Impact of Weight Loss on Inflammatory Proteins and Their Association With the Insulin Resistance Syndrome in Morbidly Obese Patients

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Objective—Obesity is closely linked to the insulin resistance syndrome (IRS), type 2 diabetes, and cardiovascular disease, the primary cause of morbidity and mortality in these patients. Elevated levels of C-reactive protein (CRP) and interleukin-6 (IL-6), indicating chronic subclinical inflammation, have been associated with features of the IRS and incident cardiovascular disease.

Methods and Results—We studied the cross-sectional and longitudinal relation of CRP, IL-6, and tumor necrosis factor-α (TNF-α) with features of the IRS in 37 morbidly obese patients with different stages of glucose tolerance before and 14 months after gastric surgery. Weight loss after gastric surgery induced a significant shift from diabetes (37% vs 3%) to impaired glucose tolerance (40% vs 33%) and normal glucose tolerance (23% vs 64%). The baseline concentration of IL-6 was correlated with TNF-α (r=0.59, P<0.01) and CRP (r=0.44, P<0.05) levels. TNF-α, IL-6, and CRP were significantly correlated with insulin resistance estimated by the homeostatic model assessment (r=0.48, P<0.05; r=0.56, P<0.01; and r=0.35, P<0.05, respectively). Concentrations of CRP and IL-6 decreased after weight loss (median, 8.6 and interquartile range, 2.7/14.5 vs 2.5 and 1.2/4.1 mg/L; P<0.006, and 5.13 and 2.72/12.15 vs 3.95 and 1.97/5.64 pg/mL, P<0.02, respectively), whereas serum levels of TNF-α remained unchanged (8.6 and 6.3/18.8 vs 11.7 and 5.8/17.2 pg/mL; NS.). Multiple regression analysis revealed that the decrease in insulin resistance remained independently and significantly correlated with the decrease in IL-6 concentrations (P<0.01) and the decrease in body mass index with the decrease in CRP (P<0.05), respectively.

Conclusions—Weight loss in morbidly obese patients induces a significant decrease of CRP and IL-6 concentrations in association with an improvement of the IRS. (Arterioscler Thromb Vasc Biol. 2003;23:1042-1047.)

Key Words: obesity • weight loss • type 2 diabetes • C-reactive protein • interleukin-6

Insulin resistance and type 2 diabetes are closely related to the body mass index (BMI), a marker of overall obesity.1 Obesity is associated with an increased risk of coronary heart disease, stroke, hypertension, type 2 diabetes mellitus, dyslipidemia, and all-cause mortality.2,3 A BMI >35 kg/m² increases insulin resistance, hyperinsulinemia, and hyperglycemia,4,5 thus leading to an increased risk for diabetes by >60-fold in women and 42-fold in men.9 The insulin resistance syndrome (IRS) is associated with a high cardiovascular risk10 and is the major cause of death in patients with type 2 diabetes.11

However, pathophysiological mechanisms linking adiposity with insulin resistance and eventually cardiovascular disease remain largely elusive. One mechanism might be the enhanced production of adipose tissue–derived proteins, such as interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α).12,13 Elevated levels of C-reactive protein (CRP) and IL-6, indicating chronic subclinical inflammation, have been associated with features of the IRS14,15 and incident cardiovascular disease, including myocardial infarction, stroke, and peripheral vascular disease.16-20 Weight control is a widely accepted and recommended clinical goal in patients with type 2 diabetes and obesity.1 The impact of weight loss on mortality and morbidity, in particular from cardiovascular disease, is still a matter of debate.21 Studies investigating the impact of weight loss on cardiovascular end points as well as various surrogate markers of cardiovascular disease are of particular interest. Morbidly obese subjects and their marked weight loss after gastroplastic surgery emerge as a valuable model for studying the impact of changes in body weight and the associated cardiovascular risk factors.

We therefore investigated the relation of circulating levels of inflammatory proteins, including CRP and IL-6, to features
of the IRS cross-sectionally and also longitudinally in morbidly obese diabetic and nondiabetic subjects undergoing gastroplastic surgery.

Methods
Thirty-seven severely obese patients selected to undergo gastroplastic surgery participated in the study. Clinical characteristics of the study participants are shown in Table 1. Surgery had been indicated according to the guidelines of the National Institutes of Health consensus statement for surgery in severe obesity. Patients were systematically referred to a multidisciplinary team for medical, psychological, nutritional, and surgical expertise. Surgery was indicated for patients with a history of repeated failures with conservative nonsurgical techniques and whose BMI was >40 kg/m². None of the subjects had a history of stroke, transient ischemic attack, myocardial infarction, angina pectoris, or electrocardiographic abnormalities. Patients with overt eating disorders, heavy alcohol consumption, major psychiatric disease, hepatic or renal failure, Cushing syndrome, thyroid dysfunction, or other major endocrine disorders were excluded. Patients with clinically overt infectious diseases were omitted from the study. All subjects were carefully instructed about the aims of the study, and written, informed consent was obtained.

Changes in Dietary Intake, Exercise Habits, and Medication
Dietary changes after vertical banded gastroplasty were similar to those that we have previously reported. Postoperatively, all patients were instructed to use a cycle for at least 20 minutes a day. None of the patients were taking statins or fibrates before or after surgery. Nine of 37 patients were taking angiotensin-converting enzyme inhibitors; 2, β-blockers; and 2, calcium channel blockers. In total, 10 patients were treated with antihypertensive drugs before surgery, 1 patient received sulfonamide, and 3 received metformin. Postoperatively antihypertensive and antidiabetic drug treatments could be stopped.

Statistical Analysis
Data are given as mean and SD, unless stated otherwise. Because CRP and IL-6 are not normally distributed, the data for CRP, IL-6, and also TNF-α (for easier reading) are given as medians and interquartile ranges. Correlations were explored by univariate linear regression and multiple linear regression. The logarithms of the CRP, IL-6, and TNF-α values were calculated for analysis, because the distribution of these variables is right-skewed. Comparisons before and after surgery were performed by ANOVA, with the group factor time (before and after gastroplastic surgery) and OGTT (3 levels: type 2 diabetes, impaired glucose tolerance [IGT], and normal glucose tolerance [NGT]) and the random factor patient within OGTT and the interaction between time and OGTT; post hoc tests were obtained by the method of Tukey. Bowker’s test of symmetry was used to assess differences in the frequency of glucose status before and after surgery. The reported probability values of the correlation coefficients (Table 3 and Table 4) were adjusted by the method of Bonferroni-Holm. Only SAS statistical software was used. A probability value <0.05 was considered statistically significant.
Markers of inflammation in morbidly obese subjects before and after weight loss as a result of gastric surgery.

Results

Effect of Weight Loss on Parameters of the IRS
Preoperative and postoperative clinical and metabolic characteristics of patients are shown in Table 1. Weight loss induced by gastric surgery caused a significant shift from diabetes (37% vs 3%) to IGT (40% vs 33%) and NGT (23% vs 64%; P < 0.001). Fasting, 1-hour, and 2-hour blood glucose and insulin levels, as well as BMI, blood pressure, and triglycerides, were significantly reduced 14 months after surgery (Table 1).

Effect of Weight Loss on Proinflammatory Proteins

Serum CRP Concentrations
Serum concentrations of CRP (median, 8.6; interquartile range, 2.7/14.5 vs 2.5 and 1.2/4.1 mg/L, respectively; P < 0.006) and IL-6 (5.13 and 2.72/12.15 vs 3.95 and 1.97/5.64 pg/mL, respectively; P < 0.02) were reduced significantly, whereas levels of TNF-α remained unchanged (8.6 and 6.3/18.8 vs 11.7 and 5.8/17.2 pg/mL, respectively; NS; Figure 1). Preoperatively, patients with type 2 diabetes (n = 14) and IGT (n = 16) showed higher levels of CRP than did subjects with NGT (n = 7), but postoperatively, these levels were comparable between groups (Table 2). In patients with type 2 diabetes, levels of IL-6 were twice as high compared with NGT, both preoperatively and postoperatively (Table 2). Concentrations of TNF-α showed no differences between groups and did not decrease after weight loss. The 95% confidence intervals of the mean post-minus-pre differences are (−10, −2), (−6.4, −0.7), and (−6.7, 2.5) for CRP, IL-6, and TNF-α, respectively.

Association of the IRS With Proinflammatory Proteins
Correlation analysis at baseline of TNF-α, CRP, and IL-6 and features of the IRS are shown in Table 3. The preoperative concentration of IL-6 was more closely related to glycemia (glucose levels during OGTT, glycosylated hemoglobin), whereas the concentration of CRP was more closely correlated to glucose-stimulated insulin release. All inflammatory markers (TNF-α, IL-6, and CRP) were significantly correlated with insulin resistance as estimated by HOMA. To further explore the relation between CRP and insulin as well as IL-6 and glucose, patients were stratified into 2 groups according to the baseline median cutoff levels of CRP (8.5 mg/L) and IL-6 (5.13 pg/mL). Subjects with low IL-6 concentrations had significantly lower levels of blood glucose in the OGTT at baseline than did subjects with high levels of IL-6 (fasting, 103 ± 17 vs 141 ± 79 mg/dL; NS: 1 hour, 160 ± 51 vs 249 ± 111 mg/dL, P < 0.01; 2 hour, 117 ± 38 vs 191 ± 108 mg/dL, P < 0.02). Patients within the high-CRP group at baseline had a significantly higher glucose-stimulated insulin concentration than did patients within the low-CRP group (fasting, 34 ± 19 vs 19 ± 8 μU/mL, P < 0.01; 1 hour, 153 ± 75 vs 89 ± 53 μU/mL, P < 0.01; 2 hour, 115 ± 69 vs 50 ± 35 μU/mL, P < 0.003). Patients with initially high CRP levels showed a significantly larger decrease in insulin concentrations during weight loss than did patients with low CRP values (fasting insulin, 19 ± 15 vs 8 ± 8.2 μU/mL, P < 0.02; 1 hour, 94 ± 81 vs 27 ± 38 μU/mL, P < 0.008; 2 hour, 84 ± 77 vs 27 ± 30 μU/mL, P < 0.02). The decrease in IL-6 concentrations was significantly correlated with decrease in TNF-α, blood glucose (fasting, 1 hour, and 2 hour), C-peptide, glycosylated hemoglobin, and insulin resistance, as estimated by HOMA (Table 4). The decrease in CRP was correlated with the decrease in metabolic parameters like BMI, 2-hour blood glucose, 1-hour insulin levels, and C-peptide (Table 4). Multiple linear regression analyses were performed to further assess the relation of the changes in IL-6 and CRP concentrations to the changes in variables of the IRS. Independent variables in the models were those that were significantly correlated with IL-6 and CRP in univariate analysis (Table 4). After adjusting for fasting blood glucose, glycosylated hemoglobin, and C-peptide, only the decrease in

| TABLE 2. Markers of Inflammation in Morbidly Obese Subjects According to Their Glucose Tolerance Status Before and After Gastroplastic Surgery |
|--------------------------------------------------|--------|--------|--------|
|                      | CRP, mg/L | IL-6, pg/mL | TNF-α, pg/mL |
|                      | Pre     | Post    | Pre     | Post    | Pre     | Post    |
| T2D                  | 14.5(6.6/20.3)† | 2.1(1.5/3.5)† | 8.8(3.8/18.7) | 4.5(1.7/9.6) | 8.2(5.3/14.8) | 13.7(4.9/17.4) |
| IGT                  | 8.4(2.1/11.7) | 2.6(1.1/8.1)* | 4.1(2.9/5.8) | 4.5(2.3/5.8) | 9.0(7.2/16.2) | 10.9(4.2/16.8) |
| NGT                  | 4.5(2.7/6.2) | 3.3(1.2/4.4) | 4.3(2.4/14.5) | 2.8(2.0/3.5) | 11.7(6.2/21.3) | 13.7(10.0/17.2) |

†P<0.02 for IGT preoperative versus postoperative.
*P<0.001 for IGT preoperative versus postoperative.
†P<0.05 for DM preoperative versus postoperative.
‡P<0.001 for DM preoperative versus postoperative.
§P<0.05 for DM versus NGT.

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insulin resistance remained independently and significantly associated with the decrease in IL-6 concentration \((P<0.01)\). In a similar model including the change in BMI, 1-hour insulin, HOMA, and C-peptide as independent variables, only the change in BMI was significantly related to the decrease in CRP \((P<0.05)\). In a model exploring TNF-\(\alpha\) as a dependent variable, none of these variables were significantly related with the change in the concentration of TNF-\(\alpha\).

**Discussion**

In the present study, we have shown a significant reduction in circulating CRP and IL-6 levels after weight loss after gastroplastic surgery in morbidly obese individuals. The decrease in inflammatory markers was related to improvement in insulin resistance, body-weight, and glycemia. Accordingly, a significant and impressive shift toward normalized glucose tolerance status was seen, with a reversal of overt type 2 diabetes in all subjects but 1.

In line with previous reports, we have shown an association between inflammatory proteins and parameters of the IRS by cross-sectional analysis.\(^{14,15,27-29}\) However, only limited data are available about the longitudinal effects of weight loss. In a study with 21 obese women, no significant decrease in CRP after weight loss after 3 weeks was observed,\(^{30}\) probably because of the small number of subjects and a relatively modest weight loss of \(\approx 3\) kg. Heilbronn et al\(^{31}\) reported elevated levels of CRP in obese but otherwise healthy female subjects, which declined during weight loss of \(\approx 7\) kg during a very-low-calorie diet. In another study, a significant decrease in CRP was shown in 25 obese, postmenopausal women completing a weight loss program with a reduction of 14 kg of body weight.\(^{32}\) However, because no measurements of IL-6 and TNF-\(\alpha\) were made in the latter studies, it remains speculative whether the increased concentration of CRP in these obese subjects was due to IL-6, as hypothesized.

Recently, in a study conducted with 20 obese women undergoing gastric banding, a decrease in CRP levels was found 1 year after surgery (mean weight loss, 30 kg), but TNF-\(\alpha\) levels remained unchanged and IL-6 levels moderately increased.\(^{33}\) Finally, Ziccardi et al\(^{34}\) showed decreased IL-6 as well as TNF-\(\alpha\) levels after a 1-year, multidisciplinary, weight reduction program (mean weight loss of 9.8 kg) in 56 healthy premenopausal, obese, non-diabetic women.

Taken together, the results of these studies, along with the results of the present study, suggest that weight loss results in decreased circulating levels of CRP. On the other hand, apparently conflicting results are reported on the effect of weight loss on levels of IL-6 and TNF-\(\alpha\).\(^{33,34}\) Baseline differences in the study populations (eg, glucose tolerance status or insulin resistance) might explain the differing results. In the present study, IL-6 was closely related to glycemia; however, a significant number of subjects had diabetes, whereas in the study of Laimer et al,\(^{33}\) only 1 and in the study of Ziccardi et al, none had diabetes. In addition to weight loss, the substantial change in dietary intake after gastroplasty might have contributed to the observed change in the concentrations of CRP and IL-6.

In multivariate analyses, the change in IL-6 was related to the change in insulin resistance, whereas the change in CRP was independently related to the change in BMI. Therefore, an intervention that might affect insulin resistance, glycemia, or body weight to a different degree might also affect CRP and IL-6 levels differently. In addition, the plasma half-life of IL-6 is \(< 6\) hours and thus, considerably less than the half-life of CRP;\(^{33}\) therefore, CRP might provide a more stable indicator of subclinical inflammation than IL-6. Finally, it is interesting to note that treatment with an insulin-sensitizing peroxisome proliferator-activated receptor-\(\gamma\) agonist beneficially affected CRP but not IL-6 levels in patients with type 2 diabetes, also suggesting differential effects of therapeutic interventions on CRP and IL-6 levels.\(^{35}\) A significant interrelation of inflammatory proteins, including IL-6, CRP, and TNF-\(\alpha\), has been shown in the present study as well as in previous reports.\(^{27}\) Inflammatory proteins have been related to body weight\(^{31}\) and insulin resistance.\(^{15}\) The present longitudinal study supports the view that adipose tissue, insulin resistance, and "proinflammatory cytokines" are part of an interrelated network that might eventually result in cardiovascular disease. The association of inflammatory markers with adipose tissue is supported by experimental and clinical evidence. In vivo experiments have demonstrated IL-6 mRNA expression on, as well as IL-6 release from, human adipose tissue.\(^{12,36}\)

IL-6 production derived from adipose tissue accounts for \(\approx 20\%\) to \(30\%\) of total IL-6 serum levels and is increased in adiposity.\(^{27,36}\) Obese patients have significantly higher serum concentrations of inflammatory proteins than do lean controls.\(^{37}\) IL-6 is a powerful inducer of acute-phase proteins, such as CRP.\(^{38}\) Epidemiological studies have shown a relation of CRP and IL-6 to measurements of the IRS,\(^{15,27,39,40}\) including BMI. Similar relations were found in the present study. Inflammatory proteins have also been related to

| TABLE 3. Baseline relationship of concentrations of proinflammatory cytokines and CRP with parameters of the Insulin Resistance Syndrome |
|-----------------|---------|---------|
|                 | TNF-\(\alpha\) | IL-6 | CRP |
| Site            |         |         |     |
|                 | \(0.59^\dagger\) | \(-0.13\) |     |
|                 | \(0.59^\dagger\) | \(-0.44^*\) |     |
|                 | \(0.20\) | \(0.03\) | \(0.20\) |
| Blood glucose   |         |         |     |
|                 | \(0.36^*\) | \(0.58^\dagger\) | \(0.07\) |
|                 | \(0.19\) | \(0.63^\dagger\) | \(0.20\) |
|                 | \(0.41^*\) | \(0.66^\dagger\) | \(0.21\) |
| Insulin         |         |         |     |
|                 | \(0.31\) | \(0.47^*\) | \(0.43^*\) |
|                 | \(-0.28\) | \(0.12\) | \(0.27\) |
|                 | \(-0.14\) | \(0.16\) | \(0.37^*\) |
|                 | \(0.48^*\) | \(0.56^\dagger\) | \(0.35^*\) |
|                 | \(0.32\) | \(0.36\) | \(0.56^\dagger\) |
| HbA1c           | \(0.43^*\) | \(0.61^\dagger\) | \(0.07\) |

\(\dagger P<0.05; \dagger P<0.01; \dagger P<0.001.\)

Values in bold remained significant after adjustment for multiplicity according to the method of Bonferroni-Holm.
measures of insulin resistance in the present study, as well as in previous reports. To our knowledge, this is the first study to show, by multivariate analysis, a significant correlation of changes in IL-6 with changes in insulin resistance measured by HOMA and of changes in CRP with changes in BMI after weight loss. The clinical relevance of CRP lowering associated with massive weight loss in morbidly obese patients has to be investigated in future studies. Although no specific drug treatment to reduce CRP values is available, both statins and aspirin attenuate the risk of coronary heart disease in individuals with increased CRP.

In the present study, we could demonstrate an impressive reduction in the levels of CRP and IL-6 by as much as 81% and 23%, respectively, compared with baseline levels. Interestingly, a doubling of CRP or IL-6 levels was found to be associated with a doubling in the risk for myocardial infarction in apparently healthy men. By analogy, it seems likely that a significant reduction in CRP and IL-6 levels associated with weight loss could also reduce the cardiovascular risk in morbidly obese patients.

In summary, we have shown a marked decrease in circulating levels of inflammatory markers in association with a reversal of diabetes in morbidly obese individuals after gastrosplastic surgery. Long-term studies are needed to show whether this improvement in cardiovascular risk factors will eventually translate into a significant clinical benefit in regard to cardiovascular morbidity and mortality.

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