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Effect of Massive Weight Loss on Inflammatory Adipocytokines and the Innate Immune System in Morbidly Obese Women

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Context: Obesity may be regarded as a low-grade inflammatory state.

Objective: The aim of this study was to evaluate changes in proinflammatory adipocytokines and the innate immune system, cardiovascular risk, and insulin sensitivity after massive weight loss.

Design: This was a longitudinal study.

Setting: The study was conducted at Catholic University, Rome.

Subjects and Methods: There were 10 normoglucose-tolerant obese women evaluated before and 36 months after bilio-pancreatic diversion (BPD). Glucose sensitivity (M value) was estimated using the euglycemic-hyperinsulinemic clamp. Mannan-binding lectin (MBL), bactericidal/permeability increasing protein (BPI), α-defensins, soluble CD14 receptor (sCD14), C-reactive protein, adiponectin, leptin, visfatin, IL-6, and TNF-α were assayed.

Results: After massive weight loss (53% of excess body weight), leptin ($P \leq 0.0001$), IL-6 ($P \leq 0.0001$), α-defensins ($P \leq 0.001$), and C-reactive protein ($P \leq 0.0001$) decreased significantly. Adiponectin increased significantly ($P \leq 0.001$). Of the nine subjects who lost more than 20% of body mass index, sCD14 (2.87 ± 0.5 to 2.55 ± 0.5; $P = 0.016$) and visfatin levels (12.20 ± 0.93 to 10.63 ± 1.93 ng/ml; $P = 0.045$) decreased significantly. No significant changes were observed in TNF-α, BPI, or MBL. Insulin sensitivity more than doubled after BPD ($P \leq 0.0001$). sCD14 changes were significantly associated with body mass index ($r_0 = 0.80$; $P = 0.003$) and M changes ($r_0 = -0.59$; $P = 0.03$). MBL correlated with insulin sensitivity in obese ($r_0 = 0.93$; $P = 0.0001$) and post-BPD women ($r_0 = 0.66$; $P = 0.038$). Adiponectin correlated negatively with cardiovascular risk ($r_0 = -0.709$; $P = 0.02$) and IL-6 ($r_0 = -0.634$; $P = 0.05$). Multiple linear regression analysis showed that changes in sCD14 were also significantly related to changes in insulin sensitivity.

Conclusions: Surgically induced weight loss is capable of reversing low-grade inflammation, at least partially. The relationships between sCD14, MBL, BPI, and glucose sensitivity, and the role of TNF-α in obesity warrant further investigation. **(*J Clin Endocrinol Metab* 92: 483–490, 2007)**

OBESITY MAY BE regarded as a low-grade inflammatory state (1). According to Pickup and Crook's (1) hypothesis, in individuals with an innately hypersensitive acute-phase response, long-term lifestyle and environmental stressors, such as nutrition, may produce obesity and diabetes, leading to an increased risk of cardiovascular disease. In response to chronic stressors, the system may become allostatic (*i.e.* the sustained effort to combat acutely challenges may ultimately result in an overload of the system's resources). When the allostatic load exceeds these resources, the system breaks down. Dieting or surgically induced weight loss may reduce the impact of stressors. Circulating levels of inflammatory adipocytokines, including TNF-α, and IL-6 are, in fact, lowered after weight loss (2–4).

Conversely, circulating levels of a molecule acting in the acute-phase response, mannan-binding lectin (MBL), have recently been reported to be reduced in obese, diabetic and insulin-resistant subjects (5). MBL deficiency may chronically activate the inflammatory cascade, contributing to the development of obesity and insulin resistance (5–7). MBL is a serum protein capable of activating the complement system, thereby promoting phagocytic clearance of various inflammatory agents. The protein shares structural similarities with adiponectin (8). MBL stimulates fatty acid oxidation in skeletal muscle in a manner similar to adiponectin (9), enhances phagocytosis (9), and reduces the release of TNF-α and IL-1 from monocytes (10). In a very recent study, we found that levels of MBL are reduced in obese and diabetic patients compared with normal weight control subjects, and increase after weight loss with improved insulin action in the former (5). MBL was associated with glucose metabolism in patients with gestational diabetes mellitus.

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Abbreviations: BMI, Body mass index; BP, binding protein; BPD, bilio-pancreatic diversion; BPI, bactericidal/permeability increasing protein; CRP, C-reactive protein; CV, coefficient of variation; FFA, free fatty acid; FFM, fat-free mass; FM, fat mass; HDL, high-density lipoprotein; MBL, mannan-binding lectin; sCD14, soluble CD14 receptor.

We have also recently shown that the bactericidal/permeability increasing protein (BPI), a major constituent of neutrophils (from 0.5–1% of total protein) (11), and, thus, a key component in innate immunity, is not only linked to inflammatory pathways but also seems to be associated with the action of insulin (12). Both MBL and BPI play an important role in subjects with glucose intolerance or type 2 diabetes.

The soluble CD14 receptor (sCD14) is also involved in the activation of the innate immune system. This is a multifunctional receptor that is expressed in considerable amounts on the surface of mature monocytes, macrophages, and neutrophils (13–15). Exposure to lipopolysaccharide (16) significantly influences its expression. Circulating levels of the CD14s are inversely proportional to insulin resistance in apparently healthy subjects (17).

α-Defensins belong to the family of cationic trisulfide-containing microbicidal peptides. Like MBL, BPI, and sCD14, they participate in the host defense and inflammation (18), but their action in insulin metabolism has yet to be investigated.

In the present study the working hypothesis is that a stable weight loss, as can be achieved after bariatric surgery, may restore the metabolic homeostasis disturbed by chronic stressors. Thus, levels of inflammatory molecules must be decreased, and the cardiovascular risk and insulin resistance ameliorated. In this context, changes in the molecules of the innate immune system may be of interest. To test this hypothesis, we evaluated 10 normoglucose tolerant morbidly obese women before and 24 months after bilio-pancreatic diversion (BPD). This kind of malabsorptive bariatric surgery represents an exceptional model of stable weight loss in human beings (19). Unlike the situation in Europe, this technique is not frequently performed in the United States, where the preferred type of bariatric surgery is the gastric bypass, a restrictive procedure. Nevertheless, an appreciable number of clinical studies, including more than 4000 subjects worldwide, have been conducted on malabsorptive surgery (20). It has been shown that BPD fully normalizes insulin sensitivity, as well as reduces cardiovascular risk (19) and inflammation (4).

We evaluated levels of proteins involved in the acute-phase response [MBL, sCD14, BPI, α-defensins, C-reactive protein (CRP)], and adipocytokines (adiponectin, leptin, visfatin, IL-6, and TNF-α). Insulin sensitivity and secretion were also estimated.

Subjects and Methods

Study subjects

At the Department of Internal Medicine of the Catholic University in Rome, patients who have undergone bariatric surgery are admitted to the unit monthly in the first three months after surgery for thorough follow-up and are then monitored every 3 months. From the second year on, they are admitted every 6 months or whenever necessary. Patients and physicians remain in contact by phone or E-mail. As a result, patients are strongly motivated to undergo physical examinations and laboratory tests, which routinely include evaluation of insulin-mediated glucose metabolism and body composition.

Of these subjects, 10 morbidly obese women were selected [body mass index (BMI) $\geq$ 40 kg/m^2] to be recruited into the present study. Inclusion criteria were as follows: age 20–35 yr, no smoking habits, no alcohol consumption, blood pressure 130/80 mm Hg or less, normal glucose tolerance evaluated by a standard glucose tolerance test (21), normal thyroid function and cortisol secretion, regular menses, no regular physical exercise. Women were evaluated in the early follicular phase of the ovulatory menstrual cycle, before and 24 months after BPD. Medical histories, physical examinations, electrocardiograms, and blood screening showed that all patients were in good health. None of the subjects had a history of hepatic or renal disorders, and none were taking anticonvulsant medications or corticosteroids. Hypercholesterolemia ($\geq$5.17 mmol/liter) and hypertriglyceridemia ($\geq$1.92 mmol/liter) were diagnosed according to the World Health Organization criteria (22). Cardiovascular risk was estimated as previously described (23). These subjects were not taking any medications, except after BPD, when they were prescribed oral supplementation of sulfate iron 525 mg/d, calcium carbonate 1 g/d, multivitamins (Supradyn Roche, Milan, Italy), one tablet a day, and ergocalciferol 400,000 IU im (Ostelin fl; Teofarma, Valle Salimbene, Italy) every 2 wk.

The Catholic University Ethical Committee approved the study, and all subjects signed an informed consent form before participation.

Methods

The isotopic dilution method was used to estimate body composition (24). Fat-free mass (FFM) (in kilograms) was obtained by dividing total body water by 0.73.

Subjects underwent BPD, which is essentially a malabsorptive surgical procedure (25). It consists of an approximate 60% distal gastric resection with stapled closure of the duodenal stump. The residual volume of the stomach is around 300 ml. The small bowel is transected at 250 cm from the ileocecal valve, and its distal end is anastomosed to the remaining stomach. The proximal end of the ileum, comprising the remaining small bowel carrying the bilio-pancreatic juice and excluded from food transit, is anastomosed in an end-to-side fashion to the bowel 50-cm proximal to the ileocecal valve. The total length of absorbing bowel is brought to 250 cm, the final 50 cm of which, the so-called common channel, represents the site where ingested food and bilio-pancreatic juices mix.

In the whole population insulin sensitivity was estimated using an euglycemic hyperinsulinemic clamp, as previously described (26). Whole-body glucose uptake (M value in mmol·kg$_{FFM}^{-1}$·min^{-1}) was determined during a primed constant infusion of insulin (at the rate of 6 pmol·min^{-1}·kg^{-1}). The fasting plasma glucose concentration was maintained throughout the insulin infusion by a method of a variable glucose infusion and blood glucose determinations every 5 min. Whole-body peripheral glucose use was calculated during the last 40-min period of the steady-state insulin infusion.

Analytical assay

Serum samples were collected in tubes with aprotinin (500 U/liter) in an ice bath and frozen immediately at −80 C. Plasma glucose was measured by the glucose oxidase method (Beckman Coulter, Inc., Fullerton, CA). Free fatty acids (FFAs) were measured spectrophotometrically. Hormones were all assayed in duplicate. Plasma insulin was assayed by microparticle enzyme immunoassay (Abbott, Pasadena, CA) with a sensitivity of 1 μU/ml and an intraassay coefficient of variation (CV) of 6.6%. IGF-Is were measured by RIA using a commercial kit (INCSTAR Corp., Stillwater, MN), and IGF binding proteins (IGFBPs)-1 and -3 were measured by a RIA commercial kit with intraassay and interassay CVs of 8% or less for both proteins (Mediagnost, Tubingen, Germany). Leptin and adiponectin were measured by RIA kits (Linco Research Inc., St. Charles, MO) with a CV less than 5%. IL-6 and TNF-α were measured by the enzyme-linked immunosorbent assay method (R&D System, Minneapolis, MN), and CRP was assessed by routine laboratory tests (Beckman Coulter, Inc.). Circulating sCD14 was measured by the sCD14 EASIA (Biosource Europe SA, Zoning Industriel B-62220, Fleunes, Belgium), a solid phase enzyme amplified sensitivity immunoassay performed on microliter plates. The minimum detectable concentration is estimated to be 1 ng/ml and is defined as the sCD14 concentration corresponding to the average OD of 20 replicates of the zero SD. The intraassay and interassay CVs were <5.2% and 7.8%, respectively. Plasma levels of MBL were determined using the MBL ELISA kit (AntibodyShop, Copenhagen, Denmark). The lower detection

limit was 5 ng/ml for undiluted samples. Intraassay and interassay CVs were <8%. Visfatin was measured using a commercial EIA kit (Phoenix Pharmaceuticals, Inc., Belmont, CA), with a sensitivity of 2 ng/ml, interassay CV 10%, and intraassay CV 5%. Plasma EDTA BPI and α-defensin concentrations were measured by a sandwich ELISA (human BPI ELISA kit; HyCult Biotechnology, Uden, The Netherlands) according to the manufacturer's instructions. The assays had sensitivities of 250 and 50 pg/ml, respectively. Intraassay and interassay CVs were less than 5% for both.

Statistical methods

Data are presented as mean ± SD. Before statistical analysis, normal distribution and homogeneity of the variances were tested. Parameters that did not fulfill these tests (MBL) were log transformed. Comparisons between groups were performed using the paired *t* test. Relationships between variables were sought by linear correlation analysis (Spearman's r) and regression analysis performed using standard techniques. Levels of statistical significance were set at $P < 0.05$. Data analyses were performed with SPSS statistical software (SPSS V12.0, Inc., Chicago, IL).

Results

Body composition and analytical parameters

Patients lost an average of 53% of their excess body weight. BMI ($P \leq 0.0001$), weight ($P \leq 0.0001$), and fat mass (FM) ($P \leq 0.0001$) were significantly reduced 2 yr after BPD (Table 1). At baseline, the prevalence of hypercholesterolemia and hypertriglyceridemia was 50% and 20%, respectively. After surgery, values of total cholesterol and triglycerides were within normal ranges in all patients. The significant improvement in the lipid profile (Table 1) led to a dramatic reduction of the cardiovascular risk (from 2.00 ± 2.21 to −2.70 ± 2.11; $P \leq 0.0001$). Leptin was significantly reduced from 63.35 ± 9.11 to 18.49 ± 5.20 ng/ml ($P \leq 0.0001$). Circulating adiponectin increased by 41% (10.07 ± 2.64 *vs.*18.49 ± 5.20 mg/dl; $P \leq 0.001$). Both IL-6 ($P \leq 0.0001$) and CRP ($P \leq 0.0001$) decreased significantly. α-Defensins decreased from 1396 ± 887 to 558 ± 388 pg/ml after surgery ($P = 0.005$). No changes were found in circulating levels of visfatin, TNF-α, MBL, bactericidal/permeability-increasing protein, and sCD14 (Table 1). However, in the nine subjects who lost more than 20% of BMI, there were less than significant reductions in sCD14 (from 2.87 ± 0.5 to 2.55 ± 0.5; $P = 0.016$) and visfatin (from 12.20 ± 0.93 to 10.63 ± 1.93 ng/ml; $P = 0.045$) after weight reduction. Fig. 1 shows individual changes in serum fasting adipocytokines and molecules of the innate immune system.

Data relating to insulin metabolism and the IGF-I axis are reported in Table 2. Values of whole-body insulin mediated glucose uptake for each patient before and after BPD are also presented in Fig. 1.

TABLE 1. Anthropometrical characteristics, blood pressure, and serum markers of inflammation in women at the baseline and after weight loss

	Obese normoglucose-tolerant women (n = 10)	
	Baseline	After BPD
Age (yr)	38 ± 13	41 ± 12[d]
Body weight (kg)	114 ± 13	85 ± 14[d]
BMI (kg/m^2)	42 ± 6	32 ± 5[d]
FFM (kg)	71 ± 10	63 ± 8[d]
FM (kg)	44 ± 10	23 ± 6[d]
WHR	0.96 ± 0.04	0.90 ± 0.03[b]
SBP (mm Hg)	146 ± 13	114 ± 7[d]
DBP (mm Hg)	89 ± 2	80 ± 8[d]
Fasting glucose (mmol/liter)	5.98 ± 1.90	3.86 ± 0.30[b]
Fasting insulin (pmol/liter)	93.60 ± 56.50	41.16 ± 24.01[a]
Total cholesterol (mmol/liter)	5.36 ± 1.19	3.34 ± 0.66[c]
HDL cholesterol (mmol/liter)	1.14 ± 0.27	1.42 ± 0.29[a]
Triglycerides (mmol/liter)	1.45 ± 0.60	1.0 ± 0.45[a]
FFAs (mM)	0.42 ± 0.10	0.20 ± 0.07[d]
TNF-α (pg/ml)	1.97 ± 0.92	2.39 ± 1.33
CRP (mg/l)	1.03 ± 0.24	0.22 ± 0.08[d]
IL-6 (pg/ml)	3.38 ± 0.55	1.66 ± 0.55[d]
sCD14 (μg/ml)	2.50 ± 0.56	2.53 ± 0.57
BPI (pg/ml)	19532 ± 8634	16437 ± 8126
α-Defensins (pg/ml)	1396 ± 887	558 ± 338[b]
MBL (mg/ml)	3.95 ± 0.61	3.74 ± 0.21

To convert glucose to mg/dl, divide by 0.05551. To convert insulin to μUI/liter, multiply by 7.175. To convert values for triglycerides to mg/dl, divide by 0.01129. To convert values for cholesterol to mg/dl, divide by 0.02586. DBP, Diastolic blood pressure; HDL, high-density lipoprotein; SBP, systolic blood pressure; WHR, waist to hip ratio.

Data are mean ± SD. Levels of significance at paired *t* test: [a] $P \leq 0.05$; [b] $P \leq 0.01$; [c] $P \leq 0.001$; and [d] $P \leq 0.0001$.

Linear correlations

In the nine subjects who lost more than 20% of BMI, the change in circulating sCD14 was significantly associated with changes in BMI ($r_0 = 0.80$; $P = 0.003$) and insulin sensitivity ($r_0 = -0.59$; $P = 0.03$).

In obese women, circulating levels of MBL correlated with insulin sensitivity ($r_0 = 0.93$; $P = 0.0001$) and with circulating levels of IL-6 ($r_0 = -0.695$; $P = 0.04$); CRP correlated with BMI ($r_0 = 0.681$; $P = 0.03$) and body weight ($r_0 = 0.687$; $P = 0.03$). Levels of α-defensins correlated with triglyceride concentrations ($r_0 = 0.714$; $P = 0.05$). Leptin correlated with both FM ($r_0 = 0.768$; $P = 0.04$) and insulin levels ($r_0 = 0.45$; $P = 0.001$). Adiponectin correlated negatively with cardiovascular risk ($r_0 = -0.709$; $P = 0.02$), levels of IL-6 ($r_0 = -0.634$; $P = 0.05$), fasting glucose ($r_0 = -0.711$; $P = 0.02$), and insulin ($r_0 = -0.823$; $P = 0.05$), and positively with high-density lipoprotein (HDL) cholesterol ($r_0 = -0.675$; $P = 0.03$). Visfatin correlated positively with total cholesterol ($r_0 = 0.857$; $P = 0.014$) and negatively with fasting insulin secretion ($r_0 = -0.821$; $P = 0.02$). Glucose uptake correlated positively with the concentration of IGFBP-1 ($r_0 = 0.774$; $P = 0.001$).

A significant direct correlation of circulating levels of MBL with insulin sensitivity ($r_0 = 0.66$; $P = 0.038$) and an inverse correlation with fasting insulin secretion ($r_0 = -0.697$; $P = 0.02$) were observed. Furthermore, sCD14 correlated with both levels of fasting insulin ($r_0 = 0.93$; $P = 0.0001$) and triglycerides ($r_0 = 0.93$; $P = 0.0001$). CRP correlated with insulin ($r_0 = 0.762$; $P = 0.010$). Levels of visfatin were related to fasting glucose ($r_0 = -0.783$; $P = 0.02$), FFAs ($r_0 = -0.838$; $P = 0.009$), adiponectin ($r_0 = -0.810$; $P = 0.015$), and BPI ($r_0 = -0.90$; $P = 0.037$). α-Defensins correlated with levels of CRP ($r_0 = 0.829$; $P = 0.042$) and triglycerides ($r_0 = 0.886$; $P = 0.019$), and BPI with fasting glucose ($r_0 = 0.975$; $P = 0.005$) and insulin ($r_0 = 0.90$; $P = 0.037$). Again the M value and IGFBP-1 were significantly related ($r_0 = 0.802$; $P = 0.005$).

Changes in circulating levels of sCD14 correlated with those of insulin-mediated glucose uptake ($r_0 = -0.721$; $P = 0.019$), BPI ($r_0 = -0.97$; $P = 0.0001$), IL-6 ($r_0 = 0.721$; $P = 0.019$), with changes in BMI ($r_0 = 0.794$; $P = 0.006$) and body weight ($r_0 = 0.782$; $P = 0.008$). Changes in MBL were related

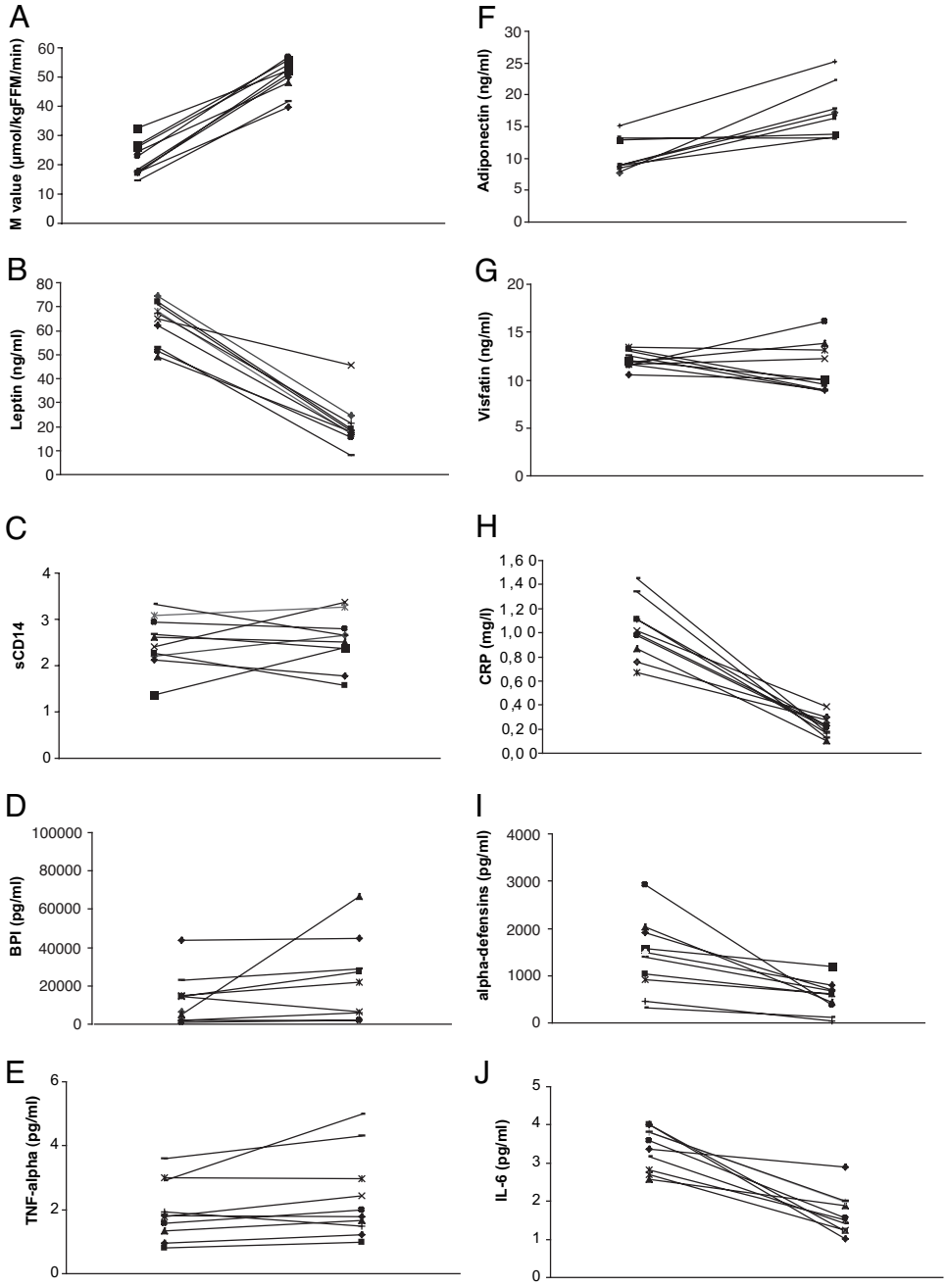

FIG. 1. Changes in whole-body insulin sensitivity (A), fasting leptin (B); sCD14 (C), BPI (D), TNF-α (E), adiponectin (F), visfatin (G), CRP (H), α-defensins (I), and IL-6 (J) before and after BPD for each patient.

to changes in CRP ($r_0 = -0.750$; $P = 0.02$), fasting ($r_0 = 0.667$; $P = 0.050$), and total insulin secretion ($r_0 = 0.883$; $P = 0.005$).

Changes in IL-6 and BPI were also significantly correlated ($r_0 = -0.96$; $P = 0.0001$).

Changes in circulating insulin correlated with those of adiponectin ($r_0 = -0.811$; $P = 0.004$) and leptin ($r_0 = 0.520$; $P = 0.02$).

Table 3 shows predictors of changes in glucose disposal as dependent variables among adipocytokines and molecules of the innate immune system in a multiple step linear regression.

Discussion

Circulating levels of IL-6, leptin, and PCR were reduced in morbidly obese women after bariatric surgery. Adiponectin increased as the cardiovascular risk decreased. No significant changes were observed in circulating TNF-α or in levels of circulating MBL and bactericidal/permeability-increasing protein. Significant changes were observed in the levels of sCD14 and visfatin in patients who lost more than 20% of their initial BMI. α-Defensin levels were significantly decreased after BPD. Of the molecules of the innate immune system, there was a positive correlation between MBL and whole-body glucose uptake before and after weight loss. Changes in sCD14 were significantly related to changes in BMI and insulin sensitivity.

According to the present report, elevation of CRP, TNF-α, and IL-6 characterizes morbid obesity as a condition of a low-grade inflammatory process (27–30). Bariatric surgery

TABLE 2. Data on the IGF system, insulin sensitivity, and secretion before and after BPD

	Obese normoglucose-tolerant women (n = 10)	
	Baseline	After BPD
IGF-I (ng/ml)	130.9 ± 28.79	127.6 ± 29.75
IGFBP-1 (ng/ml)	25.23 ± 4.46	23.35 ± 3.63
IGFBP-3 (ng/ml)	6430 ± 1628	6359 ± 1232
M (μmol/kg$_{FFM}$/min)	14.92 ± 1.23	39.13 ± 0.98[b]
M (μmol/kg$_{BW}$/min)	13.62 ± 3.89	36.94 ± 4.67[b]
Insulin at the EHC steady state (pmol/liter)	618 ± 67	614 ± 67
Fasting insulin secretion (pmol·min^{-1}·m^2)	152 ± 83	89 ± 46[a]
Total insulin output (nmol·m^{-2})	52 ± 17	31 ± 16[a]
β-cell glucose sensitivity (pmol·min^{-1}·m^2·mM^{-1})	80 ± 5	98 ± 5[a]
Rate sensitivity (nmol·m^{-2}·mM^{-1})	0.88 ± 0.27	0.32 ± 0.50
Potentiation factor (fold)	0.87 ± 0.10	0.83 ± 0.3

BW, Body weight; EHC, euglycemic hyperinsulinemic clamp; M, insulin-mediated glucose uptake.

Data are mean ± SD. Levels of significance at paired *t* test: [a] $P \leq 0.05$; [b] $P \leq 0.0001$.

can cause these adipocytokines partly to decline, thus suggesting that resolution of low-grade inflammation occurs. This observation is in line with those reports showing that: 1) IL-6 production by adipose tissue can only account for 10–30% of the entire circulating IL-6 concentration (27); 2) a significant reduction of FM is associated with a considerable reduction of IL-6 (4); 3) IL-6 is the main stimulating factor for hepatocyte synthesis and secretion of CRP in human beings (31); and 4) changes in either FM or weight cannot translate into changes in circulating TNF-α.

Changes in IL-6 concentration reflect the reduction of the degree of obesity more than circulating TNF-α (27). As far as the whole-body glucose uptake is concerned, as described by Ryan and Nicklas (3), we report that IL-6 levels before and after BPD do not correlate with insulin resistance, but when in a multi-step regression analysis percentage changes in adipocytokines are considered, IL-6 and leptin are strongly associated with changes in the amount of whole-body glucose disposal (Table 3). A negative relationship between plasma glucose use and levels of IL-6 has been described in Pima Indians (32). Indeed, for the first time, we show an inverse *in vivo* correlation between levels of circulating IL-6 and adiponectin. The action of IL-6 reduces adiponectin gene expression (33).

TABLE 3. Predictors of changes in metabolized glucose (M value) as dependent variables among adipocytokines and molecules from the innate immune system

Adipocytokines (changes %)	β	P	Molecules of innate immune system (changes %)	β	P
Adiponectin	0.019	0.330	α-Defensins	−0.161	0.315
IL-6	−0.642	**0.0001**	CRP	−0.799	**0.001**
Leptin	−0.377	**0.014**	MBL	0.339	**0.047**
TNF-α	−0.09	0.930	BPI	−0.157	0.330
Visfatin	−0.042	0.668	sCD14	0.280	**0.041**

The models explain the 95% and 85% of the variance for changes in M value (insulin-mediated glucose uptake), respectively. Values that are statistically significant are in *bold* font.

The finding that significant weight loss does not influence circulating TNF-α is not surprising. Levels of TNF-α did not decrease in short-term (34) and long-term patient follow-ups after bariatric surgery (6, 35), and in very active weight-losing patients (36). A number of hypotheses have been formulated to explain the absence of changes in circulating TNF-α after bariatric surgery. The relative starvation of post-BPD patients leads to the persistence of a stressful condition (37). Alternatively, after bariatric surgery, patients generally remain relatively obese, and a certain amount of adiposity is likely to be lost before any effect of body weight on circulating TNF-α is observed, although the expression of TNF-α is locally reduced 2 yr after BPD (38). Moreover, a local rather than a systemic effect of TNF-α may be more important for insulin resistance (3). Finally, the increase in systemic TNF-α might be related to the presence of nonalcoholic steatohepatitis, both in the obese and the post-obese state (39).

Adipocytokines showed a different trend. Although adiponectin and leptin levels decreased after surgery independently of the amount of weight loss, a decrease in visfatin levels did not occur in all patients (Fig. 1). The meaning of this heterogeneous response to surgery in terms of weight loss (19) and changes in hormonal milieu is unclear. The experimental evidence that visfatin does not decrease uniformly in all patients after weight loss suggests that the improvement in the obesity related low-grade inflammatory state cannot be explained only in terms of a reduction of visceral FM. Contrasting results have been reported so far in the literature concerning the relationship between visfatin/pre-B-cell colony enhancing factor and insulin sensitivity in obesity and after weight loss (40); but, to the best of our knowledge, this is the first report investigating the effect of massive weight loss on visfatin levels with regard to changes in markers of inflammation and innate immune system activity. In a prospective study concerning 31 morbidly obese patients, visfatin and leptin were reduced, and adiponectin concentrations increased 6 months after gastric banding, but no significant changes occurred in insulin sensitivity (41). In our series we found a negative correlation between visfatin and fasting insulin secretion in obese women and with fasting glucose after weight loss. As for the role of visfatin, insulin-mimetic actions are to be expected, as supported by experimental animal data (41), and, thus, a higher serum visfatin concentration may respond to low insulin secretion with a compensatory mechanism aimed at improving the functional consequences of relative insulin deficiency. Very recently, Haider *et al.* (42) reported on the effects of glucose and insulin on circulating visfatin *in vivo* and on visfatin secretion *in vitro*. They found an up-regulation of visfatin by hyperglycemia both *in vivo* and *in vitro*. Although insulin administration prevented the increase in visfatin concentrations under these conditions, insulin alone had no effect on either plasma visfatin or visfatin expression (42).

The negative correlation between obesity and circulating adiponectin has been well established, and adiponectin concentrations increase concomitantly with weight loss (33). Decreased adiponectin concentrations are associated with insulin resistance and hyperinsulinemia. However, the role of insulin in the regulation of adiponectin production is far from being clarified. There are reports that insulin can either

stimulate or inhibit adiponectin gene expression or secretion in cultured adipocytes (33). In our series an inverse correlation between this hormone and fasting glucose and insulin levels was found, and its changes after weight loss were related to those of insulin and leptin. The synthesis and secretion of adiponectin are decreased in the presence of a calorie excess, presumably associated with leptin deficiency or resistance (33). Several genes linked to circulating adiponectin levels have pleiotropic genetic effects on serum HDL and triglyceride levels. Data from two large cross-sectional studies indicate that after adjusting for both sex and body adiposity, circulating adiponectin concentrations correlate negatively with triglyceride levels and positively with plasma HDL concentrations (43, 44). Our findings confirm the protective role of adiponectin against cardiovascular risk factors, as demonstrated by its positive correlation with HDL cholesterol.

Changes in leptin along with those in circulating IL-6 contribute to explaining the changes in insulin sensitivity after weight loss. Both adipocytokines may thus contribute to the accelerated atherosclerosis associated with insulin resistance, glucose intolerance, and central obesity (45). Leptin is firmly believed to be an important signal that regulates the immune response, with a special role in the up-regulation of inflammation, but we were unable to find any relationship between levels of this hormone and molecules of the acute-phase response, including CRP. Recent studies have found that high levels of leptin, along with CRP and IL-6 independently of each other, increase the risk of metabolic and cardiovascular disease (46–48).

Because the present study was not performed under conditions of acute energy restriction, IGF-I levels were unchanged after weight reduction. Constant levels of IGF-I after BPD, as previously described (49), also demonstrate the adequacy of protein supplementation, because protein depletion has been shown to reduce IGF-I concentrations. Because changes in the IGF-I axis did not occur, it is unlikely that IGF-I can significantly affect insulin sensitivity and contribute to reducing the low-grade inflammatory state after weight loss. However, we observed a positive correlation between fasting insulin and IGFBP-I, both before and after weight loss.

As far as the role of molecules of the innate immune system is concerned, changes in CD14 and MBL concentrations were related to changes in insulin sensitivity, as well as to absolute values of MBL and insulin-mediated glucose uptake (Fig. 2). In agreement with other reports (3, 32, 50), we found a significant correlation between changes in insulin-mediated glucose uptake sensitivity and changes in CRP.

The meaning of the associations between sCD14 and MBL concentrations and insulin sensitivity is unclear. In actual fact, in our own and in previous series of patients affected by chronic inflammatory diseases (51) or obesity (5), within-person variations in MBL concentration are quite small. It is feasible that the MBL concentration may increase up to 3-fold during acute phase responses (6, 52). MBL and sCD14 act as buffers against bacterial products, preventing their interaction with membrane-associated receptors of monocytes and macrophages, which are embedded within the adipose tissue, such as the Toll receptor 4 (53). The activation of Toll receptor 4 induces inflammation by the activation of the redox-sensitive pro-inflammatory transcription factor, nuclear factor-$_k$B (52), and suppresses the insulin transduction pathway by acting through the serine/threonine phosphorylation of insulin receptor 1.

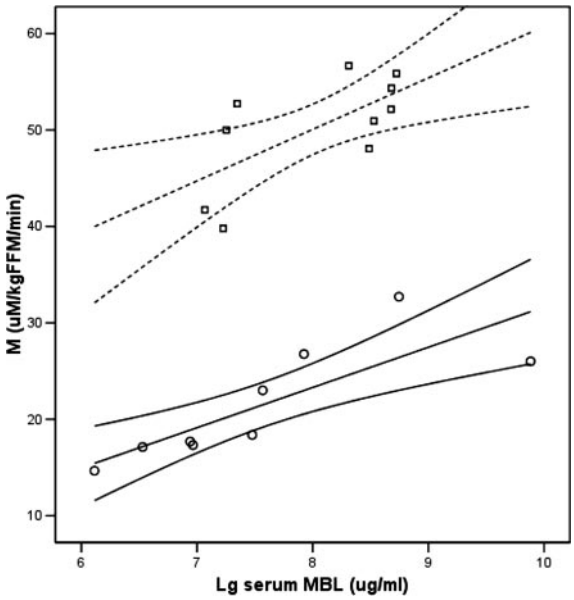

FIG. 2. Relationship between insulin-mediated glucose uptake (M value) and l g serum MBL (μg/ml). In obese women (*circles*, *continuous line*), y = 0.1594x + 4.1413 (R^2 = 0.66; P = 0.012). In post-BPD (*squares*, *dashed line*), y = 0.0839x + 3.8144 (R^2 = 0.45; P = 0.001). Lg, natural logarithm.

Our findings at least partly confirm a role of BPI in metabolic pathways (12). In a very recent study, we found that BPI concentrations were significantly different across categories of glucose tolerance, being significantly lower in patients with type 2 diabetes. Improvement of insulin sensitivity by the administration of an insulin-sensitizer drug (metformin) increased the levels of BPI (12). In the present series, we still found a correlation between BPI, fasting glucose, and insulin concentrations, but the improvement in insulin sensitivity due to the weight loss was not associated with any increase in BPI levels.

One of the most interesting findings of the present study was the significant decrease in α-defensin levels after weight loss. Activated neutrophils exclusively release this protein. Its concentration is low in healthy subjects, while it increases substantially in inflammatory diseases. It plays a key role in the host defense but also in the recruitment of the adaptive immune system in instances of infection and/or inflammation (54). The correlation we observed between levels of this protein and circulating triglycerides may suggest a role for lipids in regulating inflammatory pathways (55) and neutrophil functions. Moreover, also in obese and post-obese subjects, insulin and glucose metabolism might play an appreciable role in neutrophil regulatory pathways, as recently shown in nondiabetic healthy subjects (56). The cellular functions of human neutrophils, including bactericidal activity, require energy derived from glucose. Although insulin does not stimulate hexose transport in these immune cells, previous reports have clearly shown that this hormone is ca-

pable of regulating glucose metabolism in neutrophils (57–58).

We are aware of the limitations of this study. First of all we selected only severe obese women (BMI $\geq$ 40 kg/m^2), who were otherwise healthy apart from a mild dyslipidemia. The small number of subjects recruited and the gender specificity were due to our desire to avoid confounding factors related to sex hormones, or the presence of hypertension or impaired glucose tolerance. Moreover, the number of obese female patients who undergo bariatric surgery is three times the number of males monitored at our medical center (19), thus it is very hard to select men with the aforementioned characteristics. In a previous study, we estimated that 69% of morbidly obese women have one cardiovascular risk factor associated with the metabolic syndrome; 21% have two risk factors and 7% have three or more factors (19). We do not know to what extent the present findings can be extended to women with "simple obesity," to moderately obese male subjects and to obese subjects with comorbidities, such as hypertension or impaired glucose metabolism.

In conclusion, surgically induced weight loss is capable of partially reversing the condition of low-grade inflammation that characterizes obesity. Despite the fact that our results add new insights with regard to our understanding of the finer mechanisms linking obesity, insulin resistance, and inflammation, further investigation is needed to clarify the role of adipocytokine production and activation of the innate immune system in obesity and insulin resistance.

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