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ARTICLE

Downregulation of genes involved in NFκB activation in peripheral blood mononuclear cells after weight loss is associated with the improvement of insulin sensitivity in individuals with the metabolic syndrome: the GENOBIN study

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Abstract
Aims/hypothesis The transcription factor nuclear factor-kappa-B (NFκB) is implicated in inflammatory responses, obesity and the metabolic syndrome, while immune cells appear to play a central role in mediating insulin resistance and can be used as a model to study inflammation and its relationship with insulin resistance. In peripheral blood mononuclear cells of overweight participants with the metabolic syndrome, we evaluated (1) the effect of diet-induced weight loss on the expression of genes involved in NFκB activation and (2) their association with insulin sensitivity. The genes studied were: TNF receptors *TNFRSF1A* and *TNFRSF1B*, and *IL1R1*, *TLR4*, *TLR2*, *ICAM1*, *CCL5* and *IKBKB*.
Methods We analysed data from 34 overweight participants with abnormal glucose metabolism and the metabolic syndrome, who were randomised to a weight-reduction (n=24) or control group (n=10) for 33 weeks. The mRNA expression was measured using real-time PCR. Measures of insulin and glucose homeostasis were assessed by IVGTT and OGTT.
Results In general, the genes studied were downregulated after weight loss intervention. The changes in *TLR4*, *TLR2*, *CCL5 and TNFRSF1A* mRNA expression were associated with an increase in insulin sensitivity index independently of the change in waist circumference (p<0.05). The change in *IKBKB* expression correlated with most of the changes in gene expression in the weight-reduction group.
Conclusions/interpretation These results suggest that proteins encoded by *CCL5*, *TLR2* and *TLR4*, and *TNFRSF1A* might contribute to insulin-resistant states that characterise obesity and the metabolic syndrome.

Trial registration: ClinicalTrials.gov NCT 00621205

Keywords CCL5 · Gene expression · Inflammation · Insulin resistance · Insulin sensitivity · Metabolic syndrome ·

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Peripheral blood mononuclear cells · TLR · TNF · Weight reduction

Abbreviations

CCL5	chemokine (C-C motif) ligand
ICAM-1	intercellular cell adhesion molecule-1
IKKβ	I-kappaB-kinase β
NFκB	nuclear factor-kappa-B
PBMCs	peripheral blood mononuclear cells
s	serum-soluble
S_I	insulin sensitivity index
TLRs	toll-like receptors

Introduction

Obesity is characterised by a state of chronic low-grade inflammation [1, 2], which may contribute to the development of insulin resistance [3]. Lifestyle intervention, including weight loss, has been shown to improve insulin and glucose metabolism and to reduce abdominal obesity [4–9]. Moreover, individuals with the metabolic syndrome are at an increased risk of type 2 diabetes mellitus and cardiovascular diseases [10, 11].

We recently found that a long-term weight loss intervention in individuals with the metabolic syndrome altered gene expression of cytokines related to inflammation and the immune response in peripheral blood mononuclear cells (PBMCs). Moreover, the decrease in *IL1B* mRNA expression was associated with an improvement of insulin and glucose metabolism [9]. These findings and those of others suggest that these cells are a target for insulin action and can be used as a model to study inflammation and its relationship with insulin resistance [12–14]. Moreover, monocytes, which along with lymphocytes, are an important cell type in PBMCs, differentiate into macrophages in most tissues [15], including adipose tissue. PBMCs are also convenient for gene expression studies because they can be easily and repeatedly collected in sufficient quantities in contrast to adipose, muscle and liver tissues and could reflect the disturbance of insulin action.

The transcription factor nuclear factor-kappa-B (NFκB) is implicated in the regulation of inflammatory responses and control of the innate immune system [16, 17]. Recent studies have highlighted the role of NFκB and I-kappaB-kinase β (IKKβ) in the development of insulin resistance and type 2 diabetes mellitus [18, 19]. IKKβ is responsible for the phosphorylation and degradation of the inhibitor-kappaB complex, which enables the activation of NFκB [16]. Pro-inflammatory cytokines like TNF-α and IL1β stimulate NFκB activation through classical receptor-mediated mechanisms. Toll-like receptors (TLRs) are key regulators of immune responses. Thus TLR4, which is part of the bacterial lipopolysaccharide receptor, is an important bridge between innate and adaptive immunity and also triggers NFκB, with consequent production of cytokines such as TNF-α and IL1β [20–22].

Inflammatory processes are characterised by the migration of proliferating white blood cells from the circulation to the tissues [23]. The relevance of these activated immune cells and inflammation in the development of type 2 diabetes mellitus has recently been shown, and they appear to play a central role in mediating insulin resistance [24, 25]. In animal models, lack of IKKβ in immune cells fully protects from insulin resistance [24]. Moreover, there is speculation that nutrients can act through pathogen-sensing systems such as the TLRs, giving rise to metabolically or nutritionally induced inflammatory responses [26, 27].

Against this background, we evaluated: (1) the effect of diet-induced weight loss on the expression of genes associated with the activation of NFκB and encoding of receptors for TNF-α (*TNFRSF1A* and *TNFRSF1B*, IL1 (*IL1R1*) and TLRs 2 and 4 (*TLR2* and *TLR4)*; and (2) their association with the improvement of insulin sensitivity that occurred with weight loss. We also sought to evaluate: (1) mRNA expression of genes that are related to immunological and endothelial function, and are positively regulated by NFκB, e.g. *CCL5*, *ICAM1* [20] and the pro-inflammatory mediator *IKBKB*; and (2) their association with the improvement in insulin sensitivity.

Methods

Participant recruitment and clinical investigation Altogether 46 overweight or obese (BMI 28–40 kg/m^2) participants aged 40 to 70 years with impaired fasting glucose (fasting plasma glucose concentration 5.6–7.0 mmol/l) or impaired glucose tolerance (2 h plasma glucose concentration 7.8–11.0 mmol/l) and at least two other features of the metabolic syndrome according to the Adult Treatment Panel III criteria [28] were randomised to a diet-induced weight-reduction (n=28) or control group (n=18) as previously described [7]. Of these, 24 participants randomised to the weight-reduction and ten participants randomised to the control group, all matched for age, sex, BMI and the status of glucose metabolism, were included in the present study as previously described [9]. Briefly, the weight-reduction group underwent a 12 week intensive weight-reduction period during which they followed detailed instructions given by a clinical nutritionist and based on a 4 day dietary record and an interview [29]. During the period between weeks 12 and 33 the minimum aim was to maintain the achieved reduction in weight using the same dietary prescription. Participants in the control group were

advised to keep their dietary and lifestyle habits unchanged during the study. 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 written informed consent.

Blood samples were drawn and anthropometric measurements (body weight, height, body fat mass and percentage body fat as assessed by bioimpedance [STA/BIA Body Composition Analyzer; Akern Bioresearch, Florence, Italy], and waist circumference) were carried out at baseline (week 0) and at the end of the study (week 33).

Glucose tolerance tests A 2 h OGTT was performed with 75 g of glucose. Blood samples for plasma glucose and serum insulin were drawn at 0, 30 and 120 min. A frequently sampled IVGTT was performed according to the Minimal Model method as previously described [30]. The insulin sensitivity index (S_I) was calculated by the MINMOD Millennium software [31].

Biochemical analyses Biochemical analyses were performed in the Clinical Laboratory Centre of the Kuopio University Hospital and in the Department of Clinical Nutrition and Institute of Biomedicine of the University of Kuopio. Plasma glucose concentration was analysed by the hexokinase method (concentration range: 4–8 mmol/l; Thermo Clinical Labsystems, Vantaa, Finland). Insulin was determined by the chemiluminescence sandwich method using an automated system (ACS; Bayer, Tarrytown, NY, USA). The intra-assay and interassay CVs were 7.7% and 9.0%, respectively. Serum soluble (s) intercellular cell adhesion molecule-1 (ICAM-1) and chemokine (C-C motif) ligand 5 (CCL5) concentrations were measured by solid phase ELISA (Quantikine; R&D Systems, Minneapolis, MN, USA). The intra-assay and interassay CVs were 6.0 and 4.4%, respectively for sICAM-1 and 5.0 and 5.0%, respectively for CCL5.

Isolation of PBMCs, RNA extraction and real-time PCR analysis of gene expression Samples were available for 24 participants of the weight-reduction group and ten participants of the control group. PBMCs were isolated from anticoagulated peripheral blood by density centrifugation for 10 min at 900 *g* and for another 10 min at 300 *g* using a reagent (Lymphoprep; Axis-Shield, Oslo, Norway) and total RNA was extracted using a kit (RNeasy Mini; Qiagen, Valencia, CA, USA) as previously described [9]. RNA was reverse-transcribed into cDNA using a kit (High-Capacity cDNA Archive; Applied Biosystems, Foster City, CA, USA) and real-time PCR was performed with TaqMan chemistry using ready-made assays from Applied Biosystems. Gene expression was normalised to an endogenous control (*GAPDH* gene). Measurements were done on samples from baseline (week 0) and end of the study (week 33).

Statistical analysis Univariate general linear models were used to test changes in circulating sICAM-1 and CCL5 levels and variables of gene expression between weeks 0 and 33 with group as the fixed factor and the baseline measurement as a covariate whenever the baseline measurement had an effect on variable change and was different between groups. Because age influenced some of the changes in mRNA expression of the target genes, these variables were also included in the model. Within-group analyses were performed if either the overall variable change or treatment effect was significant. To test associations between gene expression and S_I changes in the weight-reduction group, correlations were performed using partial correlation analyses adjusted for changes in body weight or waist circumference. Multivariate linear regression analyses were carried out to test for independent effects of changes in gene expression on changes in S_I. Variables with a skewed distribution were log transformed before the analyses and are presented as medians (interquartile range). A p value of <0.05 was considered to be statistically significant. All analyses were performed using SPSS software version 14.0 for Windows (SPSS, Chicago, IL, USA).

Results

Characteristics of the participants at baseline and after the interventions As already published [9], there were no differences in age, sex and variables related to body composition, insulin and glucose homeostasis, lipids, circulating inflammatory markers and blood pressure between groups at baseline (Table 1). Improvements in mean body weight (93.0 vs 88.4 kg), BMI (33.0 vs 31.4 kg/m^2), waist circumference (108.3 vs 104.6 cm), body fat mass (34.5 vs 31.5 kg), percentage body fat (37.2 vs 35.8%) and S_I (2.40×10^{-4} vs 2.62×10^{-4} min^{-1} [mU/l]$^{-1}$; to convert values to SI units (min^{-1} [pmol/l]$^{-1}$) multiply by 0.167) were observed only in participants from the weight-reduction group after week 33 of the intervention ($p<0.05$ for all), but not in the control group (body weight 85.7 vs 85.7 kg, BMI 31.6 vs 31.5 kg/m^2, waist circumference 104.1 vs 104.3 cm, body fat mass 31.4 vs 30.9 kg, percentage body fat 36.9 vs 36.4% and S_I 2.64×10^{-4} vs 2.43×10^{-4} min^{-1} [mU/l]$^{-1}$; $p>0.05$ for all). Serum circulating levels of TNF-α, IL6 and high-sensitive C-reactive protein also decreased only in the weight loss group [9].

Changes in gene expression in PBMCs In the weight-reduction group a decrease in both *TNFRSF1A* and

Table 1 Clinical and biochemical characteristics of the participants at baseline

Characteristics	Weight reduction	Control	*p* value
N	24	10	
Age (years)	58±7	62±8	0.19
Sex (male/female)	10/14	4/6	0.62
Body weight (kg)	93±16.1	85.7±8.5	0.19
BMI (kg/m^2)	33.0±3.4	31.6±1.9	0.22
Waist circumference (cm)	108.3±9.3	104.1±3.6	0.17
Systolic blood pressure (mmHg)	136±18	135±11	0.83
Diastolic blood pressure (mmHg)	89±10	86±10	0.39
Fasting plasma glucose (mmol/l)	6.5±0.5	6.4±0.5	0.61
2 h plasma glucose (mmol/l)	6.8±2.1	8.2±2.5	0.12
Fasting serum insulin (pmol/l)	85.4±44.5	67.7±20.1	0.11
2 h serum insulin (pmol/l)	425.7 (207.7–729.9)	339.6 (230.6–725.1)	0.83
S_I ($\times 10^{-4}$ min^{-1} [mU/l]$^{-1}$)[a]	2.4 (1.4–2.9) [b]	2.6 (1.9–3.1)	0.26
Serum total cholesterol (mmol/l)	5.1±1.1	5.1±0.9	0.96
HDL-cholesterol (mmol/l)	1.2±0.2	1.2±0.2	0.87
Serum triacylglycerol (mmol/l)	1.3 (1.0–2.1)	1.5(1.1–2.4)	0.48
TNF-α (pg/ml)	1.17 (0.61–2.54)	1.47 (0.54–2.55)	0.89
IL-6 (pg/ml)	2.55±1.15	2.10±1.10	0.31
hsCRP (mg/l)	2.84 (1.38–5.79)	2.36 (1.11–3.12)	0.42

Data are mean±SD or median (interquartile range)
[a]To convert values to SI units (min^{-1} [pmol/l]$^{-1}$) multiply by 0.167
[b]n=22
hsCRP, high-sensitivity C-reactive protein
Adapted from de Mello et al. [9]

TNFRSF1B mRNA expression occurred along with a decrease in *IL1R1* and *TLR4* expression (Fig. 1a). In the control group, by contrast, *TNFRSF1A* and *TLR4* mRNA levels increased (Fig. 1b). The mRNA levels of *TNFRSF1B* did not change in the control group. However, a decrease in *IL1R1* and an increase in *TLR2* expression did occur, although for the latter conventional statistical significance was not reached (p=0.06). In the weight-reduction group, no change in *TLR2* mRNA expression was found (p=0.25). A difference in the level of change between weight loss and control groups was seen only for *TLR2* mRNA levels (p=0.01).

We also looked at whether the weight loss intervention could have modulated other genes involved in NFκB activation. Weight loss significantly decreased mRNA levels of *IKBKB*, *CCL5* and *ICAM1* in PBMCs (Fig. 2), whereas in the control group, *IKBKB* mRNA levels increased and no change in *ICAM1* and *CCL5* expression was found (Fig. 2).

Correlations among gene expression changes and with changes in insulin sensitivity after weight loss We observed that the decrease in *TNFRSF1A* expression correlated with the increase in S_I when adjusted for change in body weight (r=−0.57, p=0.007) or waist circumference (r=−0.58, p=0.008). Moreover, the change in *TLR2* also correlated with the change in S_I (r=−0.48, p=0.03). Adjustments for changes in body weight (r=−0.58, p=0.008) or waist circumference (r=−0.66, p=0.002) did not alter these associations. Furthermore, the decrease in *TLR4* and in *CCL5* mRNA expression correlated with increase in S_I after weight loss when adjusted for changes in body weight or waist circumference (*TLR4* r=−0.48, p=0.03, r=−0.50, p=0.02, respectively; *CCL5* r=−0.50, p=0.02, r=−0.43, p=0.04, respectively). Adjustment for changes in body fat mass or percentage body fat instead of changes in body weight or waist circumference did not alter any of these correlations.

To control for the possible effect of changes in body weight and waist circumference on S_I, multivariate linear regression analyses were carried out for each of these genes to test for independent effects of the fold changes in gene expression and the absolute change in body weight or waist circumference (independent variables) on changes in S_I (dependent variable). In these models, the change in waist girth and the change in mRNA expression of *TLR4*, *CCL5* or *TNFRSF1A* predicted the absolute change in S_I in the weight-reduction group (Table 2). The same was true for the effect of the change in *TLR2* mRNA expression on the change in S_I (Table 2). Replacing the change in waist circumference in the models with the change in either body fat mass or percentage body fat generated similar results.

The correlations among the mRNA expression changes of the target genes studied are depicted in Table 3. Except for *ICAM1*, the changes in mRNA expression of the genes was in general significantly correlated among each other. In particular, the changes in *CCL5* and *TLR4* were highly

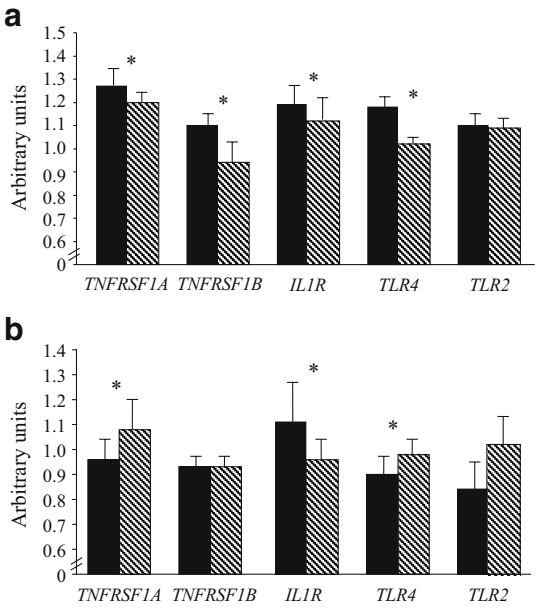

Fig. 1 mRNA expression of genes encoding surface ligands for cytokines and TLRs in the weight-reduction (n=24) (**a**) and in the control (n=10) (**b**) groups. Black bars, week 0; hatched bars, week 33. Data are means±SEM normalised to *GAPDH* mRNA expression. *p<0.05 for the change ([expression at week 33−expression at week 0]×100/expression at week 0) after univariate analyses

correlated with each other and with all changes in the expression of the other target genes. We also tested correlations between a previously noted change in *IL1B* mRNA expression [9] and the changes in mRNA expression observed in the present study (Table 3).

Changes in concentrations of circulating sICAM-1 and CCL5 Circulating levels of CCL5 and sICAM-1 were measured before and after the intervention. Circulating levels of sICAM-1 did not change in either the weight-reduction or the control group from baseline to week 33 (weight-reduction 179±41 vs 177±37 pg/ml, control 202±56 vs 212±51 pg/ml; p=0.09 and p=0.10, respectively). The change between groups, however, was of borderline significance (p=0.06). In the control group, serum levels of CCL5 were not different between week 0 and week 33 (77.61±20.92 vs 84.24±19.88 ng/ml, p=0.12); however, in the weight-reduction group, they increased significantly after the intervention (82.25±27.45 vs 85.47±23.91 ng/ml, p=0.01). The change in this serum marker was not different between groups (p=0.65).

Discussion

In the present study we show that a 9 month period of sustained diet-induced weight loss and improvement of insulin sensitivity resulted in a decrease of mRNA expression of genes encoding surface cell receptors involved in the activation of NFκB and the immune-inflammatory response as measured in PBMCs of overweight glucose-intolerant individuals with the metabolic syndrome. We found that the downregulation of some of these genes such as *TLR4*, *TNFRSF1A* and *CCL5* were independently associated with an improvement of S_I. To the best of our knowledge these findings have not been described before.

NFκB is considered to be a primary regulator of inflammatory responses [16]. It can be activated by a variety of stimuli, including TNF-α, IL-1, T cell activation signals, growth factors and stress inducers [32]. The binding of these ligands to specific receptors such as TNF, IL1 and TLRs induces a proinflammatory response leading to new expression of the genes encoding these ligands. This activation also controls the gene expression of molecules involved in the production of secondary inflammatory mediators such as *CCL5* and *ICAM1*. In the present study, the decrease in mRNA expression of *CCL5* and *ICAM1*, *IL1R1*, *TNFRSF1A* and *TNFRSF1B*, and *TLR4* after weight loss, and in particular of those genes encoding the surface cell receptors, could be interpreted as a lower state of activation of proinflammatory NFκB, although it was not our aim to measure NFκB activity itself. The fact that the decrease in *IKBKB* expression was generally highly associated with these markers supports our supposition.

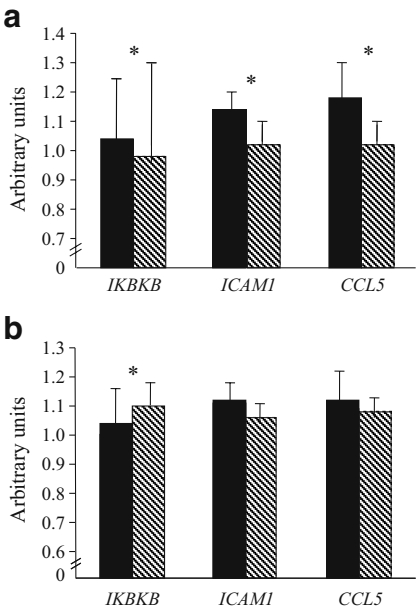

Fig. 2 mRNA expression of genes encoding surface ligands for cytokines and TLRs in the weight-reduction (n=24) (**a**) and in the control (n=10) (**b**) groups. Black bars, week 0; hatched bars, week 33. Data are median and interquartile range for *IKBKB* and mean±SEM for *ICAM1* and *CCL5* normalised to *GAPDH* mRNA expression. *p<0.05 for the change ([expression at week 33−expression at week 0]×100/expression at week 0) after univariate analyses

Table 2 Standardised regression coefficients (β) for each fold change in *TLR4*, *CCL5, TNFRSF1A* or *TLR2* mRNA expression and for each unit of the absolute change in waist circumference as determinants of the absolute change in the insulin sensitivity index (S_I) with weight loss in the weight-reduction group (n=22)

Model	β	p value
Model 1		
TLR2	−0.63	<0.01
Waist circumference	−0.57	<0.01
Model 2		
TLR4	−0.47	0.02
Waist circumference	−0.48	0.02
Model 3		
CCL5	−0.49	0.02
Waist circumference	−0.48	0.02
Model 4		
TNFRSF1A	−0.54	<0.01
Waist circumference	−0.48	0.02

Indeed, in humans, obesity is associated with increased NFκB binding activity and *IKBKB* expression in circulating mononuclear cells [12, 13].

Macrophage infiltration in adipose tissue has been linked to obesity-related inflammation and insulin resistance [33, 34], and upregulation of *CCL5* in visceral fat of obese individuals with the metabolic syndrome has recently been associated with markers of T cells and macrophages in this tissue [35]. Although we cannot directly conclude this, the correlation between the decrease in *CCL5* expression and that in *IKBKB* and *ICAM1* expression suggests that, besides NFκB activation, an interplay occurs between markers of endothelial function in immune cells on the one hand, and insulin and glucose metabolism after weight loss on the other. Conversely, circulating levels of sICAM-1 and CCL5 did not go in the same direction as gene expression in the present study, although these findings should be interpreted cautiously due to the relatively high CVs of the assays. Also, these discrepancies could be explained by the fact that CCL5 and ICAM-1 are produced by several other tissues in addition to PBMCs. On the other hand, increased mRNA expression may not necessarily be translated to protein and there may be other conditions that affect mRNA stability and mRNA translation. However, the decrease in expression of *ICAM1* and *CCL5* in PBMCs could play a role in avoiding the detrimental effect of the production of these molecules directly at sites of inflammation.

Interestingly, we found that after diet-induced weight loss the decrease in mRNA expression of *TLR4* and *TLR2*, and *CCL5* and *TNFRSF1A* was associated with an increase in insulin sensitivity. Glucose and insulin metabolism has been implicated in NFκB activation [36–39]. No correlation was found between *IKBKB* mRNA expression and S_I changes, but the downregulation of genes associated with S_I improvement was in general highly correlated with the change in *IKBKB*. Our data also suggest that TLR4 might also have an independent effect on the improvement in S_I that was induced by weight loss.

Increasing adiposity also activates IKKβ, possibly through cytokines or even TLRs [20]. TLR4 has recently been suggested to be involved in the adverse effects of NEFA on tissues and processes that constitute the metabolic syndrome. Also NEFA seem to induce proinflammatory cytokine expression via TLR4, which appears to be required for induction of insulin resistance by NEFA [27]. Our results show that the association between the changes in *TLR4*, *TLR2*, *TNFRSF1A* and *CCL5* mRNA expression and the improvement of S_I was nonetheless independent of the diet-induced changes in waist circumference or body weight. We were unable to demonstrate a significant change in fasting serum NEFA levels after weight loss (data not shown). Nevertheless, the decrease in waist circumference and its independent effect on S_I indicate a role for this abdominal fat mobilisation or oxidation, even if not reflected by serum NEFA levels [40].

Dietary components and exercise can also play a role in S_I and possibly in TLR4 and CCL5 [6, 8, 41–47]. This study is too small to reliably assess the independent effect of physical activity and diet, but adjustment for changes in physical

Table 3 Correlations (r) among changes in mRNA expression levels of target genes in the weight-reduction group (n=24)

Gene	*TLR2*		*TLR4*		*IKBKB*		*TNFRSF1A*		*TNFRSF1B*		*IL1R1*		*ICAM1*		*CCL5*	
	r	p value	r	p value	r	p value	r	p value	r	p value	r	p value	r	p value	r	p value
TLR4	0.63	<0.001														
IKBKB	0.30	0.17	0.42	0.04												
TNFRSF1A	0.61	0.002	0.73	<0.001	0.55	0.006										
TNFRSF1B	0.43	0.04	0.73	<0.001	0.42	0.04	0.45	0.03								
IL1R1	0.52	0.01	0.66	0.001	0.43	0.04	0.73	<0.001	0.33	0.12						
ICAM1	0	1.0	0.43	0.04	0.20	0.36	0.19	0.38	0.62	0.001	0.04	0.85				
CCL5	0.42	0.04	0.68	<0.001	0.56	0.004	0.67	<0.001	0.77	<0.001	0.50	0.01	0.53	0.007		
IL1B	0.51	0.013	0.71	<0.001	0.22	0.30	0.72	<0.001	0.44	0.03	0.46	0.02	0.22	0.31	0.514	0.01

activity frequency, serum NEFA and energy-adjusted intake of total fat, saturated fat, monounsaturated fat, polyunsaturated fat or total fibre did not attenuate the independent effect of changes in waist circumference and *TLR4*, *TLR2*, *TNFRSF1A* or *CCL5* on changes in S_I (data not shown).

In obesity, the effect of weight loss on lowering circulating concentrations of TNF-α and its expression in sites of production [9, 48, 49] could have an impact on the decrease in TNF receptor mRNA expression, with consequences on the activation of NFκB [35, 50]. Moreover, the associations between both the downregulation of *IL1B* [9] and the changes in *TLR4*, *TLR2*, *TNFRSF1A* or *CCL5* mRNA expression in PBMCs and the improvement of S_I after weight loss in the present study supports this hypothesis. Immune cells are not only exposed to adipose, liver and other tissues, but also cross-talk with these tissues [26]. Consequently, gene expression in PBMCs might reflect metabolic and immune responses of adipocytes or hepatocytes, and probably also their communication with other sites such as pancreatic islets and skeletal muscle.

The major limitation of this study was the small sample size. However, the methods employed and the design of the study were complex, time-consuming and expensive, and therefore difficult to apply to a much larger group of participants. Since the control group was much smaller than the weight-reduction group, we cannot rule out the possibility that the lack of statistically significant changes might have been due to inadequate sample size. In the future, larger studies with more statistical power are needed. However, even with a small number of participants it was possible to observe significant changes and associations that are supported by the previous literature and biologically plausible. Because we could not measure markers related to oxidative stress and intracellular redox status, we cannot exclude the possibility of redox modulation of NFκB activation and other inflammatory processes in the improvement of S_I [14]. Moreover, 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 expression of the markers studied.

Our results clearly showed an association between the improvement of insulin sensitivity that occurred with weight loss and attenuation of the expression of genes encoding receptors of surface ligands (*TLR4*, *TLR2*, *TNFRSF1A*) and immune mediators (*CCL5*) that are involved in NFκB inflammatory signalling pathways in the PBMCs of men and women with disturbed glucose metabolism and the metabolic syndrome. Our findings may also be relevant with respect to migration of PBMCs to sites of inflammation such as adipose tissue, liver or endothelium. The mechanisms by which diet-induced weight loss modulated the changes in gene expression and the associations with changes in insulin sensitivity require further clarification.

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Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

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