Circulating Tumor Necrosis Factor Alpha Concentrations Are Higher in Abdominal Versus Peripheral Obesity

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Fat tissue is a significant source of endogenous tumor necrosis factor alpha (TNFα), the pluripotent cytokine that plays an important role as a mediator of the peripheral insulin resistance found in obesity. The majority of evidence for this role of TNFα is from studies in animal models of obesity. To explore further the role of TNFα in the pathogenesis of obesity-related insulin resistance in humans, we compared plasma levels of TNFα and the other main endocrine cytokine, interleukin-6 ([IL-6] both measured by enzyme-linked immunosorbent assay), in 26 obese women (body mass index [BMI] > 30 kg/m^2) and 13 female controls (BMI < 26 kg/m^2) without a history of recent or active infection. Glucose and insulin levels were measured at 0, 1, and 2 hours after a 75-g oral glucose load. There was no significant difference in plasma TNFα or IL-6 levels between obese and non-obese subjects overall (2.10 ± 0.19 *v* 1.65 ± 0.18 pg/mL and 2.06 ± 0.29 *v* 1.50 ± 0.17 pg/mL, respectively). However, TNFα levels were significantly elevated in obese subjects with a 2-hour glucose level more than 140 mg/dL (n = 8) compared with the other obese subjects (n = 18) and the non-obese controls (2.88 ± 0.46 *v* 1.75 ± 0.10 and 1.65 ± 0.18 pg/mL, respectively, $P < .01$). Furthermore, the TNFα level correlated significantly with the waist to hip ratio ([WHR] $r = .53$, $P < .01$) and fasting and post–oral glucose tolerance test (OGTT) insulin levels ($r = .47$, $P < .02$), but not with the BMI, and was higher in obese women with a WHR more than 0.90 (n = 14) in comparison to those with a WHR less than 0.90 (n = 12, 2.47 ± 0.29 *v* 1.66 ± 0.18 pg/mL, respectively, $P < .03$). The corresponding plasma leptin level was significantly higher in obese women versus the control group (41.6 ± 2.5 *v* 22.3 ± 2.9 ng/mL, $P < .001$) and was related to the BMI ($r = .60$, $P < .01$) but not to TNFα or the WHR. There were no significant differences in the corresponding IL-6 concentration between groups, and IL-6 did not correlate with TNFα, leptin, BMI, WHR, or insulin levels. In conclusion, circulating TNFα levels are higher in abdominal obesity compared with peripheral obesity, and may contribute to the insulin resistance that more commonly complicates the former pattern of fat distribution.

TUMOR NECROSIS FACTOR ALPHA (TNFα) is a pluripotent cytokine known primarily for its role in the inflammatory response.[1] TNFα also has important effects on lipid and glucose metabolism.[2] It has been shown that fat tissue is a significant source of endogenous TNFα production.[3] In fact, TNFα expression is elevated in the adipose tissue of most rodent models of obesity and appears to play a critical role as a mediator of the peripheral insulin resistance that characterizes these animals.[3,4] Recently, it has been demonstrated that TNFα expression is also elevated in human fat and muscle tissue and that it best correlates with the body mass index (BMI) and the degree of hyperinsulinemia.[5,6] In addition, weight loss caused by dietary treatment in obese subjects results in a significant decrease of TNFα expression in adipose tissue with a parallel improvement of insulin resistance.[7] This suggests that abnormal production of TNFα may also play an important role in obesity-linked insulin resistance in man. TNFα interferes with insulin action, probably by inhibiting insulin receptor signaling.[8,9] Thus, TNFα induces serine phosphorylation of insulin receptor substrate-1[10] and converts it to an inhibitor of insulin receptor tyrosine kinase activity. However, the proximal signaling pathways activated by TNFα to inhibit insulin action are largely unknown.

TNFα actions in obesity appear to occur primarily via autocrine and paracrine mechanisms involving adipose and muscle tissue, the principal targets of insulin action.[4] It is possible that TNFα may also reach targets of insulin action via the circulation, thus having an additional endocrine contribution to the pathogenesis of obesity-related insulin resistance. To explore this possibility, we examined whether TNFα secretion in the circulation is elevated in human obesity and whether it relates to markers of insulin resistance. We also examined whether obesity is associated with elevations in the circulatory level of the other main endocrine cytokine, interleukin-6 (IL-6). Recently, it was shown that IL-6, like TNFα, is also produced and released by human adipose tissue, albeit in smaller amounts that roughly correspond to 10% of total-body tissue release.[11] IL-6 increases readily in response to inflammatory and noninflammatory stressors such as trauma or exercise,[12,13] and has important endocrine and metabolic effects that may influence glucose homeostasis.[14,15] By measuring circulating IL-6 levels, we wished to control for nonadipose sources of TNFα (mainly inflammatory) and for the possibility that IL-6, independently of TNFα, also may contribute to the metabolic abnormalities found in human obesity.

SUBJECTS AND METHODS

We studied 26 obese women with a BMI more than 30 (calculated as the weight in kilograms divided by the square of the height in meters) and 13 age-matched non-obese controls with a BMI less than 26 without a history of recent or active infection or inflammatory disease. None of the subjects were on medications that affect glucose homeostasis or sympathetic nervous system activity. All subjects underwent a 75-g oral glucose tolerance test (OGTT) with measurement of plasma glucose and insulin levels at 0, 1, and 2 hours after the glucose load. A blood

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glucose level more than 140 mg/dL at 2 hours after the OGTT was used as the standard for impaired glucose tolerance. Venous blood samples were collected after a 10-hour fast, the plasma was separated immediately, and aliquots were frozen at −40°C until needed for assays. Basal plasma levels of TNFα and IL-6 were measured in duplicate in a single assay by high-sensitivity enzyme-linked immunosorbent assay kits (Quantikine; R & D Systems, Minneapolis, MN). The assay sensitivity was 0.3 pg/mL for TNFα and 0.2 pg/mL for IL-6. The intraassay coefficient of variation (CV) was less than 5% for both cytokine assays. Serum leptin was determined by a specific commercial radioimmunoassay (RIA) kit (Linco Research, St. Charles, MO). The assay sensitivity was 1.0 ng/mL and the intraassay CV was less than 5%. Serum insulin was determined by RIA and glucose by an in-house assay (glucose oxidase method).

Data are expressed as the mean ± SE. Comparisons between groups were made by the Mann-Whitney test, and the relationship between individual variables was tested by Spearman's rank correlation analysis.

RESULTS

Clinical characteristics of the obese and control patients are summarized in Table 1. There were no overall significant differences between obese and control subjects for either plasma TNFα (2.10 ± 0.19 *v* 1.65 ± 0.18 pg/mL) or plasma IL-6 (2.06 ± 0.29 *v* 1.50 ± 0.17 pg/mL), although the levels of both cytokines tended to be higher in obese women.

However, after grouping obese patients on the basis of the OGTT response (2-hour post-OGTT glucose >140 mg/dL, n = 8; 2-hour post-OGTT glucose <140 mg/dL, n = 18), TNFα levels were significantly higher ($P < .01$) in the former (2.88 ± 0.46 pg/mL) compared with the latter (1.75 ± 0.10 pg/mL) and with non-obese controls (1.65 ± 0.18 pg/mL). The corresponding IL-6 levels showed no significant differences between the three groups (Fig 1).

Similarly, when obese patients were grouped on the basis of the WHR as subjects with abdominal fat distribution (WHR >0.90, n = 14) and subjects with peripheral fat distribution (WHR <0.90, n = 12), plasma TNFα levels were significantly higher in the former group (2.47 ± 0.29 pg/mL) compared with the latter (1.66 ± 0.13 pg/mL, $P < .03$). In contrast, IL-6 levels remained comparable between the groups (1.90 ± 0.21 *v* 2.25 ± 0.44 pg/mL) (Fig 2).

Consistent with these data, the TNFα levels correlated

Table 1. Clinical Characteristics of the Obese and Control (lean) Subjects

Characteristic	Lean (n = 13)	Obese		
		Total Group (n = 26)	IGT (n = 8)	NGT (n = 18)
Age (yr)	46.5 ± 3.1	42.1 ± 2.6	49.1 ± 4.6	39.5 ± 2.9
BMI (kg/m²)	24.4 ± 1.6	39.5 ± 1.3§	39.3 ± 1.5§	39.9 ± 1.9§
WHR	0.85 ± 0.03	0.90 ± 0.02‡	0.99 ± 0.01†	0.86 ± 0.02
Glucose (mg/dL)				
Basal	93 ± 3	91 ± 4	107 ± 8	83 ± 3
2 h post-OGTT	119 ± 4	135 ± 9‡	177 ± 12†	110 ± 4
Insulin (mIU/mL)				
Basal	19 ± 4	28 ± 4‡	31 ± 3*	26 ± 4
2 h post-OGTT	109 ± 26	139 ± 22	199 ± 30*	108 ± 25

Abbreviations: IGT, impaired glucose tolerance; NGT, normal glucose tolerance.

*$P < .05$, †$P < .01$ *v* lean and obese NGT.

‡$P < .05$, §$P < .01$ *v* lean.

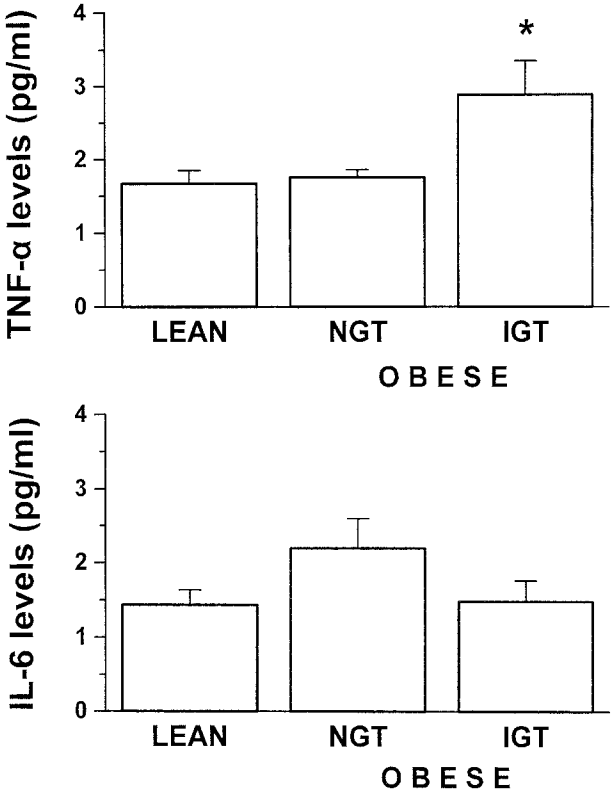

Fig 1. Plasma TNFα levels were significantly higher ($P < .01$) in obese women with impaired glucose tolerance (IGT) compared with obese women with normal glucose tolerance (NGT) and lean controls. The corresponding plasma IL-6 levels were not significantly different between groups.

significantly with the WHR ($r = .53$, $P < .01$) and with fasting, post-OGTT, and integrated (area under the curve [AUC]) serum insulin levels ($r = .47$, $P < .02$), as well as the ratio for the AUC of insulin to the AUC of glucose ($P < 0.05$), in obese patients (Fig 3). In contrast, TNFα did not correlate with the BMI ($r = .30$, $P = .12$) or with leptin levels in these patients, the latter being clearly elevated compared with the value in the lean controls (41.6 ± 2.5 *v* 22.3 ± 2.9 ng/mL, $P < .001$) directly proportionately to the BMI ($r = .60$, $P < .01$).

There were no differences in TNFα or leptin levels between premenopausal (n = 18) and postmenopausal (n = 8) obese women (2.01 ± 0.23 pg/mL and 40.8 ± 3.6 ng/mL *v* 2.07 ± 0.20 pg/mL and 42.4 ± 5.3 ng/mL, respectively).

DISCUSSION

This study shows that circulating TNFα levels are clearly elevated in obese women with abdominal obesity, most of whom also have some degree of insulin resistance and/or glucose intolerance. In contrast, TNFα levels are not elevated in women with peripheral obesity. This differential relationship between TNFα and the two patterns of fat distribution is also reflected in the significant positive relationship of TNFα to the WHR, a good, albeit indirect, marker of visceral fat mass, but not to the BMI, a marker of total adiposity that cannot account for fat distribution.

The circulating levels of TNFα found in the present study

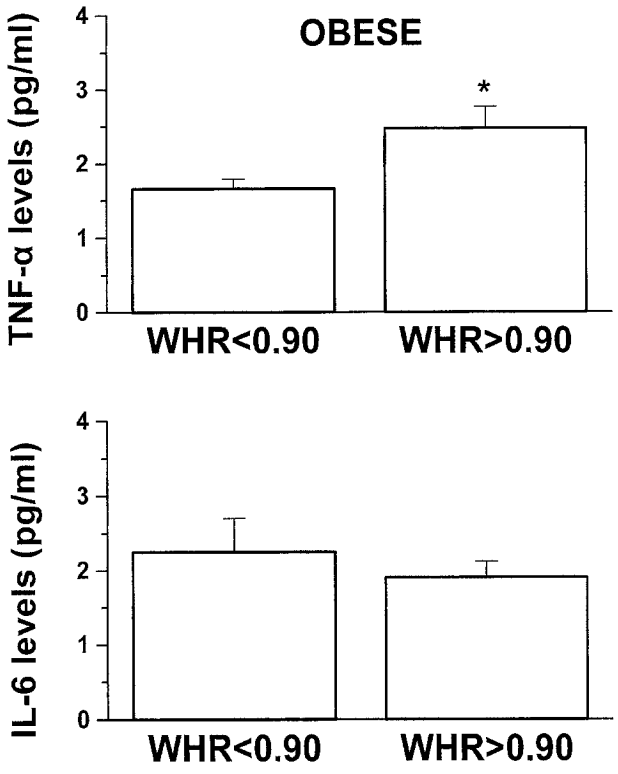

Fig 2. TNFα, but not IL-6, was higher in obese women with a WHR >0.90 versus a WHR < 0.90.

were relatively low, and it could be argued that they may not have significant biological activity on insulin target tissues. However, it is possible that circulating TNFα acts synergistically with locally produced TNFα in fat and skeletal muscle. The bioavailability and/or action of circulating TNFα in obesity might further depend on the circulating amount of soluble TNFα receptors, which can either inhibit or enhance the activity of TNFα.[16,17] Moreover, it is believed that in the absence of an inflammatory process, circulating TNFα levels reflect TNFα message expression and protein synthesis in adipose tissue and its constitutive secretion.[4]

The potential contribution of elevations in circulating TNFα to the development or deterioration of insulin resistance and glucose intolerance is supported by the fact that TNFα levels correlated significantly with both the 2-hour post-OGTT glucose level and the fasting, post-OGTT, and AUC insulin levels in our obese subjects. Such a role for circulating TNFα is also suggested by the reported significant decrease of TNFα in obese type 2 diabetic patients after weight loss with dietary treatment and exercise in parallel with an improvement in insulin sensitivity (decrease in insulin).[18] Interestingly, in the same study, consistent with our findings, basal TNFα levels correlated with the visceral fat area and not with the subcutaneous fat area (both measured by computed tomography) or BMI.[18] In another study, serum TNFα bioactivity was increased in obese and type 2 diabetic patients and correlated positively with basal serum C-peptide levels.[19] Also, Dandona et al[20] have shown that serum TNFα decreases with weight loss in obese patients.

In addition to the direct inhibitory effect of TNFα on insulin signaling, there is evidence that elevations of circulating TNFα may also contribute to the increase in fibrinogen and plasminogen activator inhibitor-1 production and to the lipid abnormalities (high total cholesterol and triglycerides and low high-density lipoprotein cholesterol) that, together with hyperinsulinemia, are important components of the metabolic syndrome.[21,22]

It is of note that TNFα did not correlate with serum leptin, despite in vitro and in vivo evidence that TNFα may contribute to obesity-related hyperleptinemia by stimulating leptin release from adipocytes.[23,24] This suggests that the regulation of leptin production is largely under the control of different factors.

Unlike TNFα, circulating IL-6 did not differ between obese and lean individuals and did not relate to fat distribution or to indices of insulin resistance. This suggests that in the absence of infection or inflammation, circulating IL-6 does not have an appreciable contribution to obesity-related insulin resistance, and TNFα and IL-6 expression may be subject to different regulatory mechanisms. Further, the lack of correlation between IL-6 and the presence or degree of obesity in our patients suggests that the recently reported increases in serum IL-6 in obese sleep-apneic patients[25] are most likely a correlate of the sleep apnea and related hypoxia rather than obesity per se.

In conclusion, circulating TNFα, but not IL-6, is higher in abdominal compared with peripheral obesity and may contribute to the insulin resistance that more commonly complicates the former pattern of fat distribution.

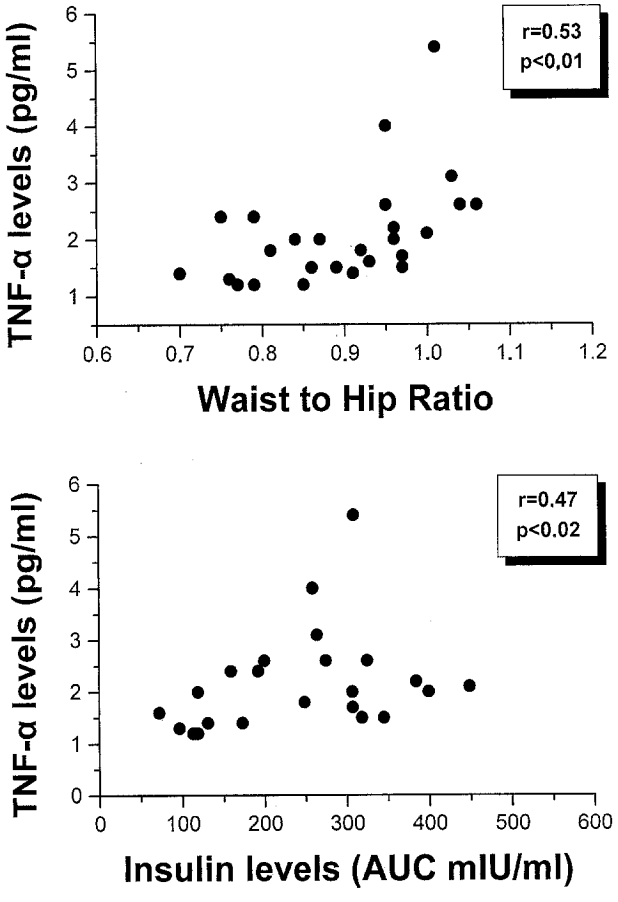

Fig 3. Plasma TNFα correlated significantly with the WHR and the integrated insulin level (AUC) in obese patients.

REFERENCES

1. Akira S, Hirano T, Taga T, et al: Biology of multifunctional cytokines: IL-6 and related molecules (IL-1 and TNF). FASEB J 4:2860-2867, 1990

2. Grunfeld C, Feingold KR: The metabolic effects of tumor necrosis factor and other cytokines. Biotherapy 3:143-158, 1991

3. Hotamisligil GS, Shargill NS, Spiegelman BM: Adipose expression of tumor necrosis factor-alpha: Direct role in obesity-linked insulin resistance. Science 259:87-91, 1993

4. Hotamisligil GS, Spiegelman BM: TNFα: A key component of obesity-diabetes link. Diabetes 43:1271-1278, 1994

5. Hotamisligil GS, Arner P, Caro JF, et al: Increased adipose expression of tumor necrosis factor-a in human obesity and insulin resistance. J Clin Invest 95:2409-2415, 1995

6. Saghizadeh M, Ong JM, Garvey WT, et al: The expression of TNFα by human muscle: Relationship to insulin resistance. J Clin Invest 97:1111-1116, 1996

7. Kern PA, Saghizadeh M, Ong JM, et al: The expression of tumor necrosis factor in adipose tissue: Regulation by obesity, weight loss, and relationship to lipoprotein lipase. J Clin Invest 95:2111-2119, 1995

8. Hotamisligil GS, Murray DL, Choy LN, et al: Tumor necrosis factor a inhibits signalling from the insulin receptor. Proc Natl Acad Sci USA 91:4854-4858, 1994

9. Liu LS, Spelleken M, Rohrig K, et al: Tumor necrosis factor-a acutely inhibits insulin signalling in human adipocytes. Diabetes 47:515-522, 1998

10. Hotamisligil GS, Peraldi P, Budavari A, et al: IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-a- and obesity-induced insulin resistance. Science 271:665-668, 1996

11. Fried SK, Bunkin DA, Greenberg AS: Omental and subcutaneous adipose tissues of obese subjects release interleukin-6: Depot difference and regulation by glucocorticoids. J Clin Endocrinol Metab 83:847-850, 1998

12. Besedovsky HO, Del Ray A: Immune-neuro-endocrine interactions: Facts and hypotheses. Endocr Rev 17:64-102, 1996

13. Papanicolaou DA, Petrides J, Tsigos C, et al: Exercise stimulates interleukin-6 secretion: Inhibition by glucocorticoids and correlation with catecholamines. Am J Physiol 271:E601-E605, 1996

14. Tsigos C, Papanicolaou DA, Defensor R, et al: Dose-effects of recombinant human interleukin-6 on anterior pituitary hormone secretion and thermogenesis. Neuroendocrinology 66:54-62, 1997

15. Tsigos C, Papanicolaou DA, Kyrou I, et al: Dose-dependent effects of recombinant human interleukin-6 on glucose regulation. J Clin Endocrinol Metab 82:4167-4170, 1997

16. Tartaglia LA, Goeddel DV: Two TNF receptors. Immunol Today 13:151-153, 1992

17. Aderka D, Engelmann H, Maor Y, et al: Stabilization of the bioactivity of tumor necrosis factor by its soluble receptors. J Exp Med 175:323-329, 1992

18. Katsuki A, Sumida Y, Murashima S, et al: Serum levels of tumor necrosis factor-α are increased in obese patients with noninsulin-dependent diabetes mellitus. J Clin Endocrinol Metab 83:859-862, 1998

19. Winkler G, Salamon F, Salamon D, et al: Elevated serum tumour necrosis factor-alpha levels can contribute to the insulin resistance in type II (non insulin-dependent) diabetes and in obesity. Diabetologia 41:860-861, 1998

20. Dandona P, Weinstock R, Thusu K, et al: Tumor necrosis factor-α in sera of obese patients: Fall with weight loss. J Clin Endocrinol Metab 83:2907-2910, 1998

21. Hsueh WA, Law RE: Cardiovascular risk continuum: Implications of insulin resistance and diabetes. Am J Med 105:4S-14S, 1998 (suppl)

22. Pickup JC, Mattock MB, Chusney GD, et al: NIDDM as a disease of the innate immune system: Association of acute-phase reactants and interleukin-6 with metabolic syndrome X. Diabetologia 40:1286-1292, 1997

23. Kirchgessner TG, Uysal KT, Wiesbrock SM, et al: Tumor necrosis factor-alpha contributes to obesity-related hyperleptinemia by regulating leptin release from adipocytes. J Clin Invest 100:2777-2782, 1997

24. Mantzoros CS, Moschos S, Avramopoulos I, et al: Leptin concentrations in relation to body mass index and the tumor necrosis factor-α system in humans. J Clin Endocrinol Metab 82:3408-3413, 1997

25. Vgontzas AN, Papanicolaou DA, Bixler Eo, et al: Elevation of plasma cytokines in disorders of excessive daytime sleepiness: Role of sleep disturbance and obesity. J Clin Endocrinol Metab 82:1313-1316, 1997