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Tumor Necrosis Factor-α in Sera of Obese Patients: Fall with Weight Loss

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ABSTRACT

In view of the recent demonstration that obesity in animals and humans is associated with an increase in tumor necrosis factor-α (TNFα) expression, that this expression falls with weight loss, and that TNFα may specifically inhibit insulin action, the possibility that TNFα may be a mediator of insulin resistance has been raised. We have undertaken this study to investigate whether serum TNFα concentrations are elevated in obese subjects, whether they fall after weight loss, and whether this fall parallels the fall in insulin release after glucose challenge. Obese patients (age range: 25–54, weight mean ± SD: 96.4 ± 13.8 kg, body mass index: 35.7 ± 5.6 kg/m^2) were started on a diet program. The mean weight fell to 84.5 ± 11.3 ($P <$ 0.0001) and body mass index to 31.3 ± 4.9 ($P < 0.0001$). Plasma TNFα concentrations were markedly elevated in the obese (3.45 ± 0.16 pg/mL), when compared with controls (0.72 ± 0.28 pg/mL), and fell significantly (2.63 ± 1.40 pg/mL) after weight loss ($P < 0.02$). The magnitude of insulin release after glucose (75 g) challenge (area under the curve) also fell significantly ($P < 0.01$) after weight loss. The magnitude of weight loss and fall in TNFα were related to basal body weight (r = 0.57, $P < 0.001$) and basal TNFα (r = 0.55, $P < 0.001$) concentrations, respectively, but not to each other or to the glucose-induced insulin release (area under the curve). We conclude that obesity is associated with increased plasma TNFα concentrations, which fall with weight loss. Because circulating TNFα may mediate insulin resistance in the obese, a fall in TNFα concentrations may contribute to the restoration of insulin resistance after weight loss, Thus, TNFα may be an important circulating cytokine, which may provide a potentially reversible mechanism for mediating insulin resistance. (*J Clin Endocrinol Metab* **83:** 2907–2910, 1998)

IT HAS recently been demonstrated that: 1) tumor necrosis factor-α (TNFα) is constitutively expressed by adipose tissue; 2) genetically obese mice (ob/ob mice) and rats (fa/fa Zucker) have increased expression of TNFα in their adipose tissue (1, 2); and 3) TNFα may be a mediator of insulin resistance (1, 2) known to occur in these animals. TNFα interferes with insulin action, probably by inhibiting tyrosine kinase activity of the insulin receptor (3). Phosphorylation of the insulin receptor by this tyrosine kinase is known to be a cardinal step in the postreceptor events that follow the binding of insulin to its receptor (2). Though this effect of TNFα may induce a resistance to the action of insulin, such a reduction of insulin action may also limit adipocyte lipogenesis, stimulate lipolysis, and reduce the magnitude of further lipid accumulation (4). In this way, TNFα may be a local modulator of adipocyte's activity and its ability to accumulate fat. Administration of soluble TNFα receptor, which binds to TNFα and serves as an inhibitor of TNFα action to insulin-resistant obese animals, normalized their insulin sensitivity (1). Following the lead of these elegant experiments, Kern *et al.* (5) and Hotamisligil *et al.* (6, 7) have recently shown that human adipocytes and adipose tissue also constitutively express TNFα and that, unlike macrophages, the adipose tissue does not respond to endotoxin by increasing the expression/synthesis of TNFα (5, 6). Furthermore, they have also shown that, in adipocytes from obese subjects, the expression of TNFα message and protein falls markedly after weight loss (5, 6).

Because TNFα probably accomplishes its insulin inhibitory action by acting at various tissue sites of insulin action, it is possible that the obese have a higher-than-normal concentration of TNFα in plasma/serum. In this way, TNFα may act as a circulating hormone with supranormal secretion and serum concentrations in the obese. To test this hypothesis, we measured serum concentration of TNFα in a series of obese patients before and after weight loss.

Subjects and Methods

Thirty-eight obese female patients (age range: 25–54 yr; weight: 69.3–112 kg) were included in this study. All patients signed an informed consent. They were started on low-calorie diets (925–1150 kcal) and behavior modification for a period of 1–2 yr. Behavior modification included a program of aerobic exercise, starting with 12 min per day, increasing by 2 min every week, reaching a maximum of 40 min over 14 weeks.

Controls were 30 normal women (age range: 20–60 yr) with a mean body weight of 65 ± 5 kg and a body mass index (BMI) of 24 ± 3 kg/m^2. Fasting blood samples were obtained from the antecubital vein before and at the end of the treatment period. Blood samples were allowed to clot and were centrifuged. Serum was separated and frozen at −70 C until the time of the assay. Serum samples were assayed for lipids, insulin, and TNFα.

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All patients were challenged with 75 g glucose (dissolved in 300 mL water) administered orally. Blood samples were collected at 0, 30, 60, 90, and 120 min. Serum was separated and frozen at −70 C, as above. Total cholesterol, triglycerides, low-density-lipoprotein cholesterol, and high-density-lipoprotein cholesterol were measured by standard techniques. Insulin was assayed by RIA using a kit from Linco Laboratories (St. Louis, Mo.) TNFα was measured by a kit from R & D Systems (Minneapolis, MN). This enzyme-linked immunosorbent assay kit (Quantikine HS) has a sensitivity of 0.3 pg/mL TNFα in serum. This allows the measurement of TNFα in the normal range in human plasma/serum. The coefficient of variation for this kit is 7% at high concentrations and 10% at low concentrations.

Statistical analysis

Basal serum TNFα concentrations in the obese patients and normal subjects were compared by Student's *t* test for unpaired data, whereas the serum concentrations of TNFα and lipids before and after weight loss in the obese were compared by *t* test for paired data. Linear regression analysis was used to assess the degree of association between various indices.

Results

Basal (pretreatment) TNFα concentration was significantly greater in the obese (3.45 ± 0.16 pg/mL) than in a group of normal subjects (0.82 ± 0.25 pg/mL); normal range: 0.3–1.3 pg/mL (Fig. 1). TNFα concentrations were not related to body weight or BMI. TNFα concentrations were not correlated with fasting insulin concentrations. After the treatment period, the mean weight fell from 96.4 ± 13.8 kg to 84.5 ± 11.3 kg ($P < 0.001$) (Table 1), and the BMI fell from 35.7 ± 5.6 kg/m^2 to 31.3 ± 4.9 kg/m^2 ($P < 0.001$). The magnitude of weight loss was significantly related to basal weight (r = 0.57; $P < 0.001$). Fasting TNFα concentrations in the obese fell from 3.45 ± 0.16 to 2.63 ± 1.40 pg/mL ($P < 0.02$) (Fig. 2).

The magnitude of fall in TNFα was not related to basal body weight or to the degree of weight loss but was related to basal TNFα concentration (r = 0.55; $P < 0.001$). (Fig. 3)

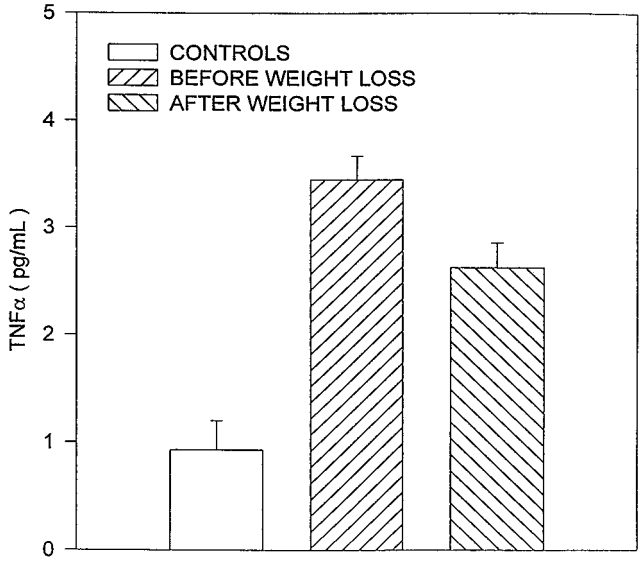

FIG. 1. Serum TNFα in the controls and in obese subjects, before and after weight loss. The TNFα in the obese was significantly greater than in the controls ($P < 0.001$). TNFα concentrations fell significantly after weight loss ($P < 0.02$). The data are presented as mean ± SD.

TABLE 1. Body weight and BMI before and after the period of dieting

	Before	After
Body weight (kg)	96.4 ± 13.8	84.5 ± 11.3[a]
BMI (kg/m^2)	35.7 ± 5.6	31.3 ± 4.9[a]

[a] $P < 0.01$.

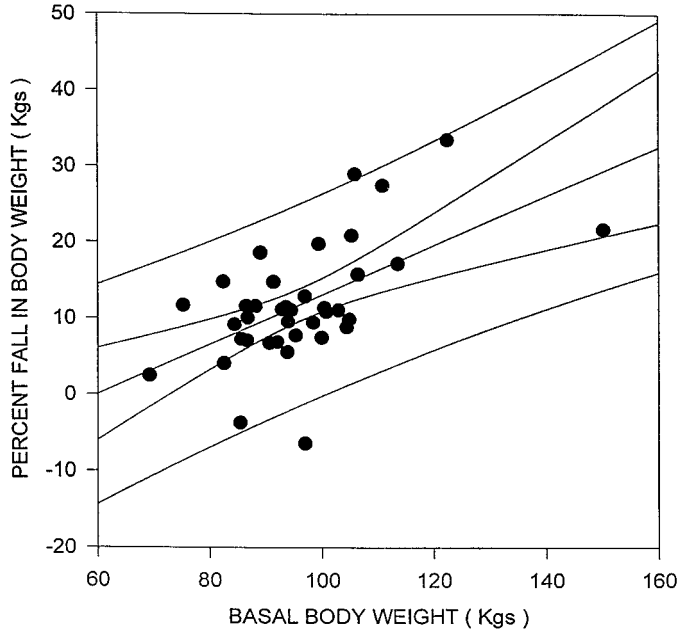

FIG. 2. Regression analysis of basal body weight *vs.* percent fall in body weight. The magnitude of the weight loss was significantly related to basal weight (r = 0.57, $P < 0.001$). The data are presented as the regression line, with 2 SD of the confidence interval.

The fall in serum TNFα concentrations after weight loss was not related to the fall in fasting insulin concentrations. Area under the curve (AUC) for insulin, after glucose challenge, fell significantly after weight loss (9355.59 ± 1407.07 *vs.* 6591.56 ± 1508.52; $P < 0.01$) (Fig. 4), but there was no correlation between the fall in TNFα and the decrease in AUC. The changes in lipid concentrations were as follows: total cholesterol fell from 5.7 ± 1.06 to 5.01 ± 0.01 mmol/L ($P < 0.01$); low-density-lipoprotein cholesterol fell from 3.53 ± 0.80 to 3.13 ± 0.61 mmol/L ($P < 0.01$). High-density-lipoprotein cholesterol increased from 1.43 ± 0.32 mmol/L to 1.48 ± 0.33 mmol/L (not significant). Serum triglycerides fell from 1.62 ± 0.90 to 1.13 ± 0.52 mmol/L ($P < 0.01$).

Discussion

Our data show clearly, for the first time, that serum TNFα concentrations in the obese are significantly greater than those in normal subjects and that after weight loss, TNFα concentrations fall significantly. In our patients, they fell by approximately 25%. The fall in serum TNFα concentrations after weight loss parallels the data on the diminution in the expression of TNFα in adipose tissue (40%), as demonstrated by Kern *et al.* (5) and Hotamisligil *et al.* (6, 7). It is possible that TNFα in serum reflects the level of expression of TNFα message and protein synthesis in adipose tissue.

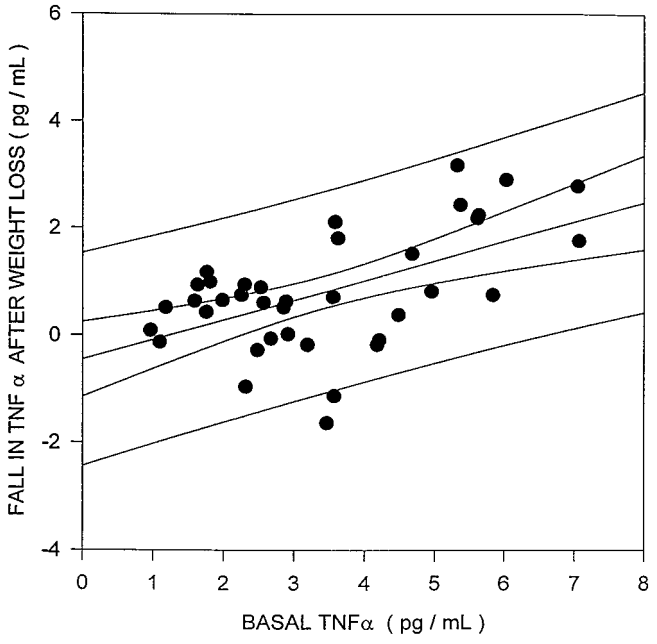

FIG. 3. Regression analysis of basal TNFα *vs.* fall in TNFα after weight loss. The magnitude of fall in TNFα was related to basal TNFα concentration ($r = 0.55$, $P < 0.001$) The data are presented as the regression line, with 2 SD of the confidence interval.

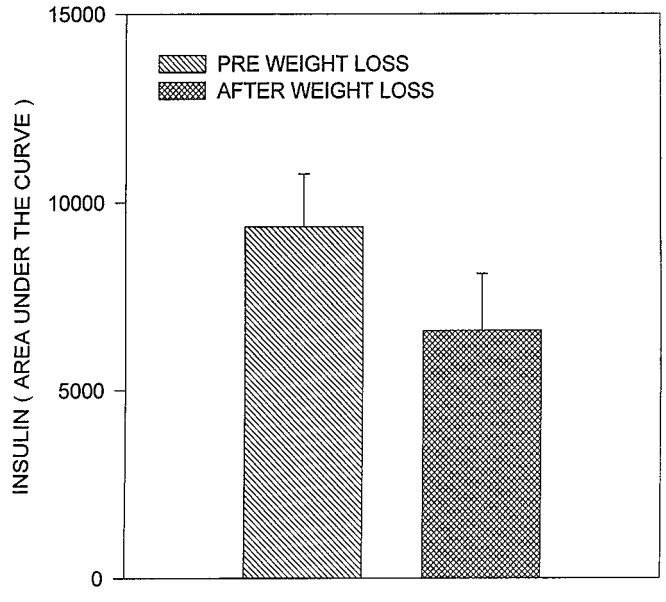

FIG. 4. AUC for insulin concentrations, before and after weight loss. AUC for insulin fell significantly after weight loss ($P < 0.001$). The data are presented as mean ± SD.

Although we have no evidence for the source of plasma TNFα in the obese, it is probable that the adipose tissue is the immediate source of TNFα in these patients, because there is an overexpression of the TNFα gene in the obese.

The fall in serum TNFα concentrations after weight loss may contribute to the restoration of insulin sensitivity in obese patients who lose weight (8). The concomitant fall in AUC for insulin after glucose challenge in these patients is consistent with concept. Because there is no direct correlation between the fall in serum TNFα and the AUC for insulin, there are probably other factors that also contribute to the fall in insulin resistance after weight loss.

Weight loss in the obese leads to a fall in metabolic rate (9, 10); although this may be accounted for by a parallel decrease in lean body mass, it is tantalizing to suggest that TNFα may be the modulator of metabolic rate that is elevated in the obese and falls with weight loss. Experimental data from animals, however, suggests that very high TNFα infusion rates and plasma concentrations are required for inducing weight loss and cachexia (11–13). These concentrations may be relevant to clinical cachexia of malignancy and chronic infection. However, smaller increases in TNFα may be sufficient to induce changes in metabolic rates observed in obesity and after weight loss.

It has been suggested that insulin resistance at the adipose tissue level may be a mechanism to prevent lipid deposition in this tissue and that it may be a way to limit obesity (lipostat function) (4). This effect may be achieved through a combination of inhibition of lipogenesis and stimulation of lipolysis: in both of these actions, TNFα may play a role through antagonism of insulin action. It has also been observed that, whereas some obese patients have remarkable resistance to weight loss, some lean subjects have an equally remarkable inability to gain weight (14, 15).

In view of previous experimental data reported by Hotamisligil *et al.* (1, 2) and data on obese patients from Kern *et al.* (4), allied to our data reported in this paper, we would suggest that: 1) TNFα is a peptide constitutively expressed and secreted by adipose tissue, which increases in the obese; 2) this increased expression of TNFα is reflected in supranormal TNFα concentrations in serum and may contribute to insulin resistance; 3) after weight loss, TNFα expression and secretion fall, in association with a fall in serum TNFα concentration and a decrease in insulin secretion after glucose challenge, reflecting a fall in insulin resistance. Because TNFα, constitutively secreted by adipose tissue, may arrive through circulation at distal sites (like the skeletal muscle, the liver, and the heart) to induce an effect antagonistic to insulin, through the inhibition of the insulin receptor tyrosine kinase, TNFα may well be qualified to be termed a hormone, whose constitutive source, the adipose organ, may thus be termed an endocrine organ.

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