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Effect of weight loss on cytokine messenger RNA expression in peripheral blood mononuclear cells of obese subjects with the metabolic syndrome

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Abstract

Inflammation is associated with obesity, the metabolic syndrome, and diabetes. No data are available on the effect of weight reduction on the gene expression of cytokines in immune cells in obesity and the metabolic syndrome. We assessed how long-term weight loss affects expression of cytokines in peripheral blood mononuclear cells (PBMCs) in individuals with impaired glucose metabolism and the metabolic syndrome. Data from 34 subjects randomized to either a weight reduction or a control group for a 33-week period were analyzed. The messenger RNA (mRNA) expression of interleukins (ILs) in PBMCs was measured using real-time polymerase chain reaction. Measures of insulin and glucose metabolism (intravenous and oral glucose tolerance tests), body composition, and circulating adipokines and inflammatory markers were also assessed. Weight reduction resulted in a decrease in the mRNA expression of IL-1β (IL1B), IL-1 receptor antagonist, and tumor necrosis factor α ($P < .001$) and an increase in expression of IL-6 (IL6) and IL-8 ($P < .01$). The increase in IL6 expression was associated with a decrease in fasting glycemia ($r = -0.53$, $P < .01$). Interestingly, the decrease in IL1B expression was correlated with an increase in insulin sensitivity index ($r = -0.68$, $P < .01$). In general, a decrease in circulating levels of adipokines and inflammatory markers was also observed after weight loss. Weight loss altered gene expression of cytokines related to inflammation and the immune response in PBMCs. Changes in IL6 mRNA expression were associated with changes in fasting glycemia. The decrease in IL-1 receptor antagonist expression after weight loss and the strong correlation between the decrease in IL1B expression and the increase in insulin sensitivity suggest a contribution of these genes to insulin-resistant states found in obesity and the metabolic syndrome.

1. Introduction

Intensive lifestyle intervention has substantially reduced the risk of type 2 diabetes mellitus in overweight or obese individuals with impaired glucose tolerance [1,2] even after active counseling stops [3]. Lifestyle intervention, including weight reduction, has been shown to improve insulin and glucose metabolism [1,4-6]. Moreover, subjects with the metabolic syndrome are at increased risk for type 2 diabetes mellitus and cardiovascular disease [7,8].

Obesity is characterized by a state of chronic low-grade inflammation [9,10]. Long-term activation of proinflammatory pathways may be a mechanism for the development of insulin resistance [11]. Serum concentrations of various cytokines are increased in obese individuals and may decrease after weight loss [12-15]. Obesity is also associated with alterations in immune function, but the effect of obesity itself on immune system function is variable and may be masked by hyperglycemia [16]. Obese subjects seem to

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primarily have an impairment in immune response that is reversible with weight reduction [16-18].

Peripheral blood mononuclear cells (PBMCs) are convenient for gene expression studies because they can be easily and repeatedly collected in sufficient quantities in contrast to adipose, muscle, and liver tissues. Some evidence in humans indicates that obesity and insulin resistance are associated with activation of these cells and with increased expression of a variety of cytokines [15,19], although they are not necessarily the primary source for elevated circulating inflammatory cytokines in obesity [15,20]. However, little is known about how weight loss modulates gene expression of cytokines in these cells.

The aim of the present study was to evaluate how long-term weight reduction affects expression of genes related to inflammation in PBMCs of overweight individuals with abnormal glucose metabolism and the metabolic syndrome. We studied the messenger RNA (mRNA) expression of genes encoding interleukin (IL)-1β (IL1B), IL-1 receptor antagonist (IL1RN), IL-6 (IL6), IL-8 (IL8), IL-10 (IL10), IL-18 (IL18), and tumor necrosis factor (TNF) α because these cytokines have been associated with obesity and metabolic syndrome [12-15,21-23]. Specifically, we wanted to explore genes that could be associated with insulin sensitivity.

2. Subjects, materials, and methods

2.1. Subject recruitment and clinical investigation

Altogether, 46 overweight or obese (body mass index [BMI], 28-40 kg/m^2) subjects aged 40 to 70 years with impaired fasting glucose (fasting plasma glucose concentration, 5.6-7.0 mmol/L) or impaired glucose tolerance (2-hour plasma glucose concentration, 7.8-11.0 mmol/L) and at least 2 other features of the metabolic syndrome according to the Adult Treatment Panel III criteria [24] were studied. Subjects were randomized to a weight reduction (n = 28) or a control group (n = 18) matched for age, sex, BMI, and the status of glucose metabolism. The proportion of subjects engaged in lifestyle or fitness physical activity and its frequency were not different between groups. The study lasted between 32 and 38 weeks (mean duration, 33.1 ± 1.7 weeks). The intervention was performed in accordance with the standards of the Helsinki Declaration. The Ethics Committee of the District Hospital Region of Northern Savo and Kuopio University Hospital approved the study plan, and all participants gave a written informed consent.

The weight reduction group underwent a 12-week intensive weight reduction period during which they followed detailed instructions given by a clinical nutritionist based on a 4-day dietary record and an interview. Adjustments on subjects' diet were made to encourage a higher intake of fruits and vegetables and up to 30% of total fat intake with a balanced fatty acid composition according to the American Heart Association [25], resulting in a mean deficit in caloric intake of about 500 kJ/d. During the period between weeks 12 and 33, the minimum aim was to maintain the reduced weight achieved based on the same dietary prescription. Subjects in the control group were advised to keep their dietary and lifestyle habits unchanged during the study. In both groups, subjects were advised not to change their exercise habits and medications during the study.

Blood samples analyzed were taken, and blood pressure assessment and anthropometric measurements (body weight, height, body fat percentage [STA/BIA Body Composition Analyzer; Akern Bioresearch, Florence, Italy], and waist circumference) were performed at baseline (week 0) and at the end of the study (week 33).

2.2. Glucose tolerance tests

A 2-hour oral glucose tolerance test was performed with 75 g of glucose. Blood samples for plasma glucose and serum insulin were drawn at 0, 30, and 120 minutes. A frequently sampled intravenous glucose tolerance test was performed according to the minimal-model method as previously described [26]. The insulin sensitivity index (S_I) and acute phase insulin response (AIR) were calculated by the MINMOD Millennium software [27].

2.3. Biochemical analyses

Insulin was determined by chemiluminescence sandwich method using an ACS automated system (Bayer, A/S, Tarrytown, NY). A commercial radioimmunoassay kit was used for the analysis of serum leptin (Linco Research, St Louis, MO). Adiponectin, TNF-α, IL-6, IL-1 receptor antagonist, and IL-1β concentrations were measured by solid-phase enzyme-linked immunosorbent assay (Quantikine; R&D Systems, Minneapolis, MN). High-sensitivity enzyme-linked immunosorbent assay kits were used for TNF-α, IL-6, and IL-1β measurements. High-sensitivity C-reactive protein (hsCRP) was determined by Immage Immunochemistry System (Immulite 2000; DPC, Los Angeles, CA).

2.4. Isolation of PBMCs

Samples were available for 24 subjects of the weight reduction group and 10 subjects of the control group. The PBMCs were isolated from anticoagulated peripheral blood by density centrifugation using Lymphoprep reagent (Axis-Shield, Oslo, Norway) according to the manufacturer's instructions and thereafter stored in RNAlater RNA stabilizing solution (Ambion, Austin, TX) according to the manufacturer's protocol in −80°C until used for RNA isolation.

2.5. RNA isolation and real-time polymerase chain reaction analysis of gene expression

Total RNA was extracted using RNeasy Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. The RNA concentration and the A_{260}/A_{280} ratio were measured using a NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, DE), an acceptable ratio being

1.9 to 2.1. The integrity of the RNA was assessed using agarose gel electrophoresis.

The RNA was reverse transcribed into complementary DNA (cDNA) using High-Capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA). Real-time polymerase chain reaction was performed with TaqMan chemistry using ready-made assays from Applied Biosystems. The amplification was performed and relative quantities were analyzed in triplicates using ABI Prism 7500 SDS software (TaqMan Gene Expression Assays; Foster City, CA). A standard curve with the points of 0.025, 0.075, 0.3, 0.9, and 1.8 ng/μL of cDNA (total RNA equivalent), respectively, and a calibrator at a concentration of 0.3 ng/μL were used on every plate. The cDNA pool for the standard curve was created by combining cDNA from a representative number of subjects. Quantities on each plate were first corrected by the calibrator on the plate. Furthermore, gene expression was normalized to an endogenous control, which was the glyceraldehyde-3-phosphate dehydrogenase gene.

2.6. Statistical analysis

Baseline variables were compared between groups using Student *t* test or Fisher exact test as appropriate. Univariate general linear model was used for testing changes in body composition, glycemic and insulin indices, circulating adipokines and inflammatory marker levels, and gene expression variables between weeks 0 and 33 with group as the fixed factor and baseline measurement as a covariate whenever the baseline measurement had an effect on variable change. Because age and/or sex influenced some of the changes in cytokine mRNA expression, these variables were also included in the model. Within-group analyses (general linear model) were performed if either overall variable change or treatment effect was significant. Correlations were performed using Pearson correlation coefficient (r) adjusted by partial correlation for changes in body weight, waist circumference, or body fat percentage. One-way analysis of variance (ANOVA) was used to test differences among tertiles of variables correlated to changes in gene expression followed by Bonferroni adjustments for multiple comparisons test. Multivariate linear regression analyses were carried out to test for independent effects of changes in gene expression on changes in relevant variables. Data are presented as mean ± SD for absolute values and mean (95% confidence interval [CI]) for changes. Variables with a skewed distribution were log transformed before the analyses and given as median (interquartile range). A P value < .05 was considered to be statistically significant. All analyses were performed using SPSS software version 14.0 for Windows (SPSS, Chicago, IL).

3. Results

3.1. Characteristics of the patients

No differences were observed in variables related to body composition, insulin and glucose metabolism, lipids, circulating adipokines and inflammatory markers, and blood pressure parameters between groups at baseline (Table 1). Ten subjects in the weight reduction group and 5 patients in the control group were using antihypertensive drugs. Two subjects, one from each group, were using diuretics. Lipid-lowering drugs were taken by 6 subjects in the weight reduction group and by 3 subjects in the control group. Seven women were using hormonal replacement therapy (weight reduction = 5, control = 2). The use of medications were not different between groups (P < .05). One subject from the control group was using antibiotic medication during the entire study.

An improvement in body composition variables was observed only in subjects from the weight reduction group.

Table 1
Clinical and biochemical characteristics of the subjects at baseline

	Weight reduction	Control	*P*
n	24	10	
Age (y)	58 ± 7	62 ± 8	.19
Sex (male/female)	10/14	4/6	.62
Body weight (kg)	93 ± 16.1	85.7 ± 8.5	.19
BMI (kg/m^2)	33.0 ± 3.4	31.6 ± 1.9	.22
Waist circumference (cm)	108.3 ± 9.3	104.1 ± 3.6	.17
Body fat (%)	37.2 ± 7.5	36.9 ± 5.8	.91
Systolic blood pressure (mm Hg)	136 ± 18	135 ± 11	.83
Diastolic blood pressure (mm Hg)	89 ± 10	86 ± 10	.39
Fasting plasma glucose (mmol/L)	6.5 ± 0.5	6.4 ± 0.5	.61
2-h plasma glucose (mmol/L)	6.8 ± 2.1	8.2 ± 2.5	.12
Fasting serum insulin (mU/L)	12.3 ± 6.4	9.75 ± 2.89	.11
2-h serum insulin (mU/L)	61.3 (29.9-105.1)	48.9 (33.2-104.4)	.83
S_I ([mU/L]$^{-1}$ × min^{-1})	2.0 (1.4-2.9)[a]	2.6 (1.9-3.1)	.26
AIR ([mU/L]$^{-1}$ × min^{-1})	4.0 (2.2-6.9)[b]	2.4 (1.3-3.2)	.29
Serum total cholesterol (mmol/L)	5.1 ± 1.1	5.1 ± 0.9	.96
HDL cholesterol (mmol/L)	1.2 ± 0.2	1.2 ± 0.2	.87
Serum triglycerides (mmol/L)	1.3 (1.0-2.1)	1.5(1.1-2.4)	.48
TNF-α (pg/mL)	1.17 (0.61-2.54)	1.47 (0.54-2.55)	.89
IL-6 (pg/mL)	2.55 ± 1.15	2.10 ± 1.10	.31
IL-1 receptor antagonist (pg/mL)	193.4 (167.0-605.3)	271.3 (192.9-350.5)	.23
IL-1β (pg/mL)	0.202 (0.129-0.265)	0.268 (0.179-0.334)	.18
hsCRP (mg/L)	2.84 (1.38-5.79)	2.36 (1.11-3.12)	.42
Serum leptin (ng/mL)	20.9 ± 11.3	19.2 ± 10.3	.71
Serum adiponectin (μg/mL)	5.1 (3.0-7.5)	5.1 (4.1-6.8)	.68

Data are mean ± SD or median (interquartile range). S_I indicates insulin sensitivity index; AIR, acute phase insulin response to glucose; IL, interleukin; hsCRP, high-sensitivity C-reactive protein.

[a] n = 22.

[b] n = 21.

Table 2
Changes (Δ) in body composition, glucose, and insulin metabolism in the weight reduction and control groups

	Δ (95% CI)	*P* for the effect of group	*P* for the change within group
Body weight (kg)			
Control (n = 10)	−0.0 (−1.1; 1.0)	<.01	.25
Weight reduction (n = 24)	−4.6 (−6.4; −2.9)		.03
BMI (kg/m^2)			
Control	−0.04 (−0.44; 0.35)	<.01 *	.39
Weight reduction	−1.65 (−2.26; −1.04)		.02
Waist circumference (cm)			
Control	0.2 (−1.7; 2.1)	.01 *	.26
Weight reduction	−3.7 (−5.2; −2.2)		<.001
Body fat %			
Control	−0.5 (−1.3; 0.3)	.27 *	.16
Weight reduction	−1.5 (−2.6; −0.4)		.01
Fasting plasma glucose (mmol/L)			
Control	−0.2 (−0.6; 0.2)	.04 *	.17
Weight reduction	−0.6 (−0.8; −0.4)		<.001
Fasting serum insulin (mU/L)			
Control	1.7 (−0.1; 3.5)	.11 *	.54
Weight reduction	−1.1 (−2.8; 0.7)		<.001
2-h serum insulin (mU/L)			
Control	0.6 (−30.4; 31.6)	.53 *	.14
Weight reduction	−15.6 (−52.3; 21.0)		<.001
S_I ([mU/L]$^{-1}$ × min^{-1})			
Control	−0.21 (−0.98; 0.55)	.25 *	.14
Weight reduction (n = 22)	0.22 (−0.13; 0.57)		.03
AIR ([mU/L]$^{-1}$ × min^{-1})			
Control	0.1 (−1.4; 1.6)	.78	
Weight reduction (n = 21)	0.1 (−0.7; 0.9)		
Serum leptin (ng/mL)			
Control	−0.6 (−2.5; 1.3)	.33 *	.50
Weight reduction	−3.2 (−5.2; 1.1)		<.001
Serum adiponectin (μg/mL)			
Control	0.4 (−0.7; 1.4)	.46 *	.43
Weight reduction	1.0 (0; 2.0)		<.001

Δ was calculated as week 33 − week 0. S_I indicates insulin sensitivity index; AIR, acute phase insulin response.
* $P < .05$ for overall change.

Table 3
Baseline mRNA expression of cytokines from PBMCs

	Weight reduction	Control	*P*
n	24	10	
IL1B	0.70 (0.55-1.18)	1.00 (0.63-1.45)	.27
IL1RN	1.14 ± 0.24	0.98 ± 0.24	.08
IL6	0.83 ± 0.40	0.92 ± 0.44	.54
IL8	0.71 (0.46-1.05)	0.91 (0.45-1.23)	.66
IL10	0.67 (0.53-1.14)	0.68 (0.50-1.17)	.93
IL18	1.03 ± 0.24	1.09 ± 0.30	.57
TNF	1.09 ± 0.29	1.16 ± 0.35	.51

Messenger RNA expression is the gene mRNA expression to glyceraldehyde-3-phosphate dehydrogenase mRNA expression ratio in arbitrary units. Data are mean ± SD or median (interquartile range).

Furthermore, serum adipokine concentrations and all measurements related to glucose and insulin homeostasis also improved after weight reduction, except for AIR (Table 2).

3.2. Changes in concentrations of circulating cytokines and hsCRP

In the weight reduction group, the concentrations of TNF-α (1.17 [0.61-2.54] vs 0.73 [0.51-2.55] pg/mL, $P < .001$), IL-6 (2.55 ± 1.15 vs 2.37 ± 0.32 pg/mL, $P < .001$), and hsCRP (2.84 [1.38-5.79] vs 1.66 [0.80-3.56] mg/L, $P < 0.001$) were lower after week 33 than at baseline. In the control group, TNF-α concentration increased from baseline to week 33 (1.47 [0.54-2.55] vs 1.74 [1.04-2.41] pg/mL, $P = .01$); but hsCRP concentration did not change (week 0: 2.36 [1.11-3.12] mg/L, week 33: 3.41 [0.82-4.11] mg/L; $P < .05$). The changes in plasma TNF-α, however, were not different between groups ($P = .19$). No significant

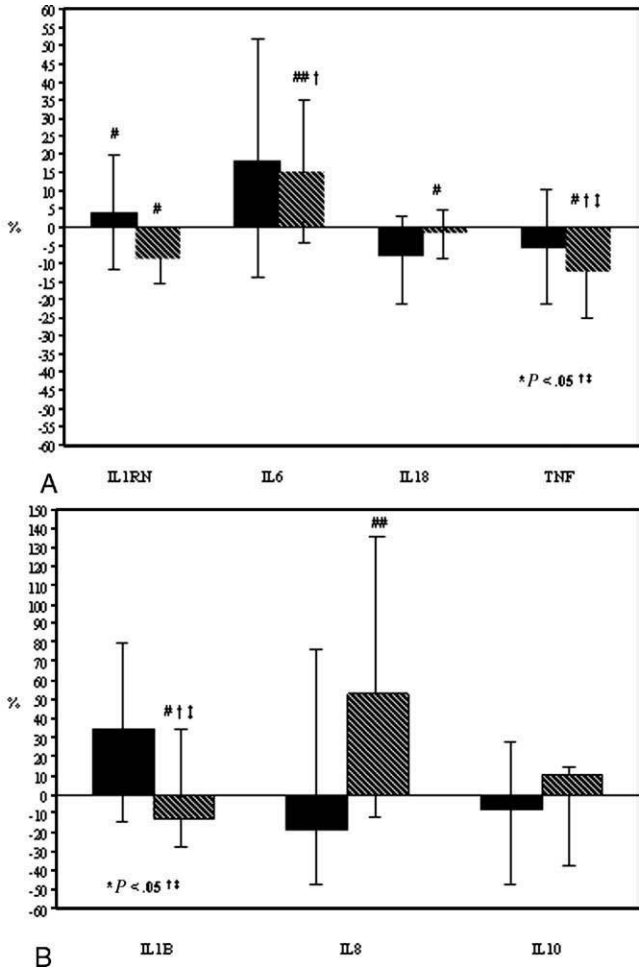

Fig. 1. A, Mean and 95% CI. B, Median and interquartile range. Changes in expression of cytokines in the control (black bars: n = 10) and weight reduction (hashed bars: n = 24) groups. Percentages of change are expressed as (week 33 − week 0) × 100/week 33 and log transformed as appropriate. **P* for the effect of group; $^{\#}P < .001$ and $^{\#\#}P < .05$ for the within-group analysis; $^{\dagger}P < .05$ for age effect; $^{\ddagger}P < .05$ for sex effect.

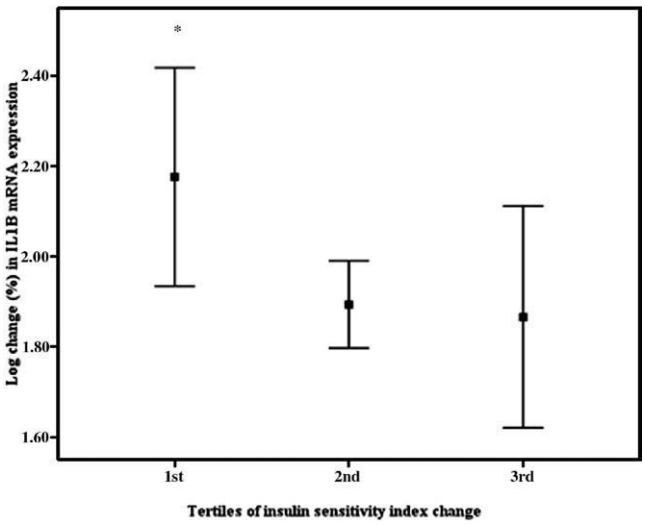

Fig. 2. Changes in IL1B mRNA expression according to tertiles of insulin sensitivity index change in the weight reduction group. First (n = 7): −1.25 − −0.21 $(mU/L)^{-1} \times min^{-1}$; second (n = 8): −0.19 − 0.48 $(mU/L)^{-1} \times min^{-1}$; third (n = 7): 0.76 − 1.82 $(mU/L)^{-1} \times min^{-1}$. One-way ANOVA, $P < .01$; *vs second tertile, $P < .05$; and vs third tertile, $P < .01$, Bonferroni post hoc test.

changes were observed in plasma IL6 concentration between baseline and week 33 in the control group (2.10 ± 1.10 vs 2.41 ± 1.11 pg/mL, $P < .05$).

Serum concentration of IL-1β and IL-1 receptor antagonist did not change either in the weight reduction (IL-1β: 0.202 [0.129-0.265] vs 0.200 [0.142-0.291] pg/mL; IL-1 receptor antagonist: 193.4 [167.0-605.3] vs 201.8 [150.7-350.3] pg/mL) or in the control group (IL-1β: 0.268 [0.179-0.334] vs 0.278 [0.217-0.355] pg/mL; IL-1 receptor antagonist: control: 271.3 [192.9-350.5] vs 249.5 [165.7-467.5] pg/mL, $P < .05$).

3.3. Changes in gene expression in PBMCs

Baseline mRNA expression of the target genes was not different between groups (Table 3). Changes in gene expression are illustrated in Fig. 1. An increase in IL6 mRNA expression was observed after weight reduction. Age affected the change in IL6 expression. Analysis selection based in median cutoff point for age (59 years) showed that the older (n = 12) and younger (n = 12) groups of subjects had different mean changes in IL6 expression (−2.2% [95% CI: −30.2 to 25.7] vs 35.4% [95% CI: 7.7-63.2], respectively; $P = .02$). No change in IL6 expression was found in the control group.

A decrease in TNF mRNA expression also occurred after weight reduction. The decrease in TNF expression observed in the control group was also significant, but it was lower than that in the weight reduction group. Weight reduction decreased IL1B mRNA expression. However, its increase in the control group was not significant. The IL1RN mRNA expression decreased after weight loss, but it increased in the control group.

An increase in IL8 mRNA expression occurred only in the weight reduction group. A decrease in IL18 mRNA expression was observed in both study groups. No significant changes were observed in IL10 mRNA expression in both study groups.

3.4. Correlations between changes in cytokine mRNA expression and changes in body composition and in variables of glucose and insulin metabolism after weight loss

The decrease in mRNA IL1B expression was correlated with an increase in S_I ($r = -0.68$, $P < .01$). According to tertiles of change in S_I, subjects who had a greater increase in S_I after weight loss had a greater decrease in IL1B expression (Fig. 2). In a multivariate regression analysis, only the change in IL1B expression ($\beta = -0.72$, $P < .01$) predicted the increase in S_I when age, sex, and change in body weight were also considered in the model. In a model where change in body fat percentage or in waist circumference was also used as a covariate, both changes in body fat percentage in one model ($\beta = -0.42$, $P < .05$) and waist circumference in the other model ($\beta = -0.43$, $P < .05$), along with the change in IL1B expression ($\beta = -0.61$ and $\beta = -0.59$, $P < .01$), significantly predicted S_I variation after weight loss. These results remained the same when change in serum adiponectin was included in the model along with age; sex; and change in body weight, body fat percentage, or waist circumference. Because the change in serum leptin correlated with both S_I ($r = -0.46$, $P < .05$) and body weight ($r = 0.38$, $P < .05$) changes, serum leptin was also included. Inclusion of the change in leptin did not affect the results. Although the decrease in S_I and the increase in IL1B expression observed

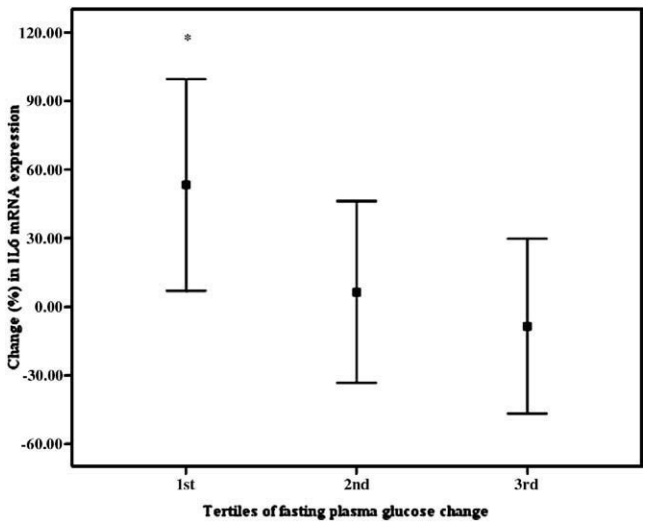

Fig. 3. Changes in IL6 mRNA expression according to tertiles of fasting plasma glucose change in the weight reduction group. First (n = 7): −1.70 − −0.90 mmol/L; second (n = 9): −0.70 − −0.40 mmol/L; third (n = 8): −0.30 − 0.10 mmol/L. One-way ANOVA, $P < .05$; *vs second tertile, $P = .10$; and vs third tertile, $P < .05$, Bonferroni post hoc test.

in the control group were nonsignificant, these changes were also correlated ($r = -0.46$, $P < .01$).

No correlation was found between the change in IL1RN mRNA expression and the change in S_I after weight loss. However, IL1RN baseline expression was inversely correlated with baseline S_I ($r = -0.43$, $P = .04$) even after adjustments for body composition, sex, or age. The change in mRNA expression of the other cytokines measured in the study were not significantly correlated with the change in S_I ($P > .05$).

The increase in IL6 mRNA expression was correlated with the decrease in fasting plasma glucose concentration ($r = -0.53$, $P < .01$). According to tertiles of the change in fasting plasma glucose (Fig. 3), individuals who had a greater reduction in fasting plasma glucose concentration had a greater increase in IL6 expression. The increase in IL6 expression also correlated with changes in body fat percentage ($r = -0.46$, $P = .02$), but adjustment for age attenuated the association such that only a trend for significance was then observed ($r = -0.39$, $P = .06$).

Finally, the increase in IL8 mRNA expression after weight loss was correlated with the decrease in hsCRP concentration ($r = -0.51$, $P = .01$).

4. Discussion

The present study shows that cytokines involved in inflammatory conditions are expressed in PBMCs of obese or overweight individuals with abnormal glucose metabolism and the metabolic syndrome. The 9-month period of sustained weight loss achieved in the weight reduction group resulted in an increase of IL6 and IL8 mRNA expression along with a decrease of TNF, IL1B, IL1RN, and IL18 expression in PBMCs. We are not aware of other long-term lifestyle intervention studies in which expression levels of inflammatory markers were measured in circulating immune cells of obese subjects with the metabolic syndrome.

Although gene expression of cytokines in PBMCs could reflect their production by these cells, it is still not clear how much this accounts for their levels in the circulating blood. However, our study is the first to show that in the circulating mononuclear immune cells of obese individuals, which have been shown to be in a proinflammatory state [15], modulation of genes encoding pro- and anti-inflammatory markers occurs after weight loss, coupled with an improvement in glucose and insulin metabolism. It is important to note that these cells are also a target for insulin action [28]. Our findings are also relevant in terms of glucose metabolism because the immune system may be involved in the development of type 2 diabetes mellitus [29]. Because concentrations of serum hsCRP, TNF-α, and IL-6 decreased along with weight loss, the overall effect of weight reduction was anti-inflammatory.

At first glance, the increase in IL-6 expression after weight loss was unexpected. In obesity, only one study has reported increased IL-6 expression in PBMCs from obese women [15], whereas another study did not [20]. Although IL-6 is considered a proinflammatory cytokine, it is pleiotropic and also has anti-inflammatory properties [30,31]. Moreover, an insulin-sensitizing role for this cytokine has recently been shown [32]. In this regard, another interesting finding was the association between the increase in IL-6 expression and the decrease in fasting plasma glucose after the weight loss. We can speculate that if obesity-related inflammation occurs with hyperglycemia, the immune response in PBMCs may be impaired [33]. Following an improvement in glucose levels, the immune function of PBMCs should normalize.

The apparent discrepancy between IL6 mRNA expression and plasma concentration changes after weight loss could have been a consequence of the pleiotropic and cell-specific actions of IL-6. It is known that IL-6 circulating levels reflect mostly IL-6 adipose tissue production and act in a paracrine fashion, up-regulating hepatic CRP production and release [12,34,35]. In fact, baseline levels and changes in plasma IL-6 were positively correlated with baseline and changes in hsCRP concentrations in our study population (data not shown).

We also observed a consistent increase in IL8 expression after weight loss. This may reflect an improvement of the inflammatory state because it correlated with the decrease in plasma hsCRP. The decrease in hsCRP concentration in our study also suggests a reduction in low-grade inflammation and lower risk of developing type 2 diabetes mellitus [36,37]. A decrease in gene expression and plasma concentration of TNF-α was also observed. These changes are in accordance with previous findings relating TNF-α to the development of insulin resistance [38,39].

The decrease in IL1RN expression after weight loss likely reflected the improvement in insulin and glucose metabolism [40]. The decreased expression levels of this cytokine must have been a response to a less insulin-resistant environment, which in our study was not directly reflected by IL-1 receptor antagonist circulating levels. However, the lower values for both serum concentration (data not shown) and mRNA expression for this cytokine in these subjects also correlated with higher values for S_I at baseline.

Interestingly, weight loss resulted in a decrease in IL1B expression levels that was associated with an increase in insulin sensitivity assessed with the minimal-model intravenous glucose tolerance test. This test has been shown to reliably reflect insulin sensitivity in nondiabetic individuals [41]. Obesity seems to increase the production of IL-1β in PBMCs from subjects with type 2 diabetes mellitus and from healthy subjects [42]. Although lower adiponectin serum levels have been associated with insulin resistance [43], its increase observed after weight loss did not modify the association of the change in IL1B mRNA expression with the change in S_I. Leptin has also been implicated in the regulation of body weight and insulin sensitivity [44] and has been suggested to regulate cytokine balance [45] and up-regulate IL1B expression in lympho-

cytes [20]. We found that the change in serum concentrations of leptin was related to weight loss and improvement in insulin sensitivity, but changes in leptin did not modify the effect of changes in IL1B expression on the variation of the change in S_I. Taken together, the impact of weight loss on ameliorating insulin sensitivity could in part be ascribed to a decrease in IL1B expression.

One limitation of the present study was the small sample size in each group. However, even with a relatively small number of subjects, it was possible to observe highly significant changes and associations that are supported by the previous literature and are biologically plausible. The variation among individuals in the magnitude of weight loss may have obscured some of the effects of long-term weight loss on changes in cytokine expression. Our study suggests interplay between gene regulation of major cytokines and insulin sensitivity, but caution must be taken because the mRNA levels do not directly reflect protein production or secretion.

Measurement of gene expression of cytokines in PBMCs during study interventions provides novel insight into the role of inflammation in the pathogenesis of insulin-resistant states such as obesity and the metabolic syndrome. The strong correlation found between the decrease in IL1B expression and the increase in insulin sensitivity and the downregulation of the IL1RN along with the improvement of insulin and glucose metabolism suggest that the up-regulation of these genes in immune cells may play a role in insulin-resistant states that characterize obesity and metabolic syndrome.

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