# Differential Regulation of the p80 Tumor Necrosis Factor Receptor in Human Obesity and Insulin Resistance

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**Previous studies have shown that tumor necrosis factor (TNF)-α production from adipose tissue is elevated in rodent and human obesity and plays an important role in insulin resistance in experimental animal models. In this study, we examined the adipose expression of both TNF receptors (TNFR1 and TNFR2) in human obesity and demonstrated that obese female subjects express approximately twofold more TNFR2 mRNA in fat tissue and approximately sixfold more soluble TNFR2 in circulation relative to lean control subjects. In contrast, TNFR1 expression and protein levels were similar in these subjects. TNFR2 expression levels in adipose tissue were strongly correlated with BMI ($r = 0.65$, $P < 0.001$) and level of hyperinsulinemia ($P < 0.001$), an indirect measure of insulin resistance, as well as level of TNF-α mRNA expression in fat tissue ($r = 0.56$, $P < 0.001$). These results suggest that TNFR2 might play a role in human obesity by modulating the actions of TNF-α. *Diabetes* 46:451–455, 1997**

Tumor necrosis factor (TNF)-α is a potent cytokine known primarily for its role in host defense (1,2). TNF-α also has important effects on whole-body lipid and glucose metabolism (1,3). It has previously been demonstrated that fat tissue is a significant source of endogenous TNF-α production, and the expression of this cytokine in adipose tissue is elevated in most of the models of rodent obesity examined to date (4–7). This abnormal expression of TNF-α in adipose tissue plays a critical role as a mediator of peripheral insulin resistance in rodent obesity, since neutralization of TNF-α results in significant increases in peripheral insulin sensitivity (4,6,8). Recently, elevated TNF-α production in fat and muscle tissues has also been demonstrated in humans (9–11). The level of TNF-α expression in fat and muscle tissues was found to best correlate with body mass index (BMI), the level of hyperinsulinemia, and decreased LPL activity, indicating that the abnormal production of this cytokine may play an important role in metabolic disturbances that accompany this disorder (9–11).

TNF-α actions in obesity appear to occur via an autocrine-paracrine mechanism involving adipose and muscle tissues (8–12). However, neither the mechanisms that control this restricted activity nor the receptor systems that are involved in TNF-α signaling in obesity are clearly understood. TNF signals through at least two known cell surface receptors: TNFR1 (p60) and TNFR2 (p80) (13,14). These receptors are present in virtually all cells of higher mammals, including adipocytes, although their relative quantities vary among different cell types. The two receptors are structurally very different, and the exact functional role of each receptor is not clear (13,14). It appears that TNFR1 can signal for virtually all known activities of TNF, including apoptosis, differentiation, and proliferation, while the actions mediated by TNFR2 are very few and are limited to certain cell types, such as thymocytes and T-cells, where it promotes proliferation (13–17). No information is currently available concerning the effects of the two TNF receptors (TNFRs) in energy metabolism and glucose homeostasis. We have previously shown that the expression of TNFR2 was elevated in fat tissues of obese mice along with increased TNF-α (5). However, the expression of TNFRs in the adipose tissue of obese humans has not been examined to date. Here, we demonstrate that both TNFRs are present in human adipose tissue. Furthermore, the expression of TNFR2 in adipose tissue and the concentration of the soluble form of TNFR2 in circulation are significantly elevated in obese female subjects compared with lean control subjects.

## RESEARCH DESIGN AND METHODS

**Patients and control subjects.** We studied 37 premenopausal women (18 lean and 19 obese; recruited at the University of Eastern Virginia Medical School, Norfolk, Virginia, and Huddinge Hospital, Department of Medicine, Huddinge, Sweden) between the ages of 25 and 45. The characteristics of the subjects are summarized in Table 1. Inclusion criteria for subjects included: *1*) BMI (weight in kilograms divided by the square of height in meters) >30 kg/m$^2$ for obese and <25 kg/m$^2$ for lean subjects, *2*) mixed Caucasian ethnic background, *3*) absence of any systemic disease, *4*) absence of any infections, *5*) absence of current medication affecting glucose homeostasis or sympathetic nervous system, *6*) absence of weight change of >10% in the last 3 months. All subjects had a medical history and physical examination before participating in the study. For baseline studies, venous blood samples were collected after a 10-h fast; plasma was separated immediately, and aliquots were kept refrigerated at 4°C or frozen at –80°C. Subcutaneous fat tissues were obtained from the abdominal region by a fat biopsy performed under local anesthesia and were immediately frozen in liquid nitrogen for future mRNA analysis. These studies were approved by the institutional review boards, and informed consent was obtained from each subject.

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ELISA, enzyme-linked immunosorbent assay; IL, interleukin; sTNFR, soluble tumor necrosis factor receptor; TNF, tumor necrosis factor; TNFR, tumor necrosis factor receptor.

TABLE 1
Characteristics of the study group

| | Lean | Obese | *P* value |
|---|---|---|---|
| *n* | 18 | 19 | |
| Age (years) | 34.4 ± 1.2 (26–45) | 34.4 ± 1.5 (25–45) | NS |
| BMI (kg/m$^2$) | 21.38 ± 0.3 (19.6–24.1) | 39.9 ± 1.4 (31.6–53.8) | 0.0001 |
| WHR (m/m) | 0.85 ± 0.02 (0.77–0.98) | 0.90 ± 0.01 (0.73–0.96) | 0.04 |
| Insulin (μU/ml) | 13.06 ± 1.2 (5.9–25.8) | 46.4 ± 3.9 (13.3–83.1) | 0.0001 |
| Glucose (mg/dl) | 83.1 ± 1.3 (72.9–91.8) | 95.9 ± 3.1 (73.8–131.5) | 0.006 |
| Triglyceride (mg/dl) | 83.1 ± 7.3 (24.0–140.0) | 136.2 ± 14.8 (58.0–248.0) | 0.002 |
| Cholesterol (mg/dl) | 158.1 ± 8.6 (88.0–210.0) | 181.8 ± 6.9 (142.0–234.0) | 0.04 |

Data are means ± SE (range). To convert values for insulin to picomoles per liter multiply by 7.175; to convert values for glucose to millimoles per liter multiply by 0.05551; to convert values for triglycerides to millimoles per liter multiply by 0.01129; to convert values for cholesterol to millimoles per liter multiply by 0.02586. WHR, waist-to-hip ratio. *P* values are by Student's *t* test.

**Northern blot analysis and protein measurements.** Total RNA was extracted from fat tissue samples by a Cs chloride extraction protocol (18). Total RNA (20 μg) was denatured in formamide and formaldehyde at 55°C for 15 min and separated by electrophoresis in formaldehyde-containing agarose gels, as described previously (4). RNA was blotted onto Biotrans membranes, ultraviolet-crosslinked (Stratagene), and baked for 0.5 h. Hybridization and washes were performed as directed by the manufacturer. Cloning of the complementary DNA for aP2 (also known as adipocyte fatty acid binding protein [A-FABP]) has been reported previously (19). Complementary DNA probes for human TNFR1 and TNFR2 are cloned in our laboratory based on the published sequences (20–22). Type 1 and type 2 interleukin (IL)-1 receptor cDNA probes were gifts from Immunex (Seattle, WA). DNA probes were radioactively labeled to specific activities of at least $10^9$ dpm/μg with [$^{32}$P]α-dCTP (6,000 Ci/mmol) by the random priming method, as described previously (4). Quantitation of Northern blots was performed using a PhosphorImager. Differences in loading were adjusted to β-actin expression, and the amounts of TNFR1, TNFR2, and aP2 mRNA were expressed as percentages of an internal control used as a standard in all experiments. Protein concentrations of both soluble TNFRs were measured by enzyme-linked immunosorbent assay (ELISA) using a monoclonal mouse anti-human p60 and p80 TNFR antibody (Biosource International) in plasma obtained from subjects. Standard curves were simultaneously generated using human soluble TNFR (p60 and p80, Biosource International). The assays were run in duplicate, and the samples that differed by >20% of the mean were repeated. The specificity of the assay was confirmed by spiking predetermined concentrations of human soluble TNFRs into normal human plasma, which resulted in >90% recovery. Neither cross-reaction between the two types of the receptor nor interference of TNF has been observed for these ELISA assays.

**Statistical analysis.** Data are expressed as means ± SE. Individual variables were compared by using two-tailed Student's *t* test analysis. The relationship between individual variables was determined by linear correlation analysis. StatView 512$^+$ (Abacus Concepts, Berkeley, CA) statistical software was used in all analyses. All *P* values reported are two-sided.

## RESULTS

**Patients and control subjects.** Table 1 summarizes the characteristics of the 37 premenopausal subjects (18 lean and 19 obese) at the time of entry into the study. All subjects were of mixed Caucasian ethnic origin, and lean and obese subjects were similar in age (34.4 ± 1.2 and 34.4 ± 1.5, respectively). None of the subjects were severely hypertriglyceridemic based on fasting plasma measurements. Although the obese group had higher average fasting plasma glucose levels compared with those of control subjects, all values were within the normoglycemic range except one, which was borderline (131.5 mg/dl [7.5 mmol/l]). The obese subjects were significantly hyperinsulinemic compared with the lean control subjects, indicating the presence of insulin resistance. However, further analysis of insulin resistance and glucose tolerance have not been performed in the study group.

**Expression of TNFRs in adipose tissue.** We first examined the expression of the TNFR1 and TNFR2 genes in subcutaneous adipose tissue of lean and obese subjects to obtain insights into their roles in human obesity. Northern blot analysis demonstrated that both receptors are expressed in human adipose tissue (Fig. 1*A*). The level of TNFR2 mRNA expression was strikingly elevated in adipose tissue obtained from obese individuals compared with the level in age-matched lean control subjects (Figs. 1*A* and *B*). When normalized to the β-actin mRNA present in each adipose tissue sample, an approximately twofold increase was observed in the amount of TNFR2 mRNA in the obese group (41.5 ± 2.5 vs. 79.3 ± 6.5%, $P < 0.001$). The level of TNFR1 mRNA present in the fat tissues of lean and obese individuals was similar (59.2 ± 9.1 vs. 58.6 ± 8.7%). To further test the specificity of this pattern of TNFR mRNA expression, we examined several other cytokine receptors and fat-specific genes. Among those examined, IL-1 receptor type 1 and type 2 mRNA and IL-6 receptor mRNA were not detected in any of the subjects (data not shown). Moreover, the expression of a fat-specific gene, aP2, was not different between lean and obese subjects (Fig. 1). These results suggested that elevated mRNA expression was specific for TNFR2 and was not the result of a generalized aberrant expression of the cytokine receptor family or fat-specific genes. These controls also demonstrate that there was no systemic bias in quantitation when comparing mRNA from fat tissue of lean versus obese subjects.

**TNFR expression and metabolic status.** We next analyzed the relationship between the levels of TNFR mRNA in fat tissue and various metabolic parameters measured in lean and obese subjects. The results are presented only for TNFR2 because no correlation was observed between any of the variables and TNFR1 expression in the group studied. Very strong positive correlations were observed between TNFR2 mRNA expression in fat tissue and BMI (Fig. 2*A*; $r = 0.65$, $P < 0.001$) and fasting plasma insulin level (Fig. 2*C*; $r = 0.71$, $P < 0.001$). As expected, there was also a positive correlation between BMI and fasting insulin level ($r = 0.77$, $P < 0.001$). Weaker positive correlations were observed between TNFR2 expression and waist-to-hip ratio (Fig. 2*B*; $r = 0.47$, $P = 0.03$) and fasting plasma glucose level ($r = 0.34$, $P = 0.04$). The significance of these correlations is not clear, especially because all plasma glucose values were within normoglycemic range in the study group. Note also that there is a trend toward

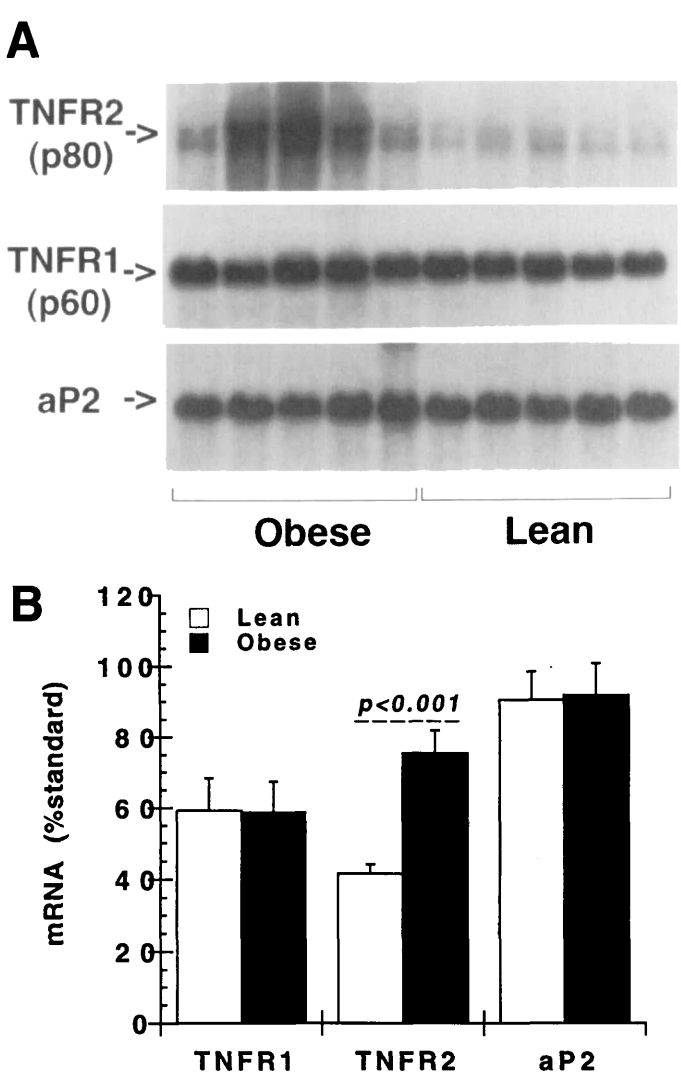


FIG. 1. Expression of TNF receptor mRNA in adipose tissue from lean and obese human subjects. *A*: a representative Northern blot showing the elevated TNFR2 mRNA expression in adipose tissues of five lean and five obese subjects. TNFR1 and aP2 mRNA expression in the same Northern blot is shown for comparison. *B*: quantitation of TNFR mRNA expression in 18 lean and 19 obese female subjects. β-actin expression was used as a standard to correct the loading differences, and mRNA is expressed as percentage of standard. Similar quantitation of aP2 is shown for comparison.

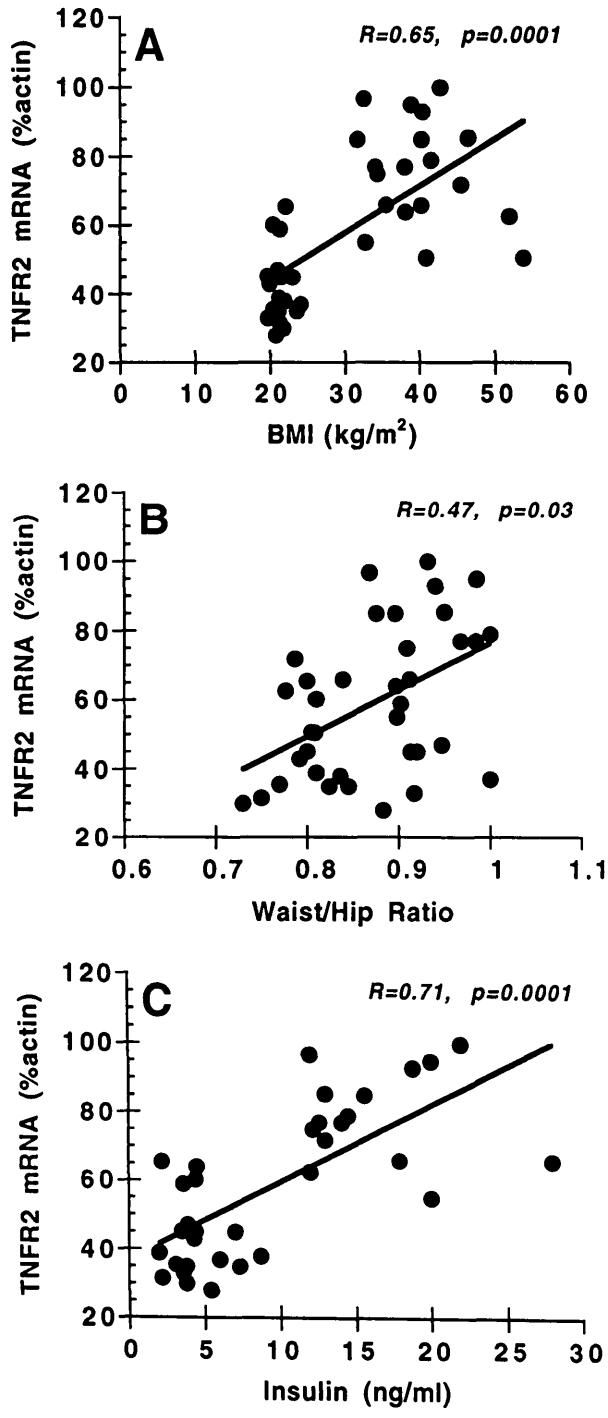


FIG. 2. Correlation of TNFR2 mRNA expression levels in adipose tissue and metabolic parameters. The TNFR2 expression levels were determined by Northern blot analysis, quantitated as described in Fig. 1, and plotted against BMI (*A*), WHR (*B*), and plasma insulin (*C*). The lines represent simple linear regression between the two variables.

bimodality in BMI values as a result of the selection criteria used to differentiate lean and obese subjects. No statistically significant correlation was present between TNFR2 expression in adipose tissue and age, plasma triglycerides, or total cholesterol level (data not shown). These latter parameters were only marginally different between the lean and obese subjects in the study group. In addition to these metabolic measures, a strong positive correlation was observed between TNFR2 and TNF-α mRNA expression levels in fat tissue ($r = 0.56, P < 0.001$).

**TNFR protein.** Both TNFR1 and TNFR2 are shed into the circulation and exist in soluble forms (13,14). Present at low levels under normal conditions, these soluble forms are believed to protect the organism from the detrimental effects of TNF-α on distant targets. We therefore measured circulating TNFR1 and TNFR2 protein concentrations in lean and obese subjects. Both soluble receptors (sTNFR1 and sTNFR2) were detectable in the plasma of lean and obese subjects. The sTNFR1 levels in serum were indistinguishable between the lean and obese subjects (Fig. 3). In sharp contrast, sTNFR2 levels were dramatically elevated in obese subjects (6.3-fold) compared with the lean controls (Fig. 3; 1.98 ± 0.6 vs. 12.85 ± 3.9, $P = 0.006$). These results indicated that there was a specific induction of TNFR2 (p80) protein levels in fat tissue and of the soluble form in circulation.

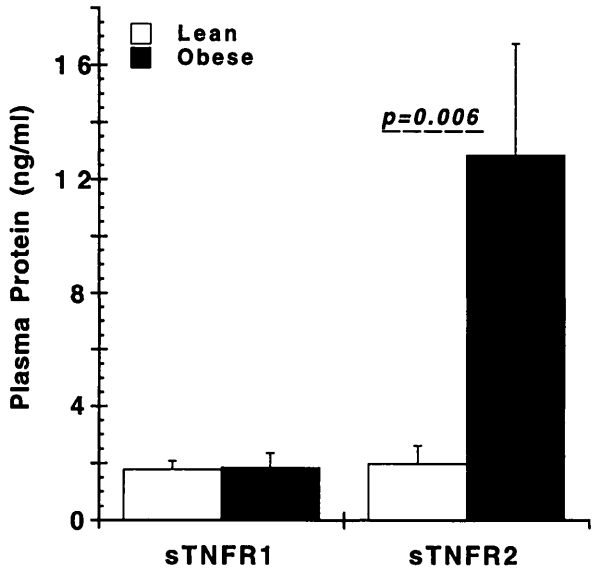


**FIG. 3. Plasma soluble TNFR protein levels in lean and obese human subjects. Plasma concentrations of sTNFR1 and sTNFR2 proteins are determined by using an ELISA assay (Biosource International).**

## DISCUSSION

Recent studies have shown that TNF-α is an important mediator of insulin resistance in experimental models of obesity (4,6,8). Overexpression of TNF-α in adipose tissue is a common correlate of obesity and insulin resistance in both obese animals and obese and euglycemic humans (4,5,7,9,10). In addition, weight loss caused by dietary treatment of obesity in humans results in a significant decrease in the amount of TNF-α expression in adipose tissue (9,10). An initial study attempting to examine the role of TNF-α elicited no detectable change in insulin sensitivity in patients with established NIDDM after a single-dose administration of a monoclonal anti-TNF antibody (23). However, these patients were not characterized with respect to either expression of TNF-α or efficiency of TNF-α neutralization. Hence, further studies and alternative regimens will be necessary to definitively determine the role of TNF-α in the insulin resistance of humans.

Studies in both cultured cells and whole animals have demonstrated that TNF-α interferes with insulin action by inhibiting insulin receptor signaling (8,12,24,25). TNF-α induces serine phosphorylation of IRS-1 (insulin receptor substrate 1) (26,27) and converts it into an inhibitor of insulin receptor tyrosine kinase activity in cultured cells and in obese animals (26). However, the proximal signaling pathways activated by TNF-α to inhibit insulin action are not known. In cultured cells, studies with agonistic antibodies have shown that both TNFR1 and TNFR2 can mediate TNF-α–induced insulin resistance, although the former appears to be more powerful (28). In this study, we have demonstrated that both the fat tissue expression levels of TNFR2 and the circulating soluble TNFR2 molecules were elevated in obesity and that the TNFR2 levels in adipose tissue were in strong correlation with BMI and fasting plasma insulin levels. Since levels of circulating insulin are an indicator of insulin resistance in obese euglycemic patients, these results suggest the involvement of TNFR2 in the insulin resistance of obesity. Although in earlier studies TNFR1 appeared to have greater biological activity in cultured cells, the relative potency of each receptor, as well as the mechanism(s) of action in regard to in vivo insulin resistance, has not been determined. A potential effect mediated by TNFR2 alone or together with TNFR1 might reflect the species-specific properties of these receptors in regard to their metabolic functions. Further experiments will be necessary to address these issues.

The biological functions mediated through the soluble form of the TNF receptor are not well understood. Since this protein has high affinity for TNF, its presence in circulation at high concentrations might alter the actions of TNF-α in humans. The dramatic increase in the systemic levels of sTNFR2 in obese individuals might serve to limit the actions of TNF-α to tissues in which it is produced and prevent the endocrine functions of this cytokine. Previous studies in both animal models and humans have also indicated that TNF-α action in obesity is unlikely to be through an endocrine mode of action (4,6,9,10). It is also possible that elevations in this form of the TNFR modulate TNF-α bioavailability and/or action in obesity. In fact, it has been reported that on binding, the soluble receptors can either inhibit or enhance the activity of TNF-α (29). The final biological outcome of the interactions between the soluble receptors and TNF-α is probably determined by the microenvironment and the type of receptor involved. For example, in animal models of sepsis, sTNFR1-based but not sTNFR2-based receptor-immunoglobulin fusion proteins have been effective in preventing lethality (30). The beneficial effects observed on administration of a sTNFR1–immunoglobulin G fusion protein to *fa/fa* rats might also be the result of the biological differences between the two forms of the soluble TNFRs. All of these factors should also be taken into account during the design of clinical trials aiming to interfere with TNF action. Further studies will be necessary to understand the exact role of TNF-α and its receptors in energy metabolism.

The strong positive correlation observed between TNFR2 and TNF-α mRNA expression levels in fat tissue ($r = 0.56$, $P < 0.001$) may suggest that both TNF-α and TNFR2 might be regulated by the same obesity-related signal. Alternatively, TNF-α itself might be the signal for elevated TNFR2 expression in obesity. In fact, TNF-α is a strong inducer of TNFR2 expression in cultured adipocytes (G.S.H., B.M.S., unpublished observations) and in other cell types in vitro (31). Accordingly, this raises the possibility that soluble TNFR might serve as a diagnostic marker for obese individuals in whom there is TNF-related insulin resistance. TNF-α measurements in plasma have been extremely difficult and uninformative in obesity, similar to many other pathophysiological conditions involving TNF-α. This may be due to both low levels of this protein and the possible interference of binding proteins (32). On the other hand, the sTNFR2 is significantly elevated in obese individuals; it is a very stable protein and quite easy to measure. Existence of such a marker should enhance the ability to quickly distinguish these patients for both diagnostic and therapeutic purposes.

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