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ORIGINAL ARTICLE

Subcutaneous adipose tissue cytokine production is not responsible for the restoration of systemic inflammation markers during weight loss

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Context: It has been suggested that weight loss can improve systemic inflammation associated with obesity by decreasing the adipose production of pro-inflammatory cytokines. This suggestion, however, remains controversial.
Objective: To analyse the effect of weight loss on peripheral inflammatory markers and subcutaneous adipocytokine production.
Design: Patients were studied at baseline, at the end of the weight loss period, and after 2 weeks of weight stabilisation.
Subjects: Nineteen morbid obese non-diabetic patients and 20 lean control subjects.
Intervention: During the weight loss period patients followed a 6-week low-calorie diet.
Measurements: Plasma levels of inflammatory markers, maximal *in vitro* whole-blood cytokine production, subcutaneous adipose tissue expression and content of several cytokines.
Results: Obese subjects had higher circulating levels of C reactive protein (CRP), serum amyloid A (SAA), interleukin IL-6, IL-1 and soluble tumor necrosis factor receptors (sTNFR). Weight loss was associated with a significant decrease in CRP, SAA, leucocytes and plasma IL-6. Maximal i*n vitro* cytokine production of IL-1 and sTNFR1 increased during this period. Weight loss did not induce significant changes in the adipose concentrations of IL-6, IL-1 or sTNF-receptors. However, adipose expression of IL-6, IL-1, TNFα, membrane cofactor protein-1 and adiponectin increased at the end of the weight loss period. During weight maintenance, circulating inflammatory parameters increased and in some cases returned to baseline.
Conclusions: A low-calorie diet is associated with an improvement in the systemic inflammatory status. This seems to be due to energy restriction rather than to adipose mass loss, since inflammatory levels return to baseline soon after weight stabilisation. Furthermore, a negative energy balance and fat mobilisation are associated with increased subcutaneous cytokine adipose expression.
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Keywords: patients; inflammation; cytokine; weight loss

Introduction

In recent years, systemic inflammation has been linked to several chronic diseases that often accompany obesity, such as cardiovascular disease, sleep apnea syndrome, lipid abnormalities or type 2 diabetes.[1–4] Consequently, the evaluation of the potential role of inflammation in the physiopathology of obesity deserves increasing attention although at present the body of evidence is still limited.

Obesity is associated with higher plasma levels of acute-phase proteins and pro-inflammatory cytokines such as tumor necrosis factor TNF-α or interleukin IL-6.[5–7] It has been suggested that this low-grade systemic inflammation found in obese patients can at least be partly explained by the increased adipose tissue expression of some pro-inflammatory cytokines,[8] such as IL-6 or TNF-α, and the subsequent release of IL-6 into the bloodstream.[9]

It has been speculated that this overexpression of pro-inflammatory cytokines in the adipose tissue of obese patients leads to peripheral and central reactions that

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prevent fat stores from expanding further.[10,11] However, these potential protective mechanisms against obesity have the considerable drawback that they produce a low-grade systemic inflammation that can contribute to the development of various metabolic obese comorbidities and, therefore, increase the risk of mortality. However, little is known about the precise role of this adipose cytokine production in the systemic inflammatory state or its implications for the metabolic complications linked to obesity.

In this context, prospective long-term studies or studies assessing the effect of weight change on local and systemic inflammatory patterns are necessary. Several studies suggest that weight loss has a restorative effect on systemic markers of chronic inflammation.[12–16] However, results about the effect of weight loss on adipose production or the secretion of pro-inflammatory cytokines are contradictory.[5,6,17]

Therefore, the aim of this study was: (a) to analyse the levels of various systemic inflammatory markers in obese and lean subjects; (b) to investigate the effect of programmed weight reduction on systemic and subcutaneous adipose tissue inflammatory response in obese patients on a low-calorie diet; and (c) to analyse the relative contribution of the subcutaneous adipose tissue to the systemic inflammatory response associated with changes in weight.

Materials and methods

Study subjects

We studied 19 weight-stable (weight change <5 kg in the last 3 months) Caucasian morbid obese patients (body mass index (BMI) >40 kg/m^2; range: 40.2–70.3 kg/m^2) and 20 lean healthy controls (BMI 19–25 kg/m^2) aged between 29 and 60 years.

Criteria for exclusion were the presence of infectious, inflammatory, neoplastic or systemic diseases, hypothyroidism, diabetes or other endocrine diseases, and the active use of antibiotics, anti-inflammatory or antiobesity drugs.

The hospital ethics committee approved the study protocol, and all subjects gave their informed written consent to participate.

Study design and protocol

The present study was divided into two substudies. One compared obese patients to healthy lean control subjects and the other analysed the effect of weight loss and maintenance in the obese group of patients.

Obese subjects were studied at three different time points: (1) at the beginning of the weight loss period (before losing weight or basal period), (2) at the end of the weight loss period, and (3) 2 weeks after the end of the active weight loss period (maintenance weight period).

On these three occasions the following procedures were performed: (a) body weight, waist circumference and impedance-derived body composition were measured, (b) blood was extracted after an overnight fast to make routine biochemical analyses (leucocyte count, lipid profile, plasma glucose, insulin and erythrocyte sedimentation rate), determine the serum concentrations of acute-phase reactants and the plasma levels of pro-inflammatory cytokines, and measure the *in vitro* leucocyte production of cytokines after stimulation, (c) the resting metabolic rate and substrate oxidation were evaluated by 30-min indirect open calorimetry and urinary nitrogen, and (d) urine was collected for 24 h to measure urinary nitrogen by the Kjeldhal's method.

Subcutaneous adipose tissue biopsy was carried out on opposite sides of the lower abdominal wall before and at the end of the weight loss period so that the cytokine expression and content could be measured.

The control subjects were studied only once, after they had been accepted on the study, and the same procedures were performed, with the exception of the adipose tissue measurements.

Weight loss and maintenance

Morbid obese patients were placed on a hypocaloric diet for 6 weeks under hospitalisation conditions. During the nutritional intervention programme their daily energy intake was 3349 kJ/day (OPTIFAST, Novartis, Basel, Switzerland) with 51.5% carbohydrates, 13.5% fat and 35% protein. Adherence to the diet was assessed by daily urinary ketonic body measurements. Patients participated in an exercise programme for 1 hour/day and also received individual guidance on increasing their physical activity and nutritional counselling for the following period (maintenance period). Recommended energy intake was estimated as $1.5\times$ measured resting energy expenditure at discharge, 15% as proteins, 55% as carbohydrates and the rest as fat. Compliance was assessed by a 3-day weighed food record.

Specific methods for evaluating systemic inflammation

Acute-phase reactant levels. Fibrinogen, albumin, pre-albumin and erythrocyte sedimentation rates were measured in blood samples by the hospital's routine chemistry methods. Serum ferritin was measured by immunochemioluminescence. Serum C reactive protein (CRP) (Alpha Diagnostic International, TX, USA) and amyloid A (Tridelta, Ireland) levels were assessed using commercial enzyme-linked immunosorbent assay (ELISA) kits. Intra- and inter-assay coefficients of variation were $<5\%$ in both measures.

Plasma cytokine concentrations. Interleukin-1 and IL-6, (Amersham, Little Chalfont, Bucks, UK), TNF-α (BD Biosciences, Mississauga, Canada), both soluble TNF receptors (sTNFR), membrane cofactor protein (MCP)-1 (BioSource, Fleunes, Belgium) and adiponectin plasma levels (Linco Research, St Chaerles, MO, USA) were determined by ELISA in duplicate. The sensitivity of these assays was 0.1 pg/ml for IL-1 and IL-6, 0.4 pg/ml for TNF-α, 1 ng/ml for both sTNFR, <20 pg/ml for MCP-1 and 0.78 ng/ml for adiponectin. Intra-assay coefficients of variation were $<10\%$.

Cytokine production in whole blood. Blood samples were diluted (1:5) with RPMI 1640 supplemented with a solution of L-glutamine (200 mM), penicillin (10.000 U/ml) and streptomycin 10 mg/ml and stimulated with bacterial lipopolysaccharide (1 mg/ml) (Sigma-Aldrich, Tres Cantos, Madrid, Spain). Samples were incubated on 5% CO_2 at 37°C for 22 h. Interleukin-1, IL-6, TNF-α and sTNFRs were determined as we have described before.

Adipose tissue RNA extraction and reverse transcriptase-polymerase chain reaction real-time analysis. Adipose tissue samples were obtained from subcutaneous abdominal depots by biopsy avoiding epinephrine. Total RNA was extracted using TriPure Reagent. Interleukin-1, IL-6, MCP-1, TNF-α, adiponectin, lipoprotein lipase and hormone-sensitive lipase expression were quantified by real-time polymerase chain reaction using the ABI Prism 5700 Sequence Detector System (Applied Biosystem, Foster City, CA, USA) and specific primers and probes. All samples were normalised to values of glyceraldehydes-3-phosphate dehydrogenase and the results were expressed as fold changes in the C_t value relative at the beginning of the weight loss.

Adipose tissue cytokine content. Of adipose tissue, 100–125 mg was minced and incubated at 37°C in a humidified atmosphere of 5% CO_2 and 95% O_2 for 3 h in 3 ml Medium 199 (M-199; with Earle's salts and 25 mM HEPES, Gibco Life Technologies, Grand Island, NY, USA) and 0.6 ml bovine serum albumin 5%.[18] Interleukin-1, IL-6, TNF-α and sTNFR 1 and 2 levels were assessed by commercial ELISA kits.

Body composition and calorimetric measurements. Whole body impedance at 50 kHz (Human-In Scan®, Dietosystem, Spain) was measured using a tetrapolar bioelectrical impedanciometer. Fat-free mass (FFM) was assessed using the gender and fat-specific equations validated by Segal *et al*.[19] Fat mass (FM) was then calculated as the difference between total body weight and FFM. Energy expenditure measurements were determined in fasting conditions by 30-min open-circuit indirect calorimetry (Deltatrac®, Datex Instrumentation, Helsinki, Finland), as previously described.[20] Resting energy expenditure (REE) was calculated using Weir's equation [21] and the results are expressed as kJ/day. The fasting respiratory quotient (RQ) was calculated as V_{CO_2}/V_{O_2}. The macronutrient oxidation rate was estimated as described elsewhere.[22]

Statistical analysis

Statistical analyses were performed with the statistical package SPSS® version 11.5 for Windows. Results are expressed as mean ± s.d.

For comparisons between groups, non-parametric tests were used for quantitative variables (*U* Mann–Whitney test) and the χ^2 test was used for categorical variables. The effect of weight loss and maintenance in the obese group was assessed using a non-parametric test for paired data (Wilcoxon test). Relationships between variables were analysed by the Pearson's correlation test, and *r* coefficients were provided. A *P*-value of <0.05 was considered to be statistically significant.

Results

Differences between obese patients and controls

Table 1 shows the general characteristics of the subjects studied. There were no differences in age or height between obese patients and controls. The distribution of genders and smoking was similar among the groups. Among the obese subjects, 35% presented with hypertension ($n=7$) and 11% with dyslipidaemia ($n=2$). None of the studied subjects had a previous history of cardiovascular disease or diabetes. Obese patients showed higher REE than controls but this difference disappeared when it was adjusted for differences in FFM. In obese patients, the RQ was significantly lower and fat oxidation higher than in control subjects.

As shown in Table 2, obese subjects were found to have significantly higher levels of glucose, very low-density lipoprotein (VLDL) cholesterol and triglycerides, and lower levels of high-density lipoprotein cholesterol than controls. In addition, obese subjects were more insulin resistant, as assessed by fasting insulin levels and Homeostasis model assessment (HOMA).

As far as the inflammatory markers are concerned, obese patients show a low-grade systemic inflammation, unlike the control subjects. In obese patients, the total number of leucocytes, CRP plasma levels, serum amyloid A (SAA) protein and erythrocyte sedimentation rates (ESRs) were higher and serum albumin was lower than in controls. Plasma levels of adiponectin were lower in obese patients

Table 1 Baseline characteristics of the study groups

	Obese patients (n = 19)	*Controls (n = 20)*	P-*value*
Age (year)	40.53 ± 9.38	37.90 ± 5.72	NS
Gender (M/F)	6/13	6/14	NS
Smokers	5 (26%)	4 (20%)	NS
Weight (kg)	125.3 ± 29.3	62.9 ± 7	<0.001
BMI (kg/m^2)	48.4 ± 9.4	23.0 ± 1.4	<0.001
Fat-free mass (kg)	61.4 ± 14.1	45.5 ± 6.2	<0.001
Body fat (%)	49.6 ± 6.3	25.2 ± 4.1	<0.001
Waist–hip ratio	0.99 ± 0.13	0.84 ± 0.06	<0.001
Waist circumference (cm)	133.6 ± 18.5	80.8 ± 6.7	<0.001
Respiratory quotient	0.78 ± 0.05	0.82 ± 0.04	<0.005
Measured REE (kJ/d)	8181 ± 1884	5442 ± 728	<0.001
Adjusted REE (kJ/d)	7368 ± 1884	6497 ± 247	NS
C (g/d)	80.8 ± 81.4	104.2 ± 50.5	NS
F (g/d)	135.3 ± 57.3	61.96 ± 26.9	<0.001
P (g/d)	76.5 ± 27.3	66.4 ± 24.1	NS

Abbreviations: BMI: body mass index; REE: resting energy expenditure; C: carbohydrate oxidation rate; F: fat oxidation rate; P: protein oxidation rate; NS: not significant.

Table 2 Differences in basal biochemical parameters and peripheral inflammatory markers between obese patients and healthy lean controls

	Obese patients (n = 19)	*Controls (n = 20)*	P-*value*
Glucose (mmol/l)	5.42 ± 0.56	5.07 ± 0.48	0.03
Insulin (μU/ml)	14.91 ± 9.34	4.06 ± 2.65	< 0.001
HOMA-IR	3.67 ± 2.63	0.95 ± 0.73	< 0.001
Total cholesterol (mmol/l)	4.92 ± 1.23	5.01 ± 0.58	NS
HDL-cholesterol (mmol/l)	1.31 ± 0.24	1.71 ± 0.36	< 0.001
LDL-cholesterol (mmol/l)	2.93 ± 1.06	2.91 ± 0.62	NS
VLDL-cholesterol (mmol/l)	0.70 ± 0.35	0.39 ± 0.21	0.001
Triglycerides (mmol/l)	1.50 ± 0.78	0.86 ± 0.45	0.003
C reactive protein (mg/l)	2.57 ± 2.27	1.46 ± 2.37	0.01
Ferritin (μg/l)	66.80 ± 59.11	45.38 ± 52.38	NS
Albumin (g/l)	40.37 ± 3.48	44.05 ± 2.50	0.001
Fibrinogen (g/l)	0.24 ± 0.05	0.27 ± 0.05	NS
Serum amiloid A protein (μg/ml)	333.80 ± 353.04	42.65 ± 56.05	< 0.001
ESR (mm/h)	20.11 ± 13.46	5.35 ± 3.94	< 0.001
Leukocytes (*10 E^9/l)	8.13 ± 1.91	5.80 ± 1.13	< 0.001
Interleukin-6 (pg/ml)	2.90 ± 7.77	0.45 ± 0.99	0.001
Interleukin-1 (pg/ml)	0.09 ± 0.15	0.10 ± 0.12	NS
TNF-α (pg/ml)	0.71 ± 0.40	0.40 ± 0.12	NS
Soluble TNFR1 (ng/ml)	1.73 ± 0.36	1.25 ± 0.38	< 0.001
Soluble TNFR2 (ng/ml)	4.84 ± 1.47	3.21 ± 0.88	< 0.001
Adiponectin (ng/ml)	23.16 ± 9.04	30.13 ± 9.45	0.019

Abbreviations: ESR: erythrocyte sedimentation rate: HDL: high-density lipoprotein; LDL: low-density lipoprotein; NS: not significant; TNFR: tumor necrosis factor receptor; VLDL: very low-density lipoprotein.

than in lean controls. In contrast, plasma levels of IL-6, sTNFR1 and sTNFR2 were significantly higher in obese patients than in lean subjects. No differences were observed in plasma levels of IL-1 between the two groups.

In the total population (lean and controls), the percentage of body fat was significantly related to Plasma CRP ($r = 0.46$), SAA protein ($r = 0.86$), fibrinogen ($r = 0.76$), ESRs ($r = 0.77$), number of leucocytes ($r = 0.32$) and plasma levels of IL-6 (0.53), sTNFR1 ($r = 0.59$) and sTNFR2 ($r = 0.49$). An inverse relationship was observed between serum albumin and plasma adiponectin levels, and such adiposity markers as BMI, waist circumference and total body fat.

Although the whole blood *in vitro* IL-6 (9.84 ± 6.59 vs 6.04 ± 3.30 pg/1000 cells/22 h), TNF-α (885 ± 77 vs 795 ± 79 pg/1000 cells/22 h) and sTNFR2 production (3.08 ± 2.30 vs 2.51 ± 1.38 pg/1000 cells/22 h) tended to be higher in obese patients than in lean controls after stimulation, no significant differences were observed in the *in vitro* overall cytokine production after stimulation between groups.

Effect of weight loss on obese patients

In the 6-week weight loss induced by a low-calorie diet, obese patients lost 8.8% of their total body weight (−11.2 ± 4.3 kg; $P < 0.001$). No differences in total body weight were observed between the end of the weight loss period and 2 weeks after (−0.11 ± 2.04 kg; NS). Table 3 shows the effect of weight loss and maintenance on the

Table 3 Effect of weight loss and maintenance on calorimetric measurements, and several biochemical parameters

	Before	*End of weight loss*	*15 days after the end*
Weight (kg)	125.3 ± 29.3	114.0 ± 27.5†	113.9 ± 27.7†
Total fat mass (%)	49.2 ± 6.3	47.5 ± 6.6*	47.0 ± 7.4*
Respiratory quotient	0.78 ± 0.05	0.75 ± 0.03*	0.77 ± 0.03‡,‡
Measured REE (kJ/d)	8181 ± 1984	6954 ± 1448†	7247 ± 1637*,‡
Adjusted REE (kJ/d)	7808 ± 1247	7130 ± 929*	7431 ± 1042*,‡
Glucose (mmol/l)	5.42 ± 1.56	4.58 ± 0.26†	5.05 ± 0.40*,‡
Insulin (μU/ml)	14.91 ± 9.34	8.91 ± 4.41*	10.13 ± 5.70*
HOMA-IR	3.67 ± 2.63	1.80 ± 0.88†	2.24 ± 1.26*,‡
Total cholesterol (mmol/l)	4.92 ± 1.23	3.58 ± 0.84†	4.23 ± 1.07*,‡
HDL-cholesterol (mmol/l)	1.31 ± 0.24	0.89 ± 0.14†	1.08 ± 0.18*,§
LDL-cholesterol (mmol/l)	2.99 ± 1.04	2.07 ± 0.92*	2.64 ± 0.90*,‡
VLDL-cholesterol (mmol/l)	0.70 ± 0.35	0.53 ± 0.16*	0.54 ± 0.28
Triglycerides (mmol/l)	1.50 ± 0.78	1.15 ± 0.35*	1.17 ± 0.63

Abbreviations: REE: resting energy expenditure; HDL: high-density lipoprotein; LDL: low-density lipoprotein; very low-density lipoprotein. *$P < 0.05$ vs before, $^{\dagger}P < 0.001$ vs before. $^{\ddagger}P < 0.05$ vs the end of weight loss period, $^{\S}P < 0.001$ vs the end of weight loss period.

calorimetric measurements and biochemical parameters. Measured REE, adjusted REE and RQ decreased significantly during the active weight loss period and tended to increase after the maintenance weight period. Levels of glucose, insulin, total and LDL cholesterol and HOMA for insulin resistance (HOMA-IR) showed the same pattern of evolution: a significant decrease during the active weight loss period and an increase after two weeks of weight maintenance (Table 3).

As far as inflammatory markers are concerned, weight loss is associated with a 36% decrease in CRP ($P < 0.05$), 56% in SAA protein ($P < 0.001$), 22% in erythrocyte sedimentation rates ($P < 0.05$), 19% in total number of leucocytes ($P < 0.001$), and 33% in plasma IL-6 ($P < 0.05$). No significant changes were observed in serum albumin, plasma IL-1, TNF-α or sTNFR1 concentrations during the active phase of weight loss (Table 4).

At the end of the 2-week maintenance weight period, almost all the inflammatory parameters increased and in some cases returned to the basal values before weight loss. Only plasma IL-6 concentrations remained significantly lower than basal values 2 weeks after weight maintenance.

Weight loss is associated with an increase in whole blood *in vitro* cytokine production after stimulation, but this increase is only significant in the case of interleukin-1 ($P < 0.05$) and sTNFR1 ($P < 0.05$). At 2 weeks after the end of the weight loss period the production of these two cytokines is still higher than basal values.

Weight loss did not induce significant changes in the subcutaneous adipose concentrations of IL-6 (3.99 ± 1.96 vs 3.79 ± 2.88 pg/ml), IL-1 (0.83 ± 0.46 vs 1.25 ± 1.44 pg/ml), TNF-α (27.0 ± 19.2 vs 37.6 ± 28.1), sTNFR1 (0.32 ± 0.28 vs 0.40 ± 0.25 pg/ml) or sTNFR2 (1.84 ± 1.46 vs 2.21 ± 1.71 pg/ml). In contrast, subcutaneous adipose tissue expression of IL-6

Table 4 Changes in leukocyte count, serum acute-phase reactants, plasma proinflammatory cytokine concentrations and whole blood *in vitro* cytokine production (after stimulation) during weight loss, and at 15 days of weight maintenance

	Before	*End of weight loss*	*15 days after the end*
Leukocytes (*10 E^9/l)	8.12±1.91	6.56±1.98†	7.83±2.41‡
Serum acute-phase reactants			
C reactive potein (mg/l)	2.56±2.27	1.65±2.22*	2.98±2.94‡
Albumin (g/l)	40.37±3.48	40.06±2.15	41.94±2.98‡
Serum amiloid A protein (μg/ml)	333.80±353.04	146.34±158.75†	307.41±496.07‡
ESR (mm/h)	20.11±13.46	15.74±11.27*	17.39±9.82‡
Plasma cytokine concentrations			
Interleukin-6 (pg/ml)	2.90±7.77	1.95±6.17*	2.20±6.64*
Interleukin-1 (pg/ml)	0.09±1.15	0.12±0.20	0.07±0.11
TNF-α (pg/ml)	0.71±0.40	0.44±0.16	0.42±0.17
Soluble TNFR1 (ng/ml)	1.73±0.36	1.73±0.34	1.64±0.39‡
Soluble TNFR2 (ng/ml)	4.84±1.47	5.88±1.69*	4.99±1.53‡
Whole blood in vitro cytokine production after stimulation			
Interleukin-6 (pg/1000 cells/22 h)	9.84±6.59	10.11±6.42	11.17±6.41
Interleukin-1 (pg/1000 cells/22 h)	0.59±0.34	0.98±0.70*	0.70±0.31*
TNF-α (pg/1000 cells/22 h)	885±78	989±73	810±106
Soluble TNFR1 (pg/1000 cells/22 h)	0.54±1.19	1.29±2.23*	0.92±2.16
Soluble TNFR2 (pg/1000 cells/22 h)	3.08±2.30	3.43±2.95	3.77±2.63

Abbreviations: ESR: erythrocyte sedimentation rates; TNFR: tumor necrosis factor receptor. *$P<0.05$ vs before, †$P<0.001$ vs before. ‡$P<0.05$ vs the end of weight loss period.

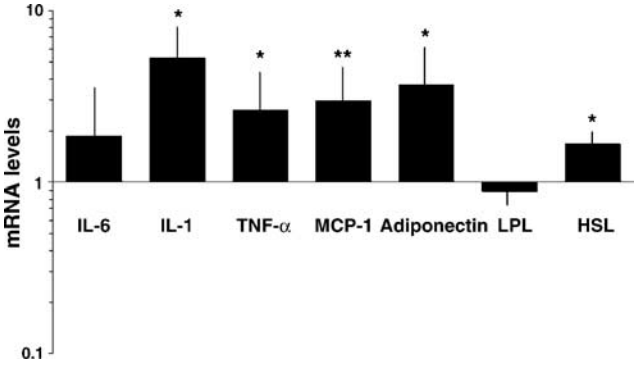

Figure 1 Adipose mRNA expression of several cytokines and enzymes during the active weight loss period. Values for mRNA levels after weight loss programme are expressed relative to baseline values before weight loss. *$P<0.05$; **$P<0.01$.

(NS) IL-1 ($P=0.028$), TNF-α ($P=0.035$), MCP-1 ($P=0.006$), adiponectin ($P=0.022$) and hormone-sensitive lipase ($P=0.021$) was higher at the end of the weight loss period than baseline values (Figure 1), whereas lipoprotein lipase mRNA expression decreased (NS) during the same period.

Discussion

The present study confirms previous observations regarding the low-grade systemic inflammatory state associated with obesity[2,7] and shows that active weight loss is accompanied by a significant reduction in peripheral levels of acute phase reactants and pro-inflammatory cytokines. This is in agreement with several studies performed in obese patients who lost weight using moderate or severe hypocaloric diets[12,14,15] or after bariatric surgery.[13,16] Since adipose tissue from obese patients overexpresses several adipocytokines,[7,11,17] some authors have suggested that adipose mass reduction after weight loss may help restore inflammatory levels by decreasing the adipose mRNA expression and secretion of these pro-inflammatory cytokines.[23]

However, results about the potential role of adipose tissue in inflammatory state are contradictory. Some studies have described a decrease in adipose mRNA TNF-α or IL-6 expression after weight loss,[6,17] whereas others have found an increase in adipose TNF-α expression,[24] or no changes in the expression of either of these cytokines.[25] These conflicting results may be due to the sign of the energy balance at the moment at which the measurements were made. The studies that reported a decrease in TNF-α expression made the adipose measurements some days after weight stabilisation, while those that reported an increase or no changes were made immediately after the active weight loss period (hence, in a negative energy balance situation). In our study, the final adipose measurements were performed in negative energy balance conditions, when lipolysis and fat mobilisation are stimulated,[26] as the increase in adipose mRNA hormone-sensitive lipase (HSL) and the decrease in mRNA lipoprotein lipase confirmed. In this situation, we observed an increase in the subcutaneous adipose tissue expression of

such cytokines as IL-1, TNF-α, MCP-1, IL-6 and adiponectin. Since these cytokines are involved in regulating different lipolytic pathways (e.g. they increase HSL activity and adipose tissue expression),[27] it can be speculated that this enhanced expression of adipocytokines stimulates the mobilisation of fat stores in a situation of calorie restriction.[28,29] Once the energy balance has been neutralised (i.e. after the weight stabilisation period) this adipose cytokine overexpression may be restored or even reduced, thus clarifying the disparity of results observed in the literature. However, the design of our study does not allow us to confirm this hypothesis since no adipose measurements were made after weight stabilisation. It should be pointed out, however, that there is a discrepancy between the observed increases in the adipose tissue mRNA levels and the protein concentrations of the measured cytokines, since adipose tissue protein levels are expected to follow the changes in gene expression. There is, however, a tendency – although not statistically significant – for the concentrations of IL-1 and both sTNFRs to increase at the end of the weight loss period. In fact the differences observed may be explained if it is taken into account that mRNA expression is a measure of what happens at a specific moment, while cytokine concentrations in adipose tissue are a reflection of cytokine production during a period of incubation and may be influenced both by mRNA translation and degradation (see the Materials and methods section).

From our results it can be speculated that, in this situation of energy restriction, subcutaneous adipose tissue does not seem to be responsible for the peripheral decrease in inflammatory markers. Certainly, after weight loss, levels of positive acute-phase reactants and IL-6 substantially decreased and in some cases were almost the same as those observed in control subjects. It should be pointed out that, after a few days of weight maintenance, systemic inflammatory levels returned to baseline values in the absence of FM or body weight changes. Hence, tissues other than FM may contribute to the variations in these inflammatory parameters throughout the study. Peripheral mononuclear cells are one of the main sources of TNF-α, IL-1 and IL-6. In our obese patients the production of TNF-α, sTNFRs, IL-6 and IL1 from peripheral blood cells after stimulation is similar to that of lean controls. The same findings have been described by other authors.[30,31] After weight loss we did not observe the expected reduction in the maximal cytokine production of the peripheral blood cells, which could have explained the restoration of the systemic levels of acute-phase reactants. On the contrary, IL-1 and sTNFR2 maximal production significantly increased after weight loss. However, the role of peripheral blood cells cannot be precluded since we have only measured *in vitro* production after stimulation, which does not reflect cytokine production in normal conditions. What is more, the total leucocyte number significantly decreases with energy restriction, a factor that could also contribute to decreased levels of pro-inflammatory proteins. The reduction in leucocyte levels with weight loss has already been described by our group[32] although the precise mechanism of this reduction and its consequences require further investigation.

Like other previously published studies evaluating the effect of weight loss on cytokine adipose tissue expression,[5,6,17,24,25] our study was performed using subcutaneous tissue biopsies. It might be possible that the expression of cytokines in visceral adipose tissue decreases during the energy restriction period, which partly explains the reduction in the systemic inflammatory levels as we have observed in our study at the end of the weight loss period. Whether non-adipocyte cells or different adipose tissue depots can modify low-grade systemic inflammation associated with obesity, however, still remains unknown.

Finally, obesity is associated with a macrophage adipose tissue infiltration[33]; and weight loss is related not only to a decrease in the number of macrophages in the subcutaneous adipose tissue of obese subjects but also to a modification in their distribution.[34] Although the extent to which these macrophages contribute to the local and systemic inflammation could not be estimated in our study, they should be considered in the future.

In conclusion, our study shows that the systemic inflammatory status is restored after weight loss. However, this effect seems to be largely due to energy restriction rather than adipose mass loss, since inflammation levels tended to return to baseline soon after the body weight stabilised. Furthermore, a negative energy balance is associated with an increase in the mRNA expression of several cytokines in subcutaneous adipose tissue, which might be considered to favour fat mobilisation. The precise mechanisms underlying the decrease in systemic inflammatory markers associated with weight loss remain to be elucidated.

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