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# Association of Low Interleukin-10 Levels with the Metabolic Syndrome in Obese Women

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**The potential role of anti-inflammatory cytokines in human obesity is unknown. We tested the hypothesis that low serum IL-10 concentrations associate with the metabolic syndrome in obese women. Compared with 50 matched nonobese women, the prevalence of the metabolic syndrome (≥3 of the following abnormalities: waist circumference, >88 cm; triglycerides, >1.69 mmol/liter; high density lipoprotein cholesterol, <1.29 mmol/liter; blood pressure, >130/85 mm Hg; glucose, >6.1 mmol/liter) was higher in 50 obese women (52% *vs.* 16%; $P < 0.01$). As a group, obese women had higher circulating levels of IL-6, C-reactive protein, and IL-10 than nonobese women. In both obese and nonobese women, IL-10 levels were lower in those with than in women without the metabolic syndrome: obese, 1.3 (0.7/2.1) pg/ml *vs.* 4.5 (4.3/7.4) pg/ml (median and quartiles; $P < 0.01$); and nonobese, 0.9 (0.7/1.3) pg/ml *vs.* 1.3 (0.9/3.3) pg/ml ($P < 0.05$). After 12 months of a lifestyle program, body weight decreased by 10.9 ± 1.7 kg and was associated with a significant decrement of IL-6, C-reactive protein, and IL-10 levels; the decrease in IL-10 levels was confined to obese women without the metabolic syndrome. These results show that circulating levels of the anti-inflammatory cytokine IL-10 are elevated in obese women and that low IL-10 levels are associated with the metabolic syndrome. (*J Clin Endocrinol Metab* 88: 1055–1058, 2003)**

OBESITY IS A risk factor for coronary atherosclerosis beyond and independent of the standard risk factors (1). This relationship suggests that much of the influence of obesity may be mediated through emerging risk factors that are usually hidden in a routine clinical work-up (2). Obese subjects typically carry a proinflammatory state that may predispose them to acute coronary syndromes (3). An excess of adipose tissue, particularly in intraabdominal depots, may play a role in the pathophysiology of the metabolic syndrome, closely linked to insulin resistance, and the increased risk for developing cardiovascular diseases (4).

Although circulating levels of proinflammatory cytokines (IL-6 and TNF$\alpha$) as well as other markers of inflammation, such as C-reactive protein (CRP), have been shown to be elevated in human obesity (3, 5, 6), nothing is known of the role of anti-inflammatory cytokines in this setting. IL-10 is a major inhibitor of cytokine synthesis; it suppresses macrophage function and inhibits the production of proinflammatory cytokines (7). Recent studies in animals have shown a protective role for IL-10 in both atherosclerotic lesion formation and stability (8). IL-10 has been shown to be released into plasma during human myocardial ischemia/reperfusion injury, possibly by lymphocytes infiltrating the reperfused myocardium, and could limit myocardial macrophage activation (9).

Until now there have been no reported studies that evaluated circulating IL-10 levels in human obesity, nor have potential associations between serum IL-10 concentrations and the metabolic syndrome been tested. In this study we tested the hypothesis that low serum IL-10 levels are associated with the metabolic syndrome in obese women. Moreover, we evaluated the effect of changes in lifestyle aimed at reducing body weight and increasing physical activity.

Abbreviations: BMI, Body mass index; CRP, C-reactive protein; HDL, high density lipoprotein; WHR, waist to hip ratio.

## Subjects and Methods

We studied 50 obese and 50 age-matched, normal weight, premenopausal women, aged 22–44 yr. Obese women were recruited from those attending the center for obesity management at the teaching hospital of the Second University of Naples (Naples, Italy). Normal weight women were recruited from the medical and paramedical staff of our department and volunteered to serve as the control group. All women were sedentary (<1 h/wk of physical activity) and were free from psychiatric problems and alcohol abuse; none smoked or took any drug. Each woman gave informed consent to participate in the study, which was approved by the institutional committee of ethical practice of our institution.

Women with three or more of the following criteria were defined as having the metabolic syndrome (10): 1) abdominal obesity (waist circumference, >88 cm), 2) hypertriglyceridemia (≥1.69 mmol/liter), 3) low high density lipoprotein (HDL) cholesterol (<1.29 mmol/liter), 4) high blood pressure (≥130/85 mm Hg), and 5) high fasting glucose (≥6.1 mmol/liter).

Obese women entered into a medically supervised program aimed at reducing body weight by 10% or more and exercised for at least 30 min/d. The prescribed daily caloric intake was 1300 kcal (55% carbohydrate, 30% lipid, and 15% protein), with intake of saturated fat less than 10% of energy, and intake of fiber at least 15 g/1000 kcal. The dietary advice was tailored to each woman on the basis of a 2-d food records completed each month. The women also received individual guidance on increasing their levels of physical activity (mainly walking, but also swimming or aerobic ball games). Women were in the program for 12 months and had monthly sessions with the nutritionist and exercise trainer.

All women were studied after a 14-h overnight fast, within the first week after the end of menstrual bleeding. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. The waist to hip ratio (WHR) was calculated as waist circumference in centimeters divided by hip circumference in centimeters.



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Routine chemical analyses were assessed in the hospital's chemistry laboratory. Plasma insulin levels were assayed by RIA (Ares Serono, Milan, Italy). Serum samples for IL-6 and IL-10 were stored at –80 C and were assayed in duplicate using a high sensitivity, quantitative sandwich enzyme assay (Quantikine HS, R&D Systems, Inc., Minneapolis, MN). The lower limit of detection was 0.7 pg/ml for both. CRP was assessed by immunonephelometry (Dade-Behring, Milan, Italy).

Data are presented as the mean ± SD and as percentages for categorical data; IL-10 values are presented as the median and quartiles (25/75%). One-sample *t* tests were used to compare values obtained before and after lifestyle changes, and two-samples *t* tests were used for between-groups comparison. Nonparametric tests (Mann-Whitney rank sum test and Wilcoxon matched test) were used to compare IL-6, CRP, and IL-10 data. Categorical data were analyzed using $\chi^2$ and McNemar's tests. The significance of the correlations was examined using the nonparametric Spearman's rank correlation test. Multivariate regression analysis tested the independent association and contribution of changes in body weight and physical activity with the dependent variable (IL-10). A value of $P < 0.05$ was considered significant. All calculations were made on an IBM PC computer (SPSS, version 9.0, SPSS, Inc., Chicago, IL).

## Results

The characteristics of the study women are shown in Table 1. Compared with nonobese women, obese women had higher fasting glucose, insulin, triglyceride, and blood pressure values, higher serum levels of IL-6 and CRP, and lower HDL cholesterol concentrations. The prevalence of the metabolic syndrome was significantly higher in the obese group (52% *vs.* 16%; $P < 0.01$). Compared with obese women without the metabolic syndrome (n = 24), obese women with the metabolic syndrome (n = 26) were more aged (38.4 ± 4.9 *vs.* 34.9 ± 5.2; $P < 0.05$) and presented a higher WHR (0.91 ± 0.08 *vs.* 0.87 ± 0.07; $P < 0.05$). High waist circumference was the most frequent abnormality found in obese women with the metabolic syndrome, whereas in nonobese women with the metabolic syndrome hypertension was the most frequent abnormality (Fig. 1). Levels of circulating IL-10 were significantly higher in the obese group than in the nonobese group (Fig. 2); however, in both obese and nonobese women IL-10 levels were significantly lower in women with the metabolic syndrome than in women without the metabolic syndrome (Fig. 3). In both groups, no women with the metabolic syndrome had IL-10 levels higher than the respective upper quartile (obese, 4.45 pg/ml; nonobese, 2.9 pg/ml).

All obese women completed the lifestyle program. After 12 months, the mean body weight decreased by 10.9 ± 1.7 kg (12.4 ± 2.1%), and the level of physical activity increased by 95 ± 21 min/wk. This was associated with significant reductions in BMI, WHR, fasting glucose, insulin, triglyceride, and blood pressure levels, and serum IL-6 and CRP, and with a significant increment in HDL cholesterol concentrations (Table 1). The prevalence of the metabolic syndrome was reduced by 46% ($P < 0.01$). After weight loss, the change in serum CRP levels correlated with the decline in serum IL-6 ($r = 0.49$; $P < 0.01$). Serum IL-10 levels decreased at 1 yr (Fig. 2); however, the decrement in IL-10 levels was only significant in obese women without the metabolic syndrome (Fig. 3). For evaluating the independent association of changes in IL-10 levels with changes in lifestyle, a multivariate analysis was performed in which the IL-10 level was the dependent variable, and BMI, WHR, level of physical activity, and plasma glucose, insulin, and triglyceride levels were the independent variables. Changes in BMI and in the level of physical activity explained 24% of the variability in IL-10 levels.

## Discussion

Our study shows for the first time that circulating levels of IL-10 are elevated in obese women and that low IL-10 levels are associated with the metabolic syndrome in both obese and nonobese women. Moreover, changes in lifestyle aimed at reducing body weight and increasing physical activity over 1 yr resulted in significant reductions in raised circulating IL-10 levels in obese women without the metabolic syndrome. Taken together, our findings suggest that obesity is associated with increased IL-10 levels and that low serum IL-10 concentrations are linked to the metabolic syndrome.

People with the metabolic syndrome are at increased risk for developing type 2 diabetes mellitus and cardiovascular

**TABLE 1.** Clinical characteristics of the study women and the effect of changes in lifestyle in obese women[a]

| | Baseline | | Obese (n = 50) (changes from baseline) |
|---|---|---|---|
| | Obese (n = 50) | Nonobese (n = 50) | |
| Age (yr) | 36.9 ± 4.6 | 35.9 ± 4.9 | |
| BMI (kg/m$^2$) | 35.5 ± 2.9 | 23.8 ± 1.2[b] | −4.1 ± 0.7[d] |
| WHR | 0.89 ± 0.07 | 0.73 ± 0.04[b] | −0.1 ± 0.02[d] |
| Glucose (mmol/liter) | 6.0 ± 0.4 | 5.2 ± 0.3[b] | −0.6 ± 0.2[d] |
| Insulin (pmol/liter) | 119 ± 39 | 59 ± 18[b] | −39 ± 18[d] |
| Cholesterol (mmol/liter) | 5.0 ± 0.7 | 4.9 ± 0.6 | −0.1 ± 0.1 |
| HDL-cholesterol (mmol/liter) | 1.2 ± 0.3 | 1.4 ± 0.3[b] | 0.3 ± 0.1[e] |
| Triglycerides (mmol/liter) | 1.5 ± 0.5 | 1.2 ± 0.4[b] | −0.3 ± 0.2[e] |
| Blood pressure (mm Hg) | 128/82 ± 9/5 | 125/79 ± 8/5[b] | −3/2 ± 1/1[d] |
| Metabolic syndrome | 26 (52%) | 8 (16%)[b] | −12 (46%)[d] |
| IL-6 (pg/ml) | 3.08 ± 0.64 | 1.25 ± 0.43[b] | −1.18 ± 0.49[e] |
| CRP (μg/ml) | 5.8 ± 1.7 | 1.4 ± 0.4[b] | −2.3 ± 0.9[d] |
| IL-10 (pg/ml) | 2.45 (1.1/4.45) | 1.2 (0.7/2.9)[c] | −0.35 (−1.45/0)[d] |

[a] Data are mean ± SD; IL-10 is presented as median (25%/75%).
[b] $P < 0.01$ compared with obese women.
[c] $P = 0.045$ compared with obese women.
[d] $P < 0.01$ compared with baseline.
[e] $P < 0.05$ compared with baseline.

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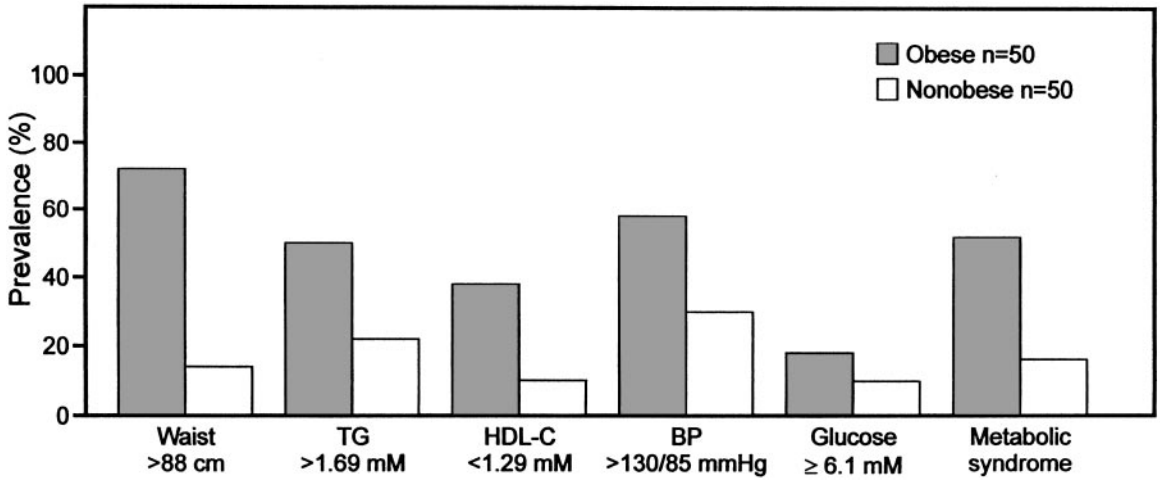


FIG. 1. Prevalence of individual metabolic abnormalities of the metabolic syndrome among 50 obese women and 50 age-matched nonobese women. TG, Triglycerides; HDL-C, HDL cholesterol; BP, blood pressure.

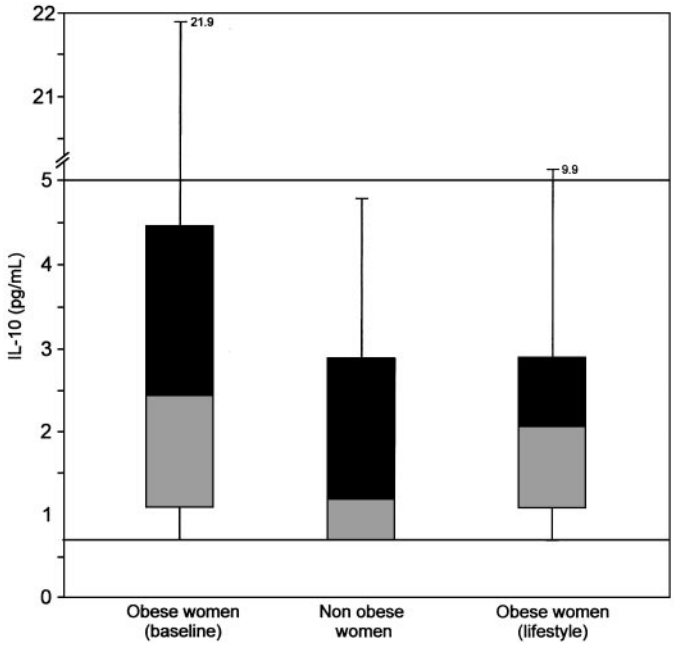


FIG. 2. IL-10 concentrations in obese women and nonobese women and in obese women after lifestyle changes. The box plot represents the median, quartiles, and extreme values of IL-10 in the three groups.

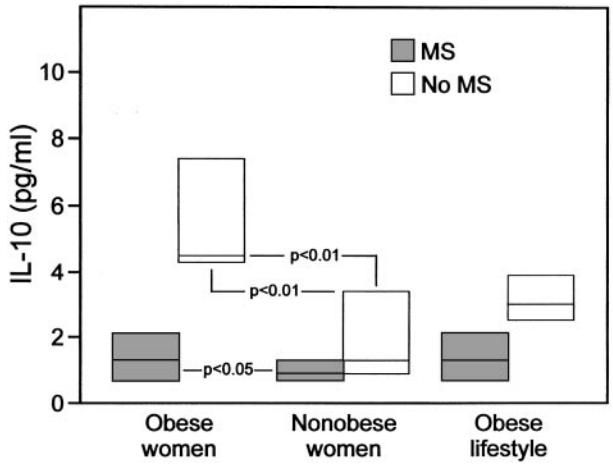


FIG. 3. IL-10 concentrations according to the presence/absence of the metabolic syndrome in the study women. The box plot represents the median and quartiles of IL-10. MS, Metabolic syndrome.

disease (4). A recent report indicates that 20–25% of the adult U.S. population has the metabolic syndrome (11). In our obese women, the prevalence of the metabolic syndrome, as detailed in the Adult Treatment Panel III (10), was as high as 52% and was associated with low circulating IL-10 levels. Visceral obesity is a key component of the metabolic syndrome: as a major source of proinflammatory cytokines, abdominal fat is thought to be causally implicated in the development of atherosclerosis and metabolic outcomes (12). Concordant with previous studies (3, 5, 6), we found that circulating levels of IL-6 and CRP were increased in obese women compared with lean women. Plasma CRP level is a sensitive marker of systemic inflammation (13) and may reflect the amount and activity of proinflammatory cytokines. In this regard, IL-6 has been proposed to play a central role in the relationship among CRP, adiposity, and cardiovascular disease (14), as CRP synthesis in the liver is largely under the control of IL-6 (15), and IL-6 is expressed in and released by adipose tissue (16). The close relationship we found between decrements in IL-6 and CRP after weight loss in obese women also supports a role for IL-6 in the elevated CRP levels of obesity. Other studies also found a decrease in serum IL-6 (17) or CRP (18) levels after weight loss in obese women.

IL-10 is a pleiotropic cytokine produced by T helper type 2 cells, B cells, monocytes, and macrophages that inhibits a broad array of immune parameters. *In vivo*, IL-10 most likely exerts its anti-inflammatory effects on the vascular system through inhibition of leukocyte-endothelial cells interactions and inhibition of proinflammatory cytokine and chemokine production by macrophages or lymphocytes (7). As IL-10 down-regulates the production of proinflammatory cytokines, it is tempting to speculate that the higher IL-10 levels observed in obese women represent an attempt to inhibit continued proinflammatory cytokine production, which,

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however, fails in those with a low innate IL-10 production. This interpretation is supported by the following: 1) about three fourths of the differences in IL-10 production in humans are derived from heritable factors (19); 2) data from animal models suggest that IL-10 deficiency predisposes to atherosclerosis (20) and that IL-10 acts in a feedback loop to inhibit the production of proinflammatory cytokines (7); and 3) long-term lifestyle changes, as obtained in our study, reduce the prevalence of the metabolic syndrome by 46% and are associated with minor changes in IL-10 levels, suggesting a role for nonheritable factors in the regulation of IL-10 levels only in obese women capable of increasing their IL-10 production capacity.

Although it was beyond the scope of the present study to assess the relationship between IL-10 levels and clinical outcomes, recent studies have demonstrated associations between lower serum IL-10 concentrations or production and clinical events. In 95 patients with angiographically documented coronary artery disease, Smith *et al.* (21) found lower serum IL-10 concentrations in patients with unstable angina compared with those who had chronic stable angina, hypothesizing that decreased IL-10 levels can contribute to atheromatous plaque instability. However, a control healthy group was lacking. The assessment of production levels of IL-10 in 599 inhabitants of the city of Leiden, led van Exel *et al.* to conclude that subjects with low IL-10 production capacity have an increased risk of stroke (22) and type 2 diabetes mellitus (23). However, only 85-yr-old subjects were studied, and the production capacity of IL-10 was assessed in a whole blood assay in which lipopolysaccharide was used as a stimulus. It is noteworthy that in IL-10-deficient mice, the induction of diabetes with streptozotocin produces greater impairment of vasorelaxation in response to acetylcholine (24), suggesting a link between low IL-10 production and endothelial dysfunction.

In summary, for the first time we have demonstrated a significant association between lower serum concentrations of the anti-inflammatory cytokine IL-10 and the metabolic syndrome in women, independent of age and body weight. Obesity *per se* is associated with increased circulating levels of IL-10, as it occurs in obese women without the metabolic syndrome, which presumably tends to limit the chronic inflammatory state associated with adiposity. A reduced capacity to produce IL-10 (low circulating levels) might help identify obese women particularly prone to the metabolic derangements (the metabolic syndrome) of visceral adiposity.

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