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# Elevated Levels of Interleukin 6 Are Reduced in Serum and Subcutaneous Adipose Tissue of Obese Women after Weight Loss*

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**ABSTRACT**

The aim of this study was to investigate the potential role of adipose cytokines in the obesity-associated insulin resistance. To that end, we compared: 1) serum concentrations of interleukin 6 (IL-6), tumor necrosis factor $\alpha$ (TNF$\alpha$), and leptin in eight healthy lean control females and in android obese female without (n = 14) and with (n = 7) type 2 diabetes; and 2) the levels of these cytokines both in serum and in sc adipose tissue in the 14 obese nondiabetic women before and after 3 weeks of a very low-calorie diet (VLCD). As compared with lean controls, obese nondiabetic and diabetic patients were more insulin resistant and presented increased values for leptin, IL-6, TNF$\alpha$, and C-reactive protein. In the whole group, IL-6 values were more closely related to the parameters evaluating insulin resistance than leptin or TNF$\alpha$ values. VLCD resulted in weight loss and decreased body fat mass (~3 kg). Insulin sensitivity was improved with no significant change in both serum and adipose tissue TNF$\alpha$ levels. In contrast, VLCD induced significant decreases in IL-6 and leptin levels in both adipose tissue and serum. These results suggest that, as for leptin, circulating IL-6 concentrations reflect, at least in part, adipose tissue production. The reduced production and serum concentrations after weight loss could play a role in the improved sensitivity to insulin observed in these patients. (*J Clin Endocrinol Metab* **85:** 3338–3342, 2000)

OBESITY, ONE OF THE most common metabolic disorders in developing countries, is characterized by a reduction in insulin sensitivity, both in animal models and in humans. However, the molecular mechanisms involved in obesity-related insulin resistance are not yet well understood (1). It has been clearly demonstrated that adipocytes are able to synthesize and to secrete several cytokines, such as leptin (2), tumor necrosis factor $\alpha$ (TNF$\alpha$) (3), and, more recently, interleukin (IL)-6 (4). In the last years, an attractive hypothesis has emerged proposing that those cytokines produced by adipose tissue may be responsible for insulin resistance in obesity (1). Indeed, the expression or production of these cytokines was shown to be directly related to the degree of obesity of the subjects (1–4) and, thus, might be involved in obesity-related insulin resistance (1). Leptin, the product of the *ob* gene (2), is primarily produced by the adipocyte and has been shown to impair the metabolic action of insulin in isolated rat adipocytes (5). However, other studies suggest that leptin could also increase insulin sensitivity (6, 7). Therefore, the role of leptin in human insulin resistance *in vivo* is still unclear. The deleterious effect of TNF$\alpha$ on insulin action has been presented in different experimental studies and is less controversial during the inflammatory process (8, 9). Its mechanism of action probably involves the phosphorylation of the insulin receptor substrate-1 on serine residues resulting in a reduction in insulin signaling into the cell (9). Increased expression of TNF$\alpha$ messenger RNA (mRNA) in the sc abdominal adipose tissue depot has been documented in obese rodents and humans (3, 10, 11), leading to the hypothesis that TNF$\alpha$ may play a crucial role in obesity-related insulin resistance (12). However, it was shown recently that human sc adipose tissue does not release a significant amount of TNF$\alpha$ *in vivo* (4) and the relationship between adipose-derived TNF$\alpha$ and the *in vivo* insulin sensitivity in human is still largely debated in the literature (13–15). Subcutaneous adipose tissue was shown to secrete IL-6, and this secretion correlated with the body mass index (BMI) of the subjects (4). The expression of IL-6 in human adipose tissue was further confirmed (16). Thus, as a cytokine, IL-6 may play a role in obesity-related insulin resistance (1). However, little is known about IL-6 expression and secretion in human adipose tissue. In particular, it is not known whether adipose tissue and plasma IL-6 levels change during weight loss and whether this could be related to change in insulin sensitivity of the subjects.

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In the present study, we aimed: 1) to compare serum concentrations of IL-6, TNFα, and leptin in healthy lean control subjects and in obese patients with and without type 2 (non-insulin dependent) diabetes mellitus; and 2) to measure the levels of these cytokines both in serum and in sc adipose tissue in 14 obese women before and after 3 weeks of a very low-calorie diet (VLCD).

## Subjects and Methods

### *Subjects*

*Study 1.* Twenty-nine Caucasian women volunteers [8 lean nondiabetic (age, 24–69 yr; BMI, 18.4–23.2 kg/m$^2$), 14 nondiabetic obese (age, 28–64 yr; BMI, 32.9–48.7 kg/m$^2$), and 7 type 2 diabetic obese women (age, 51–66 yr; BMI, 29.0–39.6 kg/m$^2$)] were included in this study. Type 2 diabetes was assessed using the new American Diabetes Association criteria (17). Apart from obesity, all nondiabetic obese subjects were in good health and had stable body weight for at least 12 months. None was engaged in any type of exercise program or was excessively sedentary. All these subjects were involved in clinical investigations (18, 19) that were approved by the ethical committee of either the Hospice Civils de Lyon or the Assistance Publique-Hôpitaux de Paris and that were performed according to the French legislation. Blood samples were drawn in the morning (between 0800 and 0900 h) after an overnight fast.

*Study 2.* The 14 nondiabetic obese women participated in a VLCD program for 21 days. The energy intake was limited to 3.9 ± 0.1 MJ (941 ± 27 kcal)/day with 45% of carbohydrate, 20% of fat, and 35% of proteins. Subcutaneous abdominal adipose tissue biopsies were taken before and after 21 days of VLCD, after an overnight fast, using the percutaneous mini-liposuction method described previously (20). The two biopsies were taken from distinct sites, at the level of umbilicus. Subjects were still consuming the low-calorie diet at the time of the second biopsy. Adipose tissue samples were immediately frozen in liquid nitrogen and stored at −80 C until protein analysis. All subjects had given their written consent after being informed of the nature, purpose, and possible risks of the study. The experimental protocol was approved by the ethical committee of Assistance Publique-Hôpitaux de Paris and performed according to the French legislation (Huriet law).

### *Analytical methods*

Venous blood samples were taken between 0800 and 0900 h, after an overnight fast, before, and at the end of the diet period and stored at −80 C before immuno-assays analysis. Blood glucose was assayed enzymatically (hexokinase) using a multiparametric analyzer (Hitachi 911; Roche Molecular Biochemicals, Meylan, France). Serum insulin concentrations were measured using commercial RIA kits (Bi-Insulin IRMA; ERIA-Pasteur, Paris, France). Serum levels of leptin, TNFα, and IL-6 were determined by enzyme-linked immunosorbent assay (Quantikine leptin; Quantikine High Sensitivity TNFα and Quantikine IL-6; R&D Systems, Oxford, UK). The sensitivity of these assays was 7.8 pg/mL, 0.18 pg/mL, and 0.70 pg/mL for leptin, TNFα, and IL-6, respectively. The same enzyme-linked immunosorbent assay kits were used to determine the immunoreactive leptin, TNFα, and IL-6 protein content in adipose tissue samples after homogenization of 200 mg frozen tissue in 400 μL of a buffer (pH 7.4) containing 10 mmol/of Tris-HCl, 250 mmol/l of sucrose, and a mixture of protease inhibitors (Complete; Roche Molecular Biochemicals). C reactive protein (CRP) was assessed by immunonephelometry on Behring Nephelometer 2 (Dade-Behring, La Défense, France). The sensitivity of the assay was 0.18 mg/L. Estimation of insulin resistance in the fasting state [fasting insulin resistance index (FIRI)] was calculated from fasting plasma glucose and insulin levels (FIRI = fasting glucose × fasting insulin/25) (21). A body composition analysis by dual x-ray absorptiometry was performed in the nondiabetic obese women only, using the QDR 1000 from Hologic, Inc. (Waltham, MA).

### *Presentation of the results*

All results are presented as mean ± SE. Nonparametric Wilcoxon's rank-sum test for paired data was used to compare values before and after diet. Differences between groups were determined using one-way ANOVA and the Kruskal-Wallis test, followed by a Fisher protected least significant test for pair-wise differences. The significance of the correlations was examined using the nonparametric Spearman's rank correlation test. Multiple regression analysis was undertaken for the continuous variable, IL-6. The threshold for significance was set at $P = 0.05$.

## Results

All the nondiabetic and diabetic obese women were characterized by android obesity (waist to hip ratio >0.90 and BMI >30 kg/m$^2$). As expected, the type 2 diabetic women had higher fasting glycemia. As indicated by the FIRI values, nondiabetic and diabetic obese women were insulin resistant whereas lean controls were not, the diabetic subjects being the most insulin resistant. Serum level of cytokines IL-6, TNFα, and leptin and of CRP were significantly higher in diabetic and nondiabetic obese women than in healthy lean subjects (Table 1).

Serum concentrations of IL-6, TNFα, and leptin were significantly correlated with BMI and fasting plasma insulin levels when the 29 subjects were analyzed together (Table 2). IL-6 and leptin concentrations were significantly correlated with serum CRP levels and waist to hip ratio, whereas serum

**TABLE 1.** Clinical and metabolic characteristics of the subjects (n = 29)

| | Lean control subjects (n = 8) | Obese nondiabetic patients (n = 14) | Obese diabetic patients (n = 7) |
|---|---|---|---|
| Age (yr) | 42 ± 5[a] | 45 ± 4[a] | 58 ± 2 |
| BMI (kg/m$^2$) | 20.6 ± 0.6 | 39.5 ± 1.1[b] | 36.6 ± 1.0[b] |
| Waist/hip ratio | 0.77 ± 0.01 | 0.96 ± 0.02[b] | 0.98 ± 0.03[b] |
| Glucose (mmol/L) | 5.3 ± 0.2[c] | 5.3 ± 0.2[c] | 11.1 ± 1.5 |
| Insulin (pmol/L) | 42 ± 6 | 84 ± 12[d] | 90 ± 18[d] |
| FIRI (mmol × mU × L$^{-2}$) | 1.39 ± 0.18 | 3.04 ± 0.37[e,f] | 6.14 ± 1.16[b] |
| Leptin (ng/mL) | 9.5 ± 1.7 | 54.9 ± 4.5[b,f] | 33.0 ± 5.6[d] |
| IL-6 (pg/mL) | 0.39 ± 0.06 | 2.78 ± 0.30[b] | 3.58 ± 0.51[b] |
| TNFα (pg/mL) | 0.74 ± 0.09 | 1.48 ± 0.15[b] | 1.08 ± 0.12 |
| CRP (mg/L) | 1.2 ± 0.3 | 6.3 ± 1.1[d] | 5.8 ± 1.1[d] |

[a] $P < 0.05$ *vs.* diabetic patients.
[b] $P < 0.001$ *vs.* control subjects.
[c] $P < 0.001$ *vs.* diabetic patients.
[d] $P < 0.01$ *vs.* control subjects.
[e] $P < 0.05$ *vs.* control subjects.
[f] $P < 0.01$ *vs.* diabetic patients.

**TABLE 2.** Correlations with leptin, TNFα, and IL-6 in all subjects (n = 29)

| | Leptin | TNFα | IL-6 |
|---|---|---|---|
| BMI (kg/m$^2$) | 0.841 | 0.480 | 0.602 |
| | $P < 0.001$ | $P < 0.05$ | $P < 0.005$ |
| Waist/hip ratio | 0.370 | 0.269 | 0.624 |
| | $P = 0.05$ | $P = 0.16$ | $P < 0.005$ |
| Glucose (mmol/L) | −0.153 | −0.096 | 0.428 |
| | $P = 0.42$ | $P = 0.60$ | $P < 0.05$ |
| Insulin (pmol/L) | 0.571 | 0.402 | 0.548 |
| | $P < 0.005$ | $P < 0.05$ | $P < 0.005$ |
| FIRI (mmol × mU × L$^{-2}$) | 0.328 | 0.256 | 0.677 |
| | $P = 0.08$ | $P = 0.18$ | $P < 0.001$ |
| Leptin (ng/mL) | | 0.674 | 0.493 |
| | | $P < 0.001$ | $P < 0.01$ |
| IL-6 (pg/mL) | 0.493 | 0.183 | |
| | $P < 0.01$ | $P = 0.34$ | |
| TNFα (pg/mL) | 0.674 | | 0.183 |
| | $P < 0.001$ | | $P = 0.34$ |
| CRP (mg/L) | 0.636 | 0.289 | 0.683 |
| | $P < 0.001$ | $P = 0.13$ | $P < 0.001$ |

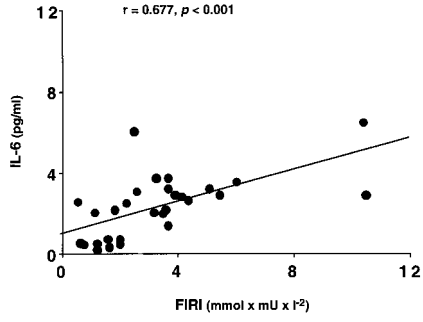


FIG. 1. Correlation between circulating IL-6 concentrations and the FIRI in the whole group.

TNFα levels were not (Table 2). Moreover, only IL-6 concentrations correlated significantly with fasting plasma glucose levels and the FIRI (Fig. 1). There was no correlation between these two parameters and either TNFα or leptin concentrations (Table 2). Therefore, IL-6 values were more strongly correlated with obesity and insulin resistance parameters than TNFα or leptin. To investigate which variables might account for the association between circulating IL-6 levels and insulin resistance, multiple regression analysis was performed. The independent variables used in this analysis were the variables significantly correlated with IL-6 in univariate analysis (*i.e.* BMI, waist to hip ratio, FIRI, fasting plasma glucose, and insulin). In the whole group, only BMI ($P < 0.05$) and FIRI ($P < 0.05$) were independently and significantly associated with IL-6 levels. Finally, when each of the three group of subjects were analyzed separately, only leptin remained significantly associated with BMI in the obese nondiabetic group (r = 0.666, $P < 0.05$).

To further study the relation between cytokine levels and obesity, the 14 obese women were studied again after 3 weeks of VLCD. The diet resulted in a mean reduction of 2.1 kg/m$^2$ in BMI and a mean reduction of 3 kg in adipose tissue mass and was associated with an improvement in the sensitivity to insulin, as estimated by the FIRI (Table 3). During VLCD, serum IL-6 concentration decreased slightly but significantly ($P = 0.05$) in all subjects whereas the changes in serum CRP levels did not reach significance ($P = 0.14$). VLCD also produced a significant decrease in serum leptin concentration ($P < 0.005$). In contrast to what was observed with IL-6 and leptin, serum TNFα concentration remained unchanged after VLCD.

**TABLE 3.** Characteristics of the nondiabetic obese women before and after diet (n = 14)

| | Before diet | After diet | *P* |
|---|---|---|---|
| BMI (kg/m$^2$) | 39.5 ± 1.1 | 37.4 ± 1.0 | <0.005 |
| Body fat mass (kg) | 47.0 ± 3.0 | 43.0 ± 3.0 | <0.005 |
| Glucose (mmol/l) | 5.3 ± 0.2 | 5.0 ± 0.1 | 0.12 |
| Insulin (pmol/l) | 84 ± 12 | 66 ± 6 | <0.05 |
| FIRI (mmol × mU × I$^{-2}$) | 3.04 ± 0.37 | 2.28 ± 0.26 | <0.05 |
| Leptin (ng/mL) | 54.9 ± 4.5 | 29.6 ± 3.1 | <0.005 |
| IL-6 (pg/mL) | 2.78 ± 0.30 | 2.32 ± 0.19 | 0.05 |
| TNFα (pg/mL) | 1.48 ± 0.15 | 1.57 ± 0.13 | 0.60 |
| CRP (mg/L) | 6.3 ± 1.1 | 4.3 ± 0.9 | 0.14 |

Finally, the cytokine level was evaluated in sc adipose tissue. We found that both IL-6 and leptin protein contents decreased significantly after VLCD (by 22 ± 9%, $P < 0.05$ and 36 ± 4%, $P < 0.01$, respectively), whereas the amount of immunoreactive adipose TNFα protein remained unchanged ($P = 0.36$, Fig. 2). These variations reflect those observed at the serum level.

## Discussion

We report in the present study that circulating concentrations of IL-6, TNFα, and leptin were increased in nondiabetic and diabetic obese women when compared with healthy lean women. Moreover, serum levels of IL-6, TNFα, and leptin were significantly correlated with the BMI of the subjects, suggesting that the circulating concentrations of these cytokines may reflect, at least in part, production by adipose tissue. Interestingly, serum CRP levels were also elevated both in the nondiabetic and diabetic obese women as compared with controls, suggesting that obesity may also influence serum CRP concentrations. CRP is an acute phase inflammatory protein in which production results from the IL-1, IL-6, and TNFα balance in human, and it was suggested recently that its serum concentration may be regulated, at least in part, by adipose tissue IL-6 secretion in the basal state (16, 22). The observed correlation between the serum concentrations of IL-6 and CRP is in line with this hypothesis. Because it was already observed by others (23, 24), we found a positive relationship between circulating IL-6 and the degree of obesity estimated by the BMI. One of the possible mechanisms whereby IL-6 level is increased in obesity could be due to the fact that adipose tissue is able to express (16, 25) and to secrete IL-6 (26) and that IL-6 production from adipose tissue is higher in obese subjects (4). However, the molecular mechanism for the increased expression of IL-6 in adipocytes from obese subjects is presently unknown.

Fasting serum concentrations of IL-6 were associated with all the markers of insulin resistance measured in the study (fasting plasma levels of insulin and glucose, FIRI, and waist to hip ratio), whereas the concentrations of TNFα were only associated with plasma insulin and those of leptin with plasma insulin and waist to hip ratio. This result may suggest, in agreement with the finding of Mo-

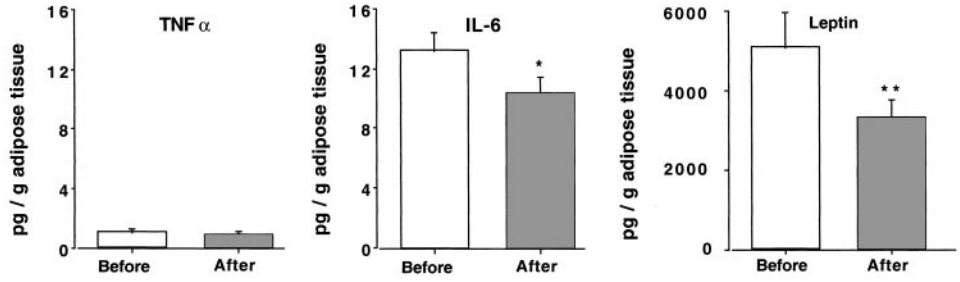


FIG. 2. Effects of VLCD on IL-6, TNFα, and leptin protein content in adipose tissue of obese women. * $P < 0.05$, ** $P < 0.01$: using the non parametric Wilcoxon's test for paired values.

hamed-Ali *et al.* (4), that IL6 concentration is more closely associated with obesity-related insulin resistance than TNFα or leptin in humans. Indeed, for the first time, we showed a direct positive correlation between insulin resistance and circulating IL-6 levels in human (Fig. 1). Some recent published works are in line with this finding, suggesting a link between insulin resistance and circulating IL-6 levels in different diseases such as sleep apnea with visceral obesity (24), patients with cancer (27), or patients with Syndrome X (28) or at risk for coronary heart disease (22, 29). The mechanisms whereby IL-6 can induce insulin resistance at the cellular level are poorly understood. However, it has been reported that IL-6 induced physiological changes reminiscent of the catabolic state with increased plasma free fatty acids and fat oxidation (30) and inhibition of adipose tissue lipoprotein lipase activity (31): all these effects are opposite to those of insulin, therefore, impairing insulin action. On the same line, IL-6 has been shown recently to have effects opposite of those of insulin on hepatic glycogen metabolism (32), and IL-6 was shown to increase glycemia (33). The role of adipose tissue IL-6 in obesity was suggested by Mohamed-Ali *et al.* (4), who have demonstrated that adipose tissue in human is able to secrete a large amount of IL-6 with an increased production in obese subjects and that this secretion may account for about 25% of the circulating IL-6 concentrations in the basal state, while no significant secretion of TNFα from sc adipose tissue was found (4). In addition, in line with previous works from others (23, 24), our results suggest a link between IL-6 and obesity. It is, thus, possible that adipose tissue-derived IL-6 may be involved in obesity-associated insulin resistance.

Leptinemia decreased significantly during VLCD in agreement with previous reports on the effect of dieting in obese subjects (19, 34, 35). This decrease in the circulating concentration of leptin was most probably a consequence of a reduction in leptin expression and production in fat as supported by the decrease in leptin protein content in adipose tissue after VLCD and also by the decrease in leptin mRNA levels that was previously described in obese patients under diet (19).

In contrast to what was observed for leptin, the serum concentrations and the protein contents in adipose tissue of TNFα remained unchanged during VLCD in our study. The regulation of TNFα levels both in serum and in adipose tissue after weight loss in human obesity is still a matter of debate (10, 11, 19, 36, 37). It was reported that a 2-yr dieting program decreased circulating serum TNFα in obese women (36). This result was in line with studies showing that diet-induced weight loss in obese patients was associated with decreased expression of TNFα in sc adipose tissue after stabilization of weight (10, 11). In contrast, we have recently shown that the dynamic period of weight loss during VLCD was associated with an increase in TNFα mRNA expression in adipose tissue of largely obese women (19). Here, we found that fasting serum levels of TNFα was unchanged after 3 weeks of VLCD. A lack of change in plasma TNFα concentration was also recently observed in obese individuals after a large weight loss (20–30 kg) 1 yr after gastroplasty (37). These discrepancies may be due to the difference in the mechanisms involved in weight loss in these studies [*i.e.* diet (10, 11, 19, 36) or gastroplasty (37)] and the period chosen to study this weight loss [*i.e.* dynamic (19) or stabilization period (10, 11, 36)]. Therefore, the contribution of adipose tissue to the circulating levels of TNFα in human obesity is still unclear.

In the present study, we report for the first time changes in IL-6 protein concentrations after VLCD both in adipose tissue and in serum in obese subjects. Adipose tissue IL-6 content decreased significantly after VLCD and was associated with a slight decrease in serum IL-6 concentrations. However, none of the observed modifications in IL-6 expression was directly associated with the loss in weight or in fat mass (data not shown). It is probable that the observed variations result from severe diet rather than from fat mass loss because IL-6 has been shown to be acutely stimulated by meals (26) and because the amount of fat loss represent only a few percentages of the total fat mass in these obese patients. Moreover, such a nutritional regulation has already been reported for leptin (31), another cytokine synthesized and secreted by adipose tissue. These results are in line with the fact that IL-6 production by adipose tissue could explain 10–30% of the whole circulating IL-6 concentration (1). IL-6 is the major stimulating factor for hepatocyte synthesis and secretion of CRP in human. Interestingly, we found that serum CRP tended to decrease during weight loss, indicating a possible link between adipose tissue secretion of IL-6, the regulation of systemic IL-6 circulating levels, and IL-6 function in the basal state without any acute inflammation.

In conclusion, this study demonstrates that elevated circulating IL-6 levels are associated with obesity in women. Moreover, adipose tissue may participate, in part, to the serum IL-6 concentrations, particularly during VLCD-induced weight loss, a situation that was associated with a decrease in both adipose tissue and serum IL-6 contents. Additional investigations are now required to verify whether modifications in the IL-6 system are involved in or are the

result of the reduction of adipose tissue mass and their role in the improvement of insulin sensitivity observed after weight loss.

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