Adipose Inflammation in Obesity: Relationship With Circulating Levels of Inflammatory Markers and Association With Surgery-Induced Weight Loss

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Context: The inflammatory state of the adipose tissue is believed to contribute to systemic low-grade inflammation in obesity.

Objective: This study assessed the relationship between adipose and circulating inflammatory markers as well as the influence of adipose inflammation on bariatric surgery-induced weight reduction.

Design: This was a cross-sectional and longitudinal study (up to 14 mo).

Setting: The study was conducted in the digestive/bariatric surgery department of the Tivoli and Jean Villar clinics, Bordeaux, France.

Patients: Thirty-seven obese patients [body mass index (BMI) $>$ 35–40 kg/m^2)] seeking bariatric surgery were included. Twenty-eight of them were successively followed up at 1–3 months after surgery and 25 between 6 and 14 months after surgery.

Main Outcome Measures: Fasting serum samples were collected before surgery to assess concentrations of inflammatory markers. Samples of visceral adipose tissue were extracted during surgery and gene expression of cytokines and immune cell markers were evaluated using quantitative RT-PCR. Pre- and postsurgery weight and BMI were collected.

Results: Gene expression of several cytokines were strongly intercorrelated in the visceral adipose tissue. Adipose expression of macrophage and T cell markers were related to adipose expression of TNF-α and IL-1 receptor antagonist ($P < .01$) and to systemic levels of TNF-α ($P < .01$) and IL-6 ($P < .05$). A higher inflammatory state of the adipose tissue predicted a lower BMI reduction after surgery ($P < .05$), notably at early stages after surgery.

Conclusions: These findings support the involvement of macrophages and T cells in adipose inflammation and provide new information regarding the role of the visceral adipose tissue in the inflammatory state of obesity and its impact on obesity treatment outcomes, such as surgery-induced weight loss. **(*J Clin Endocrinol Metab* 99: E53–E61, 2014)**

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Abbreviations: BMI, body mass index; CI, confidence interval; CRP, C-reactive protein; Ct, cycle threshold; FOXP3, Forkhead box P3; hs, high sensitivity; IL-1ra, IL-1 receptor antagonist; MCP-1, monocyte chemoattractant protein-1; TBX21, T-box 21; Th, T helper.

Obesity is characterized by a low-grade inflammatory state reflected by chronic increases in circulating concentrations of inflammatory markers, including proinflammatory cytokines (eg, IL-6, TNF-α) and C-reactive protein (CRP) (1). This chronic low-grade inflammatory state is believed to originate in part from the adipose tissue, consistent with findings showing associations between inflammatory markers and measures of central adiposity (2, 3). Of note, complementary and nonexclusive factors and/or mechanisms associated with obesity, including insulin resistance and hepatic nonalcoholic fatty liver/hepatic steatosis, may also promote chronic low-grade inflammation (4–6). Interventions to reduce weight in obese patients are associated with improved systemic inflammation (7). In addition, higher expression of inflammatory markers has been documented in the adipose tissue of obese patients compared with lean subjects, and this expression was reduced after weight loss (8–10). Altogether these data support the notion that adiposity contributes to low-grade inflammation in obesity.

Inflammation in the adipose tissue relies on the involvement of several actors, including adipocytes that have the ability to secrete adipokines, eg, cytokines and other inflammatory markers such as acute-phase proteins (11). In addition, a substantial literature establishes the role of macrophages in the inflammatory state of obesity. Weight gain is associated with a significant recruitment of macrophages in the adipose tissue contributing to the increased expression of inflammatory factors (12, 13). In addition, obesity is associated with changes in macrophage polarization, with an increase in the M1 (proinflammatory) to M2 (antiinflammatory) ratio (14, 15). Interestingly, massive weight loss was shown to result in a marked reduction in macrophage infiltration and in the proinflammatory profile of macrophage phenotypes, which might relate to significant improvement in the adipose inflammatory state (16, 17). More recently, findings have also suggested the involvement of T cells in the inflammatory state originating from the adipose tissue. In support of this, experimental studies in animals have shown that weight gain induced by a high-fat diet is associated with an infiltration of T cells in the adipose tissue preceding the recruitment of macrophages (18, 19). In obesity, the population of T cells that is believed to be increased concerns primarily cytotoxic and T helper (Th) lymphocytes, in contrast to regulatory T lymphocytes, which were found to be decreased in some but not all studies (20–22). Accordingly, a recent study found a higher proportion of regulatory T cells associated with systemic and adipose inflammation in obese patients compared with lean subjects (23). In this study, however, adipose inflammation was limited to the expression of a macrophage marker (CD68) and expression of cytokines was limited to TNF-α and the chemotactic factor for T cells, CCL-5.

Despite the admitted role of the adipose tissue in obesity-related low-grade inflammation, the relationship between adipose vs circulating markers of inflammation has been explored only in few studies. In previous investigations, circulating concentrations of CRP were found to associate with sc adipose tissue expression of IL-6 and TNF-α and with IL-6 concentrations in the portal vein (24, 25). Nevertheless, a more specific characterization of the inflammatory profile of obese subjects, associating systemic and adipose markers of inflammation, is still needed. In addition, the question whether adipose and systemic inflammatory profiles/specificities in obesity may influence the outcome of bariatric surgery on weight reduction remains to be elucidated.

The present study aimed at assessing the relationship of systemic inflammation with gene expression of cytokines and markers of macrophage and T cell subpopulations in the visceral adipose tissue of severely obese patients. Moreover, the association of systemic and adipose inflammation before bariatric surgery with the magnitude of surgery-induced weight loss was investigated.

Materials and Methods

Patients

Thirty-seven severely or morbidly obese patients [body mass index (BMI) > 35–40 kg/m^2] awaiting bariatric surgery were recruited from the services of digestive and parietal surgery at the Tivoli and Jean Villar clinics (Bordeaux, France). Patients were scheduled to receive either a sleeve gastrectomy (n = 23, mean BMI 40.6 kg/m^2) or a gastric bypass (n = 14, mean BMI 41.0 kg/m^2). Patients with infections within the last month preceding study entry and patients with chronic inflammatory conditions were excluded. Seventy-six percent of patients (n = 28) were followed up at 1–3 months after surgery (meantime 1.3 mo) and 67.6% (n = 25) after 6 months after surgery (mean time at follow-up 10.6 months, range 6–14 mo).

The study was approved by the local Committee for the Protection of Persons (Bordeaux, France). All patients provided written informed consent after reading a complete description of the study.

Circulating concentrations of inflammatory markers

Fasting blood samples for the measurement of inflammatory markers were collected the morning of the preoperative examination. Samples were centrifuged (10 min, 1000 × *g*, 4°C) after clotting, and sera were stored at −80°C. High-sensitivity (hs) serum concentrations of the inflammatory cytokines, hsIL-6, hsTNF-α, and the acute-phase protein hsCRP, as well as the main adipose derived hormone, leptin, were measured. In addition, concentrations of neopterin were assessed as marker of macro-

phage activation. Neopterin is produced by macrophages activated by Th1 cell-derived interferon-γ (26), and it was found to be elevated in subjects with overweight or metabolic disorders (27, 28). Measurements were performed by ELISA according to the manufacturer's specifications (hsIL-6, hsTNF-α, and leptin; R&D Systems; hsCRP: ref CYT298; Millipore; neopterin; IBL International). Sensitivity and intra- and interassay variability were, respectively, 0.039 pg/mL ±7.4%, and ±7.8% for hsIL-6; 0.106 pg/mL, ±5.4%, and ±8.3% for hsTNF-α; 7.8 pg/mL, ±3.2%, and ±4.4% for leptin; 0.20 ng/mL, ±4.6%, and ± 6% for hsCRP; and 0.7 nmol/L, ±4.3%, and ± 8.8% for neopterin.

Adipose tissue gene expression of inflammatory and immune cell subpopulation markers

RNA extraction and reverse transcriptase

Samples of visceral adipose tissue were taken during bariatric surgery and kept at 4°C. Samples were washed and excess of blood was removed with saline. Aliquots of visceral adipose tissue were then frozen at −80°C until RNA extraction. Total RNA from samples was extracted in TRIzol reagent and 2 μg of RNAs were reverse transcribed using Moloney murine leukemia virus-reverse transcriptase (Invitrogen).

Real-time PCR

Real-time PCR was performed on an AB7500 real-time PCR system using Taqman gene expression assays, purchased from Applied Biosystems, for adipokines: IL-6 (Hs00985641_m1); IL-1 receptor antagonist (IL-1ra; Hs00893626_m1); TNF-α (Hs99999043_m1); IL-10 (Hs99999035_m1); IL-1β (Hs01555410_m1); leptin (Hs00174877_m1); monocyte chemoattractant protein-1 (MCP-1), also referred to as chemokine ligand-2 (Hs00234140_m1); for T cell markers : CD8A (cytotoxic T cells, Hs01555600_m1); T-box 21 (TBX21; Th1 cells, Hs00203436_m1); GATA3 (Th2 cells, Hs00231122_m1); Forkhead box P3 (FOXP3; regulatory T cells, Hs01085832_m1); CD3E (T cells, Hs01062241_m1); for macrophage markers : CD11B (macrophages, Hs00355885_m1); CD11C (M1 macrophages, Hs00174217_m1); CD206 (M2 macrophages, Hs00267207_m1); and for the housekeeping gene β2-microglobulin (Hs00984230_m1).

Cycle threshold (Ct) values of β2-microglobulin were not significantly correlated with weight or BMI in obese subjects ($R = -0.09$, $P = .60$, and $R = -0.07$, $P = .70$, respectively) or with age, gender, smoke, or any obesity-related comorbidities (all $P > .05$), supporting the use of this gene as housekeeping gene. The difference between target Ct values and housekeeping β2-microglobulin Ct values (ΔCt) was calculated to normalize for differences in the amount of total nucleic acid added to each reaction and in the efficiency of the reverse transcriptase step. The expression of target gene (linear value) normalized to the housekeeping gene was determined by $2^{-(\Delta Ct)} \times 1000$. This method provides measures of mRNA levels of different target genes, normalized to the housekeeping gene. Two patients with extreme values (ie, > 4 SD above the mean) of at least one marker were considered as outliers and excluded from analyses.

Data analyses and statistics

Raw values for circulating hsIL-6, hsTNF-α, neopterin, and leptin were log transformed because of nonnormality. Pearson correlations, controlling for obesity-related comorbidities (eg, type 2 diabetes, sleep apnea syndrome, hypertension, hepatic steatosis) were performed to assess the relationship between circulating inflammatory markers and adipose inflammatory markers. Similar analyses were performed to determine associations between gene expressions of inflammatory markers and immune cell subpopulations in the visceral adipose tissue. A factor analysis with varimax rotations was performed to confirm these associations and extract distinct adipose immune/inflammatory components. The significance of changes in weight and BMI between baseline and after surgery [respectively at 1–3 months after surgery (n = 28) and after 6 months after surgery (n = 25)] was assessed with paired *t* tests. Relationships of systemic and adipose inflammation before bariatric surgery with the magnitude of surgery-induced weight loss [estimated as the difference (Δ) in weight and BMI between after surgery and baseline] were assessed using first simple linear regression analyses and then multiple linear regression analyses controlling for time at follow-up and obesity-related comorbidities. Statistical analyses were performed with Statistica (Statsoft). All probabilities were two sided with the degree of significance set at $P < .05$.

Results

Demographic and clinical characteristics of study participants

Demographics, comorbidities, and metabolic/inflammatory characteristics of obese patients before bariatric surgery are shown in Table 1. Circulating levels of inflammatory markers were comparable with levels reported earlier in similar populations (1). Concentrations of hsCRP significantly correlated with levels of hsIL-6 ($R = 0.382$, $P < .05$) and leptin ($R = 0.366$, $P < .05$). In addition, circulating concentrations of neopterin were significantly

Table 1. Characteristics of Study Participants

	Average or Proportion
Sample size, n	37
Age, y (SD)	39.4 (9.6)
Men, n (%)	9 (24.3)
Smoke, cigarettes/d (SD)	1.6 (4.1)
Weight, kg (SD)	113.7 (11.6)
BMI, kg/m^2 (SD)	40.7 (3.5)
Obesity-associated comorbidities	
Type 2 diabetes, n (%)	5 (13.5)
SAS, n (%)	17 (45.9)
Hypertension, n (%)	8 (21.6)
Hepatic steatosis, n (%)	
Absent (Grade 0)	17 (46.0)
Mild-moderate (Grades 1–2)	11 (29.7)
Severe (Grade 3)	9 (24.3)
Circulating inflammatory markers	
hsCRP, mg/L (SD)	5.5 (4)
hsIL-6, pg/mL (SD)	1.4 (0.7)
hsTNF-α, pg/mL (SD)	1.3 (0.7)
Leptin, ng/mL (SD)	54.6 (23.5)
Neopterin, nmol/L (SD)	6.4 (1.9)

Abbreviations: SAS, sleep apnea syndrome. Severity grades of hepatic steatosis were determined by ultrasonography.

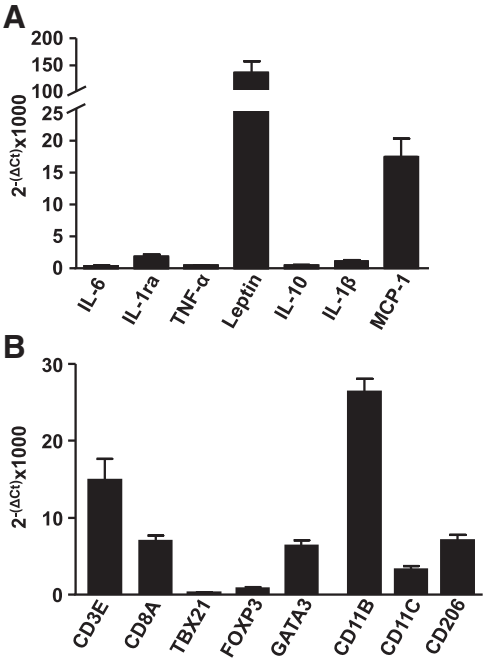

Figure 1. mRNA levels ($2^{-(\Delta Ct)} \times 1000$) of adipocytokine markers and immune cell markers in the visceral adipose tissue of obese patients. A, Adipocytokine markers. B, M1 and M2 macrophage markers and T cell subpopulation markers. CD11B, Macrophage marker; CD11C, M1 macrophage marker; CD206, M2 macrophage marker; CD3E, T cell marker; CD8A, cytotoxic T cell marker; TBX21, Th1 cell marker; GATA3, Th2 cell marker; FOXP3, regulatory T cell marker.

correlated with levels of hsTNF-α (R = 0.365, $P < .05$). Gene expressions of markers of inflammation and T cell subpopulations in visceral adipose tissue are presented in Figure 1.

Consistent with previous reports (29), associations were found between inflammatory/immune markers and anthropomorphic characteristics of patients. More precisely, circulating levels of leptin and hsCRP were positively associated with BMI (R = 0.361 and R = 0.365, $P < .05$). Consistent with these data, gene expression of leptin in the adipose tissue was positively associated with patients' BMI (R = 0.374, $P < .05$). Moreover, a significant association was found between gene expression of M2 macrophages (CD206) and BMI (R = 0.461, $P < .01$). In addition, significant relationships were found between obesity-related comorbidities and inflammatory/immune markers. In particular, obese patients with type 2 diabetes exhibited higher levels of circulating hsIL-6 (T = 2.6, $P < .05$) as well as higher gene expression of IL-1ra (T = 2.7, $P < .05$) in the adipose tissue. Higher adipose gene expressions of IL-1ra and leptin were also found in obese patients with sleep apnea syndrome (T = 3.1, $P < .01$, and T = 2.8, $P < .01$, respectively). Gene expression of T cell subpopulations was higher in patients with hypertension [T cells (CD3E) T = 2.6, $P < .05$], in particular cytotoxic T cells (CD8A, T = 3.2, $P < .01$) and regulatory T cells (FOXP3, T = 2.4, $P < .05$). Finally, hepatic steatosis was associated with increased gene expression of IL-1β (F = 4.3, $P < .05$) and Th1 cells (TBX21, F = 4.2, $P < .05$). Given the existing relationship between obesity-related comorbidities and inflammatory/immune markers, subsequent analyses on those markers were performed controlling for the effect of comorbidities.

Relationship of circulating inflammatory markers with gene expression of inflammatory and immune cell markers in the adipose tissue

As shown in Table 2, higher circulating concentrations of hsIL-6 were associated with an increased proportion of Th1 cells in the visceral adipose tissue. An inverse relationship was found between circulating levels of hsTNF-α and adipose IL-1ra mRNA, leptin mRNA, the antiinflammatory Th2 cell expression and proportion, and the antiinflammatory M2 macrophage proportion. Moreover, circulating concentrations of neopterin were inversely related to adipose gene expression of IL-1β and IL-6.

Relationship between gene expression of immune cell subpopulations and inflammatory markers in the adipose tissue

Overall, gene expressions ($2^{-(\Delta Ct)} \times 1000$) of adipocytokines were strongly correlated among each other. More specifically, IL-1β gene expression was positively associated with mRNA levels of IL-6 mRNA (R = 0.622, $P < .001$), TNF-α (R = 0.364, $P < .05$), IL-10 (R = 0.439, $P < .05$), and MCP-1 (R = 0.760, $P < .0001$). Moreover, gene expression of MCP-1 significantly correlated with IL-6 (R = 0.565, $P < .001$) and IL-10 (R = 0.518, $P < .01$) mRNAs. Finally, IL-1ra mRNA was positively associated with gene expression of leptin (R = 0.564, $P < .001$).

Significant relationships were measured between gene expression of TNF-α, IL-1ra, IL-10, leptin, and immune cell subpopulations in the adipose tissue (Table 3). More specifically, higher gene expression of TNF-α correlated with greater levels of markers of T cells and subpopulation markers of cytotoxic, Th1, and regulatory T cells. In addition, increased gene expression of IL-1ra was related to increased markers of T cells and subpopulation markers of Th2 and regulatory T cells and related proportions. Both gene expressions of TNF-α and IL-1ra were positively associated with markers of M1 macrophage expression and proportions. Higher leptin mRNA was significantly related to increased levels of T cell markers, notably Th2 and regulatory T cells, proportions of Th1 and T regulatory cells, and with expression of M1 and M2 macrophages. IL-10 mRNA correlated positively with gene expression of macrophages and negatively with proportion of M2 macrophages. Finally, IL-1β mRNA correlated negatively

Table 2. Association of Circulating Concentrations of Inflammatory Markers With Inflammatory Markers and Immune Cell Gene Expression in the Visceral Adipose Tissue

	Circulating Inflammatory Markers				
	hsCRP	**hsIL-6**	**hsTNF-α**	**Leptin**	**Neopterin**
Adipose inflammatory markers					
IL-6	−0.021	−0.187	−0.216	−0.052	−0.372[a]
IL-1ra	−0.020	0.034	−0.487[b]	0.078	0.034
TNF-α	0.059	−0.039	−0.011	0.145	−0.038
IL-10	0.179	−0.053	0.080	0.188	−0.083
IL-1β	0.058	0.101	−0.207	0.212	−0.466[b]
Leptin	−0.064	0.026	−0.384[a]	0.003	−0.253
MCP-1	0.062	0.072	−0.093	0.198	−0.339
Adipose T cell markers					
CD3E (T cells)	−0.085	−0.185	−0.045	−0.079	−0.003
CD8A (cytotoxic)	−0.117	−0.118	0.058	−0.072	−0.022
TBX21 (Th1)	0.262	0.246	0.258	−0.061	0.219
GATA3 (Th2)	−0.150	−0.203	−0.455[a]	0.035	−0.273
FOXP3 (reg.)	−0.089	−0.078	−0.149	−0.101	−0.039
Proportions					
CD8A/CD3E	−0.189	0.202	0.246	−0.137	0.037
TBX21/CD3E	0.160	0.427[a]	−0.001	−0.112	−0.029
GATA3/CD3E	−0.131	−0.042	−0.468[b]	0.047	−0.204
FOXP3/CD3E	−0.016	0.342	−0.261	−0.129	−0.056
TBX21/GATA3	0.262	0.246	0.258	−0.061	0.219
TBX21/FOXP3	0.075	0.008	0.202	−0.187	0.104
Adipose macrophage markers					
CD11B (macr.)	0.216	−0.121	0.085	0.096	−0.234
CD11C (M1)	−0.091	−0.018	−0.245	−0.119	−0.011
CD206 (M2)	0.008	−0.014	−0.305	0.039	−0.347
Proportions					
CD11C/CD11B	−0.125	0.088	−0.295	−0.162	0.103
CD206/CD11B	−0.152	0.080	−0.517[b]	−0.054	−0.245
CD11C/CD206	−0.099	−0.007	0.023	−0.186	0.238

Abbreviations: macr., macrophages; reg., regulatory. Pearson correlations controlling for obesity-related comorbidities (eg, type 2 diabetes, sleep apnea syndrome, hypertension, and hepatic steatosis). Gene expression = $2^{-(\Delta Ct)} \times 1000$. Raw values for circulating hsIL-6, hsTNF-α, neopterin, and leptin were log transformed because of nonnormality.

[a] $P < .05$.

[b] $P \leq .01$.

with proportion of Th1/Th2 cells. Apart from this association, IL-1β mRNA did not correlate with any other cell subpopulation. No significant association was found between IL-6 or MCP-1 mRNA and immune cell markers.

Consistent with these data, a factor analysis indicated that adipose immune/inflammatory markers loaded on four distinct principal components/factors. The first factor (eigenvalue = 5.4, variance = 36%) was primarily saturated by TNF-α mRNA and by markers of T cells (CD3E) and subpopulations, in particular regulatory T cells (FOXP3), cytotoxic T cells (CD8A), and Th1 cells (TBX21). The second factor (eigenvalue = 2.7, variance = 17.9%) contained primarily IL-1ra and leptin mRNAs and the subpopulations of Th2 cells (GATA3), M2 macrophages (CD206), and to a lesser extent M1 macrophages (CD11C). The third factor (eigenvalue = 1.9, variance = 13%) was saturated by MCP-1, IL-1β, and IL-6 mRNAs. Finally, the last factor (eigenvalue = 1.6, variance = 10.5%) contained IL-10 mRNA and the marker of macrophages, CD11B.

Associations between adipose and systematic inflammation before bariatric surgery and surgery-induced weight loss

Bariatric surgery was associated with a significant reduction in the weight (114.8 to 102.4 kg, T = 12.1, $P < .0001$) and BMI (40.9 to 36.6 kg/m^2, T = 13.3, $P < .0001$) of patients at 1–3 months after surgery. As expected, this reduction was even stronger after 6 months after surgery (weight 114.4 to 79.9 kg, T = 18.9, $P < .0001$; BMI 40.6 to 28.2 kg/m^2, T = 16.5, $P < .0001$). Regression analyses indicated a significant association between the fourth immune/inflammatory dimension (ie, IL-10 mRNA and CD11B) and the magnitude of weight loss at 1–3 months after surgery (Figure 2). More specifically, a higher expression of these markers before bariatric surgery pre-

Table 3. Relationship Between Gene Expressions of Inflammatory Markers and Immune Cell Subpopulations in the Visceral Adipose Tissue of Obese Patients

	Inflammatory Markers						
	IL-6	**IL-1ra**	**TNF-α**	**IL-10**	**IL-1β**	**Leptin**	**MCP-1**
T cell markers							
CD3E (T cells)	0.083	−0.368[a]	0.683[b]	−0.046	0.191	0.521[c]	−0.028
CD8A (cytotoxic)	0.016	−0.125	0.654[b]	0.084	0.204	0.259	−0.019
TBX21 (Th1)	0.022	−0.013	0.449[a]	−0.267	0.144	0.332	−0.157
GATA3 (Th2)	0.131	0.585[c]	0.331	−0.123	0.261	0.506[c]	0.039
FOXP3 (reg.)	0.100	0.451[a]	0.738[b]	−0.144	0.255	0.559[c]	−0.004
Proportions							
CD8A/CD3E	−0.050	−0.288	−0.310	0.076	−0.085	−0.323	0.023
TBX21/CD3E	−0.064	−0.056	−0.219	−0.281	−0.124	0.035	−0.130
GATA3/CD3E	0.014	0.507[c]	−0.113	−0.166	0.056	0.242	0.019
FOXP3/CD3E	0.049	0.427[a]	0.165	−0.322	0.116	0.388[a]	−0.008
TBX21/GATA3	−0.268	−0.264	−0.109	−0.290	−0.356[a]	−0.088	−0.248
TBX21/FOXP3	−0.172	−0.499[c]	−0.262	−0.017	−0.204	−0.402[a]	−0.079
Macrophage markers							
CD11B (macr.)	−0.135	−0.097	−0.002	0.529[c]	0.067	0.186	−0.042
CD11C (M1)	0.042	0.592[b]	0.553[c]	−0.184	0.156	0.564[c]	−0.149
CD206 (M2)	0.078	0.254	0.116	0.040	0.135	0.776[b]	−0.059
Proportions							
CD11C/CD11B	0.098	0.580[b]	0.511[c]	−0.355	0.115	0.382[a]	−0.124
CD206/CD11B	0.179	0.387[a]	0.126	−0.393[a]	0.086	0.714[b]	−0.039
CD11C/CD206	−0.078	0.335	0.499[c]	−0.174	0.010	−0.035	−0.200

Abbreviations: macr., macrophages; reg., regulatory. Pearson correlations controlling for obesity-related comorbidities (eg, type 2 diabetes, sleep apnea syndrome, hypertension, and hepatic steatosis). Gene expression = $2^{-(\Delta Ct)} \times 1000$.

[a] $P < .05$.

[b] $P \leq .001$.

[c] $P \leq .01$

dicted a lower re duction in BMI at 1–3 months after surgery, reflected by ΔBMI [β = .451, 95% confidence interval (CI) 0.075–0.827; $P < .05$] (Figure 2A). After adjusting for time between surgery and follow-up and obesity-related comorbidities, this relationship was still significant ($\beta = .367$, 95% CI 0.013–0.722; $P < .05$) (Figure 2B), and, albeit not significant in the unadjusted model, a new association was found between the second immune/inflammatory dimension (ie, IL-1 mRNA, leptin mRNA, and subpopulations of Th2 cells, M2 and M1 macrophages) and the magnitude of weight loss at 1–3 months after surgery ($\beta = -.434$, 95% CI −0.851 to −0.018; $P < .05$) (Figure 2, C and D). Precisely, a higher combined expression of IL-1 mRNA, leptin mRNA, Th2 cells, and M2 and M1 macrophages before bariatric surgery was associated with a greater reduction in BMI at 1–3 months after surgery. This association was also apparent at later stages after surgery, ie, between 6 and 14 months after surgery ($\beta = -.428$, 95% CI −0.838 to −0.018; $P < .05$) but did not remain significant when adjusting for time between surgery and follow-up and obesity-related comorbidities ($\beta = -.336$, 95% CI −0.887 to 0.215; $P = .2$) (Figure 2, E and F).

No significant association was found between circulating concentrations of inflammatory markers before surgery and surgery-induced weight loss.

Discussion

Results from the present study are in line with recent data suggesting the contribution of T cells, in addition to macrophages, to the inflammatory state of the visceral adipose tissue. In addition, our results indicate significant associations between the inflammatory state of the visceral adipose tissue and circulating levels of inflammatory markers in obese patients, supporting the notion that low-grade inflammation in obesity relies, at least partially, on adipose inflammation. Importantly, our results suggest that adipose inflammation may contribute to the effect of bariatric surgery on weight loss because higher adipose inflammation was found to predict lower BMI reduction after bariatric surgery, notably at early stages after surgery.

Expressions of macrophage markers and cytokines were related in the visceral adipose tissue (12, 13). Gene

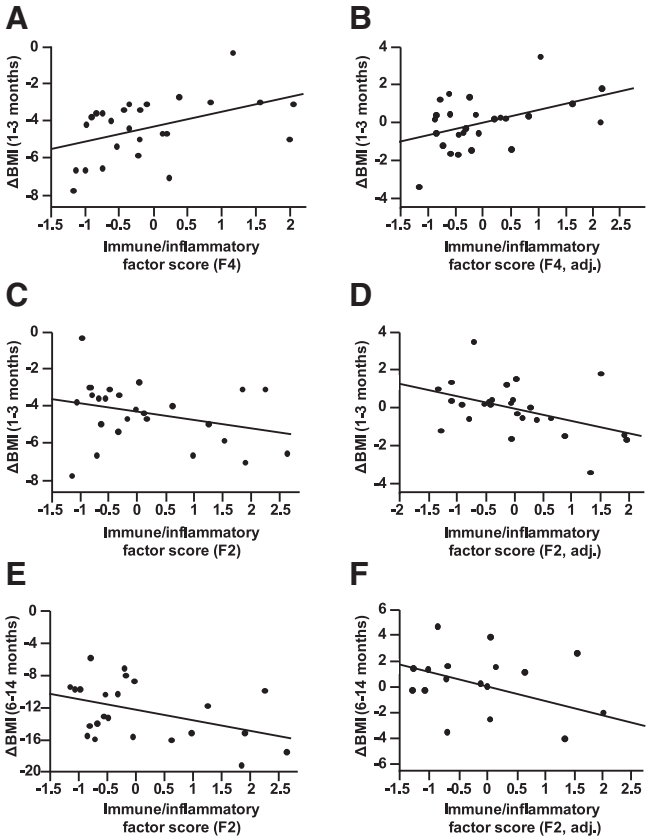

Figure 2. Relationships between adipose inflammation before bariatric surgery and surgery-induced weight loss (ΔBMI). A, Association of the immune/inflammatory dimension comprising of IL-10 mRNA and CD11B before bariatric surgery (factor 4) with decreased BMI at 1–3 months after surgery (simple regression: $\beta = .451$, $P < .05$). B, Same association controlling for time between surgery and follow-up and obesity-related comorbidities (multiple regression analysis: $\beta = .367$, $P < .05$). C, Association of the immune/inflammatory dimension comprising of IL-1 mRNA, leptin mRNA, and subpopulations of Th2 cells and M2 and M1 macrophages before bariatric surgery (factor 2) with decreased BMI at 1–3 months after surgery (simple regression: $\beta = -.297$, $P = .14$). D, Same association controlling for time between surgery and follow-up and obesity-related comorbidities (multiple regression analysis: $\beta = -.434$, $P < .05$). E, Association of the immune/inflammatory dimension comprising of IL-1 mRNA, leptin mRNA, and subpopulations of Th2 cells and M2 and M1 macrophages before bariatric surgery (factor 2) with decreased BMI at 6–14 months after surgery (simple regression: $\beta = -.428$, $P < .05$). F, Same association controlling for time between surgery and follow-up and obesity-related comorbidities (multiple regression analysis: $\beta = -.336$, $P = .2$). F, Factor.

expressions of cytokines were also associated with markers of T cells, supporting previous findings suggesting the involvement of T cells in addition to macrophages, in adipose inflammation (19–21). Significant associations were found between IL-10 mRNA and macrophage markers. In addition, the M1 macrophage marker was associated with the inflammatory cytokine, TNF-α, and the antiinflammatory cytokine, IL-1ra. Although surprising, given that M1 macrophages are assumed to be proinflammatory, this result may reflect the existence of a mix pattern of M1/M2 phenotype in the adipose tissue of obese patients, as previously suggested (30). Together with IL-1ra mRNA, leptin mRNA was associated with expression of M2 and M1 macrophages. Leptin and IL1-ra mRNAs were also associated with adipose expression of T cells, notably Th2 cells. These results are in accordance with the functional differentiation of lymphocytes, distinguishing proinflammatory, cytotoxic, and Th1 from antiinflammatory, Th2, lymphocytes (31). Moreover, the relationship of adipose leptin with macrophage and T cell subpopulation markers may reflect its ability to promote immune cell recruitment in the adipose tissue (32, 33). Adipose gene expression of TNF-α was associated with markers of T cells and subpopulations, notably cytotoxic, Th1, and regulatory T cells. Whereas some studies have found a reduced adipose expression of regulatory T cells in obesity (22), a recent report shows elevation in regulatory T cells, explained as a compensatory response to inflammation, in obese patients compared with lean subjects (23). Our finding that regulatory T cells were associated with TNF-α expression in the visceral adipose tissue is in line with this notion.

The inflammatory factors, IL-6, IL-1β, and MCP-1 strongly correlated among each other in the visceral adipose tissue, consistent with the notion that adipocytokines function as part of an integrated network (34). These markers were not associated with any specific macrophage or T cell markers, suggesting that they may primarily originate from adipocytes. Whereas MCP-1 is produced predominantly by macrophages and endothelial cells, its expression by adipocytes has been clearly demonstrated (35). MCP-1 is a chemotactic factor with a major role in the instauration and maintenance of chronic inflammation in obesity because this factor contributes substantially to macrophage infiltration into the adipose tissue but also to insulin resistance and hepatic steatosis (36).

One important finding of this study is the demonstration of a significant relationship between the visceral inflammatory state of obese patients and the magnitude of bariatric surgery-induced weight loss, notably at early stages after surgery. In particular, higher gene expression of IL-10 and M1 macrophages before bariatric surgery was found to predict a lower reduction in the BMI of patients at 1–3 months after surgery. Given that IL-10 is an antiinflammatory cytokine produced in response to inflammatory cytokine production, its increased expression in a context of increased M1 macrophage activation may be the reflection of higher inflammatory processes. In addition, a higher combined expression of IL-1 mRNA, leptin mRNA, Th2 cells, and M2 and M1 macrophages before bariatric surgery was found to predict a greater reduction in BMI at 1–3 months after surgery. This association was still apparent, but to a lesser extent, at later

stages after surgery, probably due to the additional involvement of lifestyle factors, including change in diet and exercise, with long-term effects on weight loss. Altogether these results suggest that visceral adipose inflammation may modulate the efficacy of bariatric surgery on weight loss, with increased adipose inflammatory processes associating with a reduced efficacy/success of the intervention. Identification of preoperative predictors of surgery-induced weight loss is important to understand the variability in the efficacy and success of bariatric surgery. In addition to the already identified psychiatric and psychological variables, findings from the present study reveal that biological factors may also be of particular relevance. To our knowledge, this study is the first to show that the basal inflammatory state of the visceral adipose tissue may represent one of these biological predictors of surgery-induced weight loss in obese patients. Complementary investigations on larger populations are needed to further support this notion.

Consistent with the contribution of the adipose tissue to low-grade inflammation in obesity (24, 25), the inflammatory state of the visceral adipose tissue was found to be related to systemic inflammation in the present study. In particular, significant relationships were found between adipose proportions of inflammatory Th1 cells and circulating concentrations of hsIL-6. In addition, markers of antiinflammatory Th2 cells and M2 macrophages, along with expression of leptin and IL-1ra, in the adipose tissue were inversely related to circulating concentrations of hsTNF-α, with lower expression of Th2 cells/M2 macrophages associating with higher levels of hsTNF-α. Taking together, these data support the role of visceral adipose inflammation in the instauration of the chronic systemic inflammatory state that characterizes obesity. The negative relationship found between neopterin, a marker of macrophage activation (26), and adipose gene expression of IL-1β and IL-6 suggests that activated systemic immune cells may, in turn, regulate inflammation in the adipose tissue. No significant association was found between expressions of IL-6/TNF-α in the adipose tissue and circulating levels of hsIL-6/hsTNF-α. Albeit surprising, this result may suggest that circulating concentrations of IL-6 or TNF-α do not directly reflect the release of these cytokines per se by the adipose tissue. This scenario is supported by previous findings showing no association of blood levels of IL-6 between the omental tissue and the peripheral circulation (37). Moreover, no association was found between circulating leptin and its gene expression in the visceral adipose tissue, probably due to the fact that, in contrast to cytokines (10), leptin is presumed to be secreted rather by subcutaneous than by visceral adipose tissue (38).

Limitations of the present study include primarily the small sample size. This limitation implies the need for further investigations on larger populations of obese subjects to comfort the present findings. Another limitation is that evidence of adipose inflammatory immune processes relies on adipose mRNA expression of cytokines and immune cell markers and not to protein expression. It appears therefore important to perform complementary investigations confronting the present data to protein expression of inflammatory/immune markers in the visceral adipose tissue.

In conclusion, results from the present study clearly show that visceral adipose inflammation in obesity involves macrophage and T cell activation and presents associations with systemic chronic low-grade inflammation. In addition, the present findings indicate that visceral inflammatory state may contribute, at least partially, to the efficacy of bariatric surgery-induced weight loss.

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