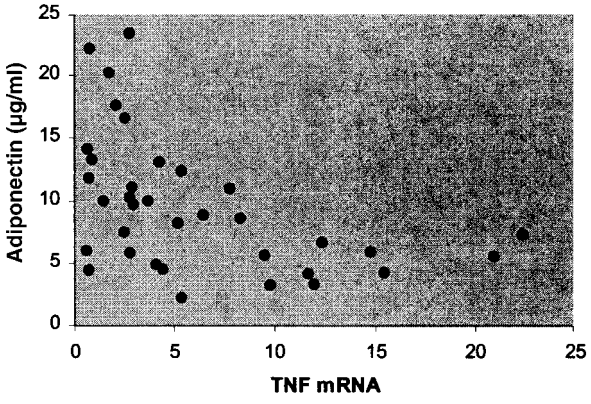

FIG. 4. Relation between plasma adiponectin and TNF-α mRNA levels.

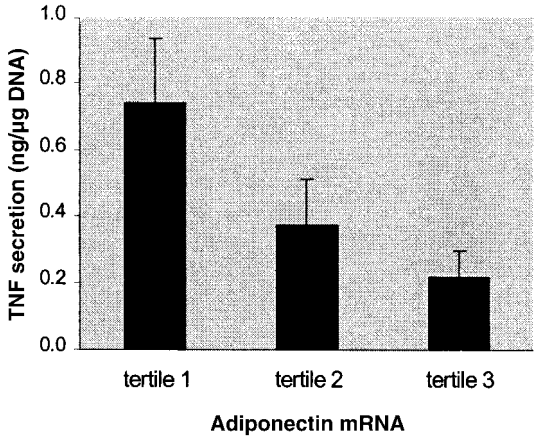

FIG. 5. TNF-α secretion with regard to adiponectin mRNA levels. Adiponectin mRNA expression was divided into tertiles of low, medium, and high expression. TNF-α secretion from other media was measured as described in RESEARCH DESIGN AND METHODS. $P < 0.05$ for tertile 1 vs. 3.

DISCUSSION

Adiponectin is a 29-kDa protein, expressed exclusively in adipocytes, that was initially called adipocyte complement–related protein of 30 kDa (ACRP30) because of its homology to complement factor C1q (29,30). Since the initial description of adiponectin, numerous animal and human studies have examined the relation of adiponectin to obesity, diabetes, and other associated syndromes. Plasma levels of adiponectin are low in obese, compared with lean, subjects; lower in diabetic, compared with nondiabetic, subjects; and lower in patients with coronary artery disease (17,31,32). In addition, plasma adiponectin levels increase following weight loss (33).

Several studies have demonstrated an association between plasma adiponectin and markers of insulin resistance. Among very obese subjects, a weak association between plasma adiponectin and insulin sensitivity has been shown using the steady-state plasma glucose method (33). In another study, plasma insulin was weakly associated with plasma adiponectin, but this relation was lost when the investigators controlled for BMI (17). Using the euglycemic clamp in a population of Pima Indians and Caucasians, Weyer et al. (34) demonstrated a strong inverse association between plasma adiponectin and insulin resistance, an association that was independent of obesity. In addition, patients and rodents treated with thiazolidinediones, which improve insulin sensitivity, demonstrated higher plasma adiponectin levels (35–37). Finally, a longitudinal study in rhesus monkeys demonstrated a significant association between insulin sensitivity and plasma adiponectin, along with a progressive decline in adiponectin with the development of obesity and insulin resistance (38).

At present, a receptor and precise target tissue for adiponectin action has not been described. However, several studies in rodents have provided evidence that adiponectin is important in tissue insulin action. In a mouse model of lipoatrophy, adiponectin levels were very low, and the administration of adiponectin in combination with leptin restored insulin sensitivity (13). That study demonstrated decreased lipid content in liver and muscle and improved lipid oxidation in muscle with adiponectin administration. The infusion of an adiponectin cleavage product containing the globular head of the protein resulted in a decrease in plasma nonesterified fatty acid, along with an increase in muscle lipid oxidation (14). Other studies have demonstrated an improvement in hepatic insulin sensitivity (15), and the infusion of mice with adiponectin during a euglycemic clamp demonstrated that the hypoglycemic effect of this cytokine was attributable to a suppression of hepatic glucose production (39). Together, these studies in rodents suggest that adiponectin may play an important role in preventing the development of lipotoxicity in insulin-sensitive tissues that commonly occurs in the metabolic syndrome (40).

This study examined the expression of adiponectin from adipose tissue in relation to insulin sensitivity in nondiabetic human subjects. Although plasma adiponectin levels were generally higher in lean compared with obese subjects, the relation was not very strong when expressed in terms of BMI. Some of the discrepancy between obesity and plasma adiponectin, however, could be accounted for by differences between men and women and by expressing obesity in terms of percent body fat. As shown in Fig. 2, the differences in plasma adiponectin between men and women were considerable, especially among subjects who were lean or mildly overweight. Among subjects with a BMI <30 kg/m^2, women predictably have a higher percent body fat. If plasma adiponectin levels were determined predominantly by fat mass, as with leptin, then one would expect women to have a lower plasma adiponectin, based on the overall inverse relation between adiponectin and obesity. However, women with a BMI <30 kg/m^2 actually express about twofold more adiponectin than men of the same BMI. It is interesting that this striking male/female difference was observed in one previous study (31), but was not observed in a study involving 144 subjects, of which 121 were Pima Indians (34). The reason for the higher adiponectin expression in women is unclear, but perhaps the higher adiponectin expression, along with differences in body composition, help explain the ability of women to maintain a normal insulin sensitivity despite higher body fat.

The relation between adiponectin and insulin resistance was stronger than the relation with obesity, and no gender differences were observed. In addition, the association between adiponectin and insulin sensitivity was independent of obesity, as demonstrated by the matching of subjects who were concordant for age and BMI but discordant for S_I. In contrast, leptin was not different in these groups. In a previous study, we demonstrated that TNF secretion and plasma IL-6 were also associated with insulin resistance, independent of obesity (23). Thus, these data suggest that adipose tissue expresses a number of cytokines that collectively are strongly associated with peripheral insulin sensitivity.

Although adiponectin levels were significantly associated with S_I, we found no evidence of an association between adiponectin levels and insulin secretion. This is of interest because of potential links between islet lipid content and insulin secretion. Troglitazone and other peroxisome proliferator–activated receptor-γ agonists increase adiponectin levels (35–37), lower islet lipid content, and improve β-cell function in Zucker diabetic rats (41). Because adiponectin has been linked to reduced lipid accumulation in tissues (13), it is possible that adiponectin also reduces islet lipid content, leading to increased insulin secretion or an improved β-cell response to insulin resistance. Although these studies did not fully test this concept, we could find no evidence for improved β-cell compensation in subjects with high adiponectin levels.

Several lines of evidence have suggested that TNF-α overproduction by adipose tissue may be involved in obesity-related insulin resistance. TNF is expressed at higher levels in the adipocytes of obese rodents and humans (26,42,43), and TNF knockout mice do not become insulin resistant with diet-induced obesity (44). In addition, the infusion of a soluble TNF-binding protein into insulin-resistant *fa/fa* rats improved insulin sensitivity and improved the defect in insulin receptor autophosphorylation (42,45). A recent study described a relation between adiponectin and TNF-α in adiponectin knockout mice (16). Although adiponectin knockout mice demonstrated normal blood glucose and insulin in the basal state,

they became highly insulin resistant when fed a high-fat diet. TNF expression was higher in the adiponectin knockout mice, and the administration of adiponectin in these mice resulted in an improvement in insulin resistance along with a decrease in TNF expression. Thus, these studies in mice suggest that TNF-α and adiponectin may be antagonists of each other or that one cytokine may control the expression of the other.

This study examined the expression of adiponectin and TNF-α in human adipose tissue and found a significant association between adiponectin and TNF-α expression. In previous studies, we found that the strongest association between TNF-α and insulin resistance was with the adipose-secreted component of TNF, and not with plasma TNF-α, suggesting a paracrine function of TNF-α in adipose tissue (23). In these studies, we found that there was a strong relation between adiponectin expression and TNF secretion from adipose tissue. In addition, TNF secretion from human adipose tissue is significantly associated with IL-6 secretion; plasma IL-6 is also associated with insulin resistance, independent of obesity (23). Taken together, these data suggest an emerging paradigm of adiponectin and TNF-α as important control points in adipose tissue that may lead to the expression of other cytokines (e.g., IL-6) and be linked to the development of tissue lipid accumulation and the insulin-resistance syndrome.

ACKNOWLEDGMENTS

This study was supported by a merit review grant from the Veterans Administration, General Clinical Research Center Grant M01-RR-14288, and National Institutes of Health Grant DK-39176.

We thank the many people who participated in these studies, as well as Denise Hargrove and Leslie Langum for assistance with subject recruitment and the nurses of the General Clinical Research Center at the University of Arkansas for Medical Sciences and Central Arkansas Veterans Healthcare System. We also thank Dr. Richard Bergman for supplying the MINMOD program and Sarah Dunn for excellent secretarial assistance.

REFERENCES

1. Flegal KM, Carroll MD, Ogden CL, Johnson CL: Prevalence and trends in obesity among US adults, 1999–2000. *JAMA* 288:1723–1727, 2002
2. Mokdad A, Serdula MK, Dietz WH, Bowman B, Marks J, Koplan J: The Spread of the obesity epidemic in the United States, 1991–1998. *JAMA* 282:1519–1522, 1999
3. Harris MI: Diabetes in America: epidemiology and scope of the problem. *Diabetes Care* 21 (Suppl. 3):C11−C14, 1998
4. Carey VJ, Walters EE, Colditz GA, Solomon CG, Willett WC, Rosner BA, Speizer FE, Manson JE: Body fat distribution and risk of non-insulin-dependent diabetes mellitus in women: the Nurses' Health Study. *Am J Epidemiol* 145:614–619, 1997
5. Kahn BB, Flier JS: Obesity and insulin resistance. *J Clin Invest* 106:473–481, 2000
6. Ludvik B, Nolan JJ, Baloga J, Sacks D, Olefsky J: Effect of obesity on insulin resistance in normal subjects and patients with NIDDM. *Diabetes* 44:1121–1125, 1995
7. DeFronzo RA, Ferrannini E: Insulin resistance: a multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. *Diabetes Care* 14:173–194, 1991
8. DeFronzo RA, Jacot E, Jequier E, Maeder E, Wahren J, Felber JP: The effect of insulin on the disposal of intravenous glucose: results from indirect calorimetry and hepatic and femoral venous catheterization. *Diabetes* 30:1000–1007, 1981
9. Flier JS: The adipocyte. Storage depot or node on the energy information superhighway? *Cell* 80:15–18, 1995
10. Flier JS: The missing link with obesity? *Nature* 409:292–293, 2001
11. Kern PA: Potential role of TNFα and lipoprotein lipase as candidate genes for obesity. *J Nutr* 127:1917S–1922S, 1997
12. Hotamisligil GS, Spiegelman BM: Tumor necrosis factor α: a key component of the obesity-diabetes link. *Diabetes* 43:1271–1278, 1994
13. Yamauchi T, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K, Mori Y, Ide T, Murakami K, Tsuboyama-Kasaoka N, Ezaki O, Akanuma Y, Gavrilova O, Vinson C, Reitman ML, Kagechika H, Shudo K, Yoda M, Nakano Y, Tobe K, Nagai R, Kimura S, Tomita M, Froguel P, Kadowaki T: The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nat Med* 7:941–946, 2001
14. Fruebis J, Tsao TS, Javorschi S, Ebbets-Reed D, Erickson MR, Yen FT, Bihain BE, Lodish HF: Proteolytic cleavage product of 30-kDa adipocyte complement-related protein increases fatty acid oxidation in muscle and causes weight loss in mice. *Proc Natl Acad Sci U S A* 98:2005–2010, 2001
15. Berg AH, Combs TP, Du X, Brownlee M, Scherer PE: The adipocyte-secreted protein ACRP30 enhances hepatic insulin action. *Nat Med* 7:947–953, 2001
16. Maeda N, Shimomura I, Kishida K, Nishizawa H, Matsuda M, Nagaretani H, Furuyama N, Kondo H, Takahashi M, Arita Y, Komuro R, Ouchi N, Kihara S, Tochino Y, Okutomi K, Horie M, Takeda S, Aoyama T, Funahashi T, Matsuzawa Y: Diet-induced insulin resistance in mice lacking adiponectin/ACRP30. *Nat Med* 8:731–737, 2002
17. Hotta K, Funahashi T, Arita Y, Takahashi M, Matsuda M, Okamoto Y, Iwahashi H, Kuriyama H, Ouchi N, Maeda K, Nishida M, Kihara S, Sakai N, Nakajima T, Hasegawa K, Muraguchi M, Ohmoto Y, Nakamura T, Yamashita S, Hanafusa T, Matsuzawa Y: Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. *Arterioscler Thromb Vasc Biol* 20:1595–1599, 2000
18. Segal KR, Gutin B, Presta E, Wang J, Van Itallie TB: Estimation of human body composition by electrical impedance methods: a comparative study. *J Appl Physiol* 58:1565–1571, 1985
19. Bergman RN, Phillips LS, Cobelli C: Physiologic evaluation of factors controlling glucose tolerance in man: measurement of insulin sensitivity and beta-cell sensitivity from the response to intravenous glucose. *J Clin Invest* 68:1456–1467, 1981
20. Bergman RN, Finegood DT, Ader M: Assessment of insulin sensitivity in vivo. *Endocr Rev* 6:45–86, 1985
21. Bergman RN, Prager R, Volund A, Olefsky JM: Equivalence of the insulin sensitivity index in man derived by the minimal model method and the euglycemic glucose clamp. *J Clin Invest* 79:790–800, 1987
22. Welch S, Gebhart SSP, Bergman RN, Phillips LS: Minimal model analysis of intravenous glucose tolerance test-derived insulin sensitivity in diabetic subjects. *J Clin Endocrinol Metab* 71:1508–1518, 1990
23. Kern PA, Ranganathan S, Li C, Wood L, Ranganathan G: Adipose tissue tumor necrosis factor and interleukin-6 expression in human obesity-associated insulin resistance. *Am J Physiol* 280:E745–E751, 2001
24. Labarca C, Paigen K: A simple, rapid, and sensitive DNA assay procedure. *Anal Biochem* 102:344–352, 1980
25. DiGirolamo M, Mendlinger S, Fertig JW: A simple method to determine fat cell size and number in four mammalian species. *Am J Physiol* 221:850–858, 1971
26. Kern PA, Saghizadeh M, Ong JM, Bosch RJ, Deem R, Simsolo RB: The expression of tumor necrosis factor in human adipose tissue: regulation by obesity, weight loss, and relationship to lipoprotein lipase. *J Clin Invest* 95:2111–2119, 1995
27. Elbein SC, Hasstedt SJ, Wegner K, Kahn SE: Heritability of pancreatic beta-cell function among nondiabetic members of Caucasian familial type 2 diabetic kindreds. *J Clin Endocrinol Metab* 84:1398–1403, 1999
28. Havel PJ: Control of energy homeostasis and insulin action by adipocyte hormones: leptin, acylation stimulating protein, and adiponectin. *Curr Opin Lipidol* 13:51–59, 2002
29. Scherer PE, Williams S, Fogliano M, Baldini G, Lodish HF: A novel serum protein similar to C1q, produced exclusively in adipocytes. *J Biol Chem* 270:26746–26749, 1995
30. Berg AH, Combs TP, Scherer PE: ACRP30/adiponectin: an adipokine regulating glucose and lipid metabolism. *Trends Endocrinol Metab* 13:84–89, 2002
31. Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, Hotta K, Shimomura I, Nakamura T, Miyaoka K, Kuriyama H, Nishida M, Yamashita S, Okubo K, Matsubara K, Muraguchi M, Ohmoto Y, Funahashi T, Matsuzawa Y: Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochem Biophys Res Commun* 257:79–83, 1999
32. Kumada M, Kihara S, Sumitsuji S, Kawamoto T, Matsumoto S, Ouchi N, Arita Y, Okamoto Y, Shimomura I, Hiraoka H, Nakamura T, Funahashi T, Matsuzawa Y, for the Osaka CAD Study Group: Association of hypoadi-

ponectinemia with coronary artery disease in men. *Arterioscler Thromb Vasc Biol* 23:85–89, 2003
33. Yang WS, Lee WJ, Funahashi T, Tanaka S, Matsuzawa Y, Chao CL, Chen CL, Tai TY, Chuang LM: Weight reduction increases plasma levels of an adipose-derived anti-inflammatory protein, adiponectin. *J Clin Endocrinol Metab* 86:3815–3819, 2001
34. Weyer C, Funahashi T, Tanaka S, Hotta K, Matsuzawa Y, Pratley RE, Tataranni PA: Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. *J Clin Endocrinol Metab* 86:1930–1935, 2001
35. Yang WS, Jeng CY, Wu TJ, Tanaka S, Funahashi T, Matsuzawa Y, Wang JP, Chen CL, Tai TY, Chuang LM: Synthetic peroxisome proliferator–activated receptor-γ agonist, rosiglitazone, increases plasma levels of adiponectin in type 2 diabetic patients. *Diabetes Care* 25:376–380, 2002
36. Maeda N, Takahashi M, Funahashi T, Kihara S, Nishizawa H, Kishida K, Nagaretani H, Matsuda M, Komuro R, Ouchi N, Kuriyama H, Hotta K, Nakamura T, Shimomura I, Matsuzawa Y: PPAR-γ ligands increase expression and plasma concentrations of adiponectin, an adipose-derived protein. *Diabetes* 50:2094–2099, 2001
37. Phillips SA, Ciaraldi TP, Kong APS, Bandukwala R, Aroda V, Carter L, Baxi S, Mudaliar SR, Henry RR: Modulation of circulating and adipose tissue adiponectin levels by antidiabetic therapy. *Diabetes* 52:667–674, 2003
38. Hotta K, Funahashi T, Bodkin NL, Ortmeyer HK, Arita Y, Hansen BC, Matsuzawa Y: 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* 50:1126–1133, 2001
39. Combs TP, Berg AH, Obici S, Scherer PE, Rossetti L: Endogenous glucose production is inhibited by the adipose-derived protein ACRP30. *J Clin Invest* 108:1875–1881, 2001
40. Unger RH: Lipotoxic diseases. *Annu Rev Med* 53:319–336, 2002
41. Higa M, Zhou YT, Ravazzola M, Baetens D, Orci L, Unger RH: Troglitazone prevents mitochondrial alterations, beta cell destruction, and diabetes in obese prediabetic rats. *Proc Natl Acad Sci U S A* 96:11513–11518, 1999
42. Hotamisligil GS, Shargill NS, Spiegelman BM: Adipose expression of tumor necrosis factor-α: direct role in obesity-linked insulin resistance. *Science* 259:87–91, 1993
43. Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM: Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. *J Clin Invest* 95:2409–2415, 1995
44. Uysal KT, Wiesbrock SM, Marino MW, Hotamisligil GS: Protection from obesity-induced insulin resistance in mice lacking TNF-alpha function. *Nature* 389:610–614, 1997
45. Hotamisligil GS, Budavari A, Murray D, Spiegelman BM: Reduced tyrosine kinase activity of the insulin receptor in obesity-diabetes: central role of tumor necrosis factor-α. *J Clin Invest* 94:1543–1549, 1994