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Published in:

Diabetes, Obesity and Metabolism

DOI:

[10.1046/j.1463-1326.2003.00264.x](https://doi.org/10.1046/j.1463-1326.2003.00264.x)

2003

[Link to publication](#)

Citation for published version (APA):

Samuelsson, L., Gottsäter, A., & Lindgärde, F. (2003). Decreasing levels of tumour necrosis factor alpha and interleukin 6 during lowering of body mass index with orlistat or placebo in obese subjects with cardiovascular risk factors. *Diabetes, Obesity and Metabolism*, 5(3), 195-201. <https://doi.org/10.1046/j.1463-1326.2003.00264.x>

Total number of authors:

3

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Decreasing levels of tumour necrosis factor α and interleukin 6 during lowering of body mass index with orlistat or placebo in obese subjects with cardiovascular risk factors

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Aim: Obesity is associated with increased levels of inflammatory mediators. The objective of this study was to evaluate changes in the leucocyte derived inflammatory mediators tumour necrosis factor alpha (TNF- α), interleukin 6 (IL-6) and the isoprostane 8-epi-prostaglandin (PG) F_{2 α} during BMI lowering with orlistat (Xenical[®], Roche) or placebo.

Methods: TNF- α , IL-6, and 8-epi PGF_{2 α} evaluated in 376 subjects aged 18–75 years with BMI 28–38 kg/m² before and after 1 year of double-blind, randomized treatment with orlistat 120 mg or placebo three times daily.

Results: Weight reduction was associated with decreasing ($p < 0.001$) levels of TNF- α and IL-6 in both orlistat and placebo groups. After 12 months, TNF- α was lower ($p < 0.05$) in the orlistat compared with the placebo group. In the orlistat group, the change in TNF- α correlated with change in s-glucose ($r = 0.22$; $p = 0.01$), and the change in 8-epi-PGF_{2 α} correlated with changes in s-cholesterol ($r = 0.27$; $p < 0.001$) and s-LDL-cholesterol ($r = 0.28$; $p < 0.001$).

Conclusion: Weight reduction was associated with decreasing levels of both TNF- α and IL-6. After 12 months of treatment, TNF- α levels were lower in orlistat than in placebo-treated subjects. Whether these results translate into reduced incidence of cardiovascular disease remains to be elucidated.

Keywords: inflammation, TNF- α , IL-6, isoprostane, 8-epi-PGF_{2 α}

Received 2 December 2002; returned for revision 7 January 2003; revised version accepted 10 February 2003

Introduction

Excess adiposity increases the risk of atherosclerotic cardiovascular disease, independently of other risk factors, such as diabetes mellitus, arterial hypertension and dyslipidemia [1–4]. In atherosclerosis, there is a low level, chronic inflammatory process, with enhanced plasma levels of inflammatory mediators [5], e.g. C-reactive protein (CRP) [6], a hepatic acute-phase protein. In apparently healthy individuals, increased levels of CRP predict atherosclerotic manifestations during long time follow up [7]. C-reactive protein is largely regulated by

circulating levels of interleukin 6 (IL-6) [7,8]. Both IL-6 and tumour necrosis factor alpha (TNF- α) are expressed [9,10], and released [9,11] from adipose tissue. Strong relations have been found between body mass index (BMI), per cent body fat and levels of IL-6 [5,9,12], TNF- α [5], and CRP [5]. Weight reduction has recently been demonstrated to reduce IL-6 and TNF- α levels and subsequently improve endothelial function in a small female population without cardiovascular risk factors [13], supporting the concept that obesity is linked to a low-level chronic inflammatory state that might be related to cardiovascular disease.

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Isoprostanes are prostaglandin F₂-like compounds, derived from arachidonic acid through lipid peroxidation on cell membranes and LDL-particles, catalysed by oxygen free particles in a non-cyclooxygenase pathway [14,15]. Isoprostanes are measured as markers for lipid peroxidation. Isoprostane levels are increased in high age [16], hypercholesterolemia [16] diabetes mellitus [14,17] and smoking [18]. 8-epi-prostaglandin (PG) F_{2 α} is an isoprostane with biological effects such as platelet activation and smooth muscle cell proliferation [18], believed to contribute to atherosclerosis and thrombosis [18]. Furthermore, 8-epi-prostaglandin (PG) F_{2 α} levels are of special interest in this context as they are increased in several rat models of obesity [19–21].

Several pharmacological compounds for treatment of obesity are available [22]. One such drug is orlistat, which by inhibition of gastrointestinal lipases lowers absorption of dietary fat [22]. Orlistat has been shown to reduce weight in obese subjects at increased risk for cardiovascular disease [23]. In this study, we tested the hypothesis that BMI lowering during orlistat and diet treatment in obese subjects was associated with decreasing levels of the leucocyte derived inflammatory mediators TNF- α and IL-6, or of 8-epi-PGF_{2 α} .

Methods

Subjects

This work is part of a larger study of 376 men and non-pregnant women aged 18–75 years (mean 53.5 years) with BMI 28–38 kg/m² (table 1 [23]). All patients had at least one of the following obesity-associated risk factors for cardiovascular disease: fasting serum (s-) glucose \geq 6.7 mmol/l, or type 2 diabetes treated with sulphonylurea

or metformin but not thiazolidines or insulin; total s-cholesterol \geq 6.5 mmol/l and/or low density lipoprotein (LDL) cholesterol \geq 4.2 mmol/l on at least two occasions, or lipid-lowering medication; diastolic blood pressure \geq 90 mm Hg on at least two occasions, or anti-hypertensive medication. Numbers of patients receiving medication for cardiovascular risk factors were similar in both groups (table 1).

Exclusion criteria were prior myocardial infarction within 3 months, surgery for weight reduction, active gastrointestinal disorders, pancreatic disease, post-surgical adhesions, excessive alcohol intake or substance abuse, systemic steroid treatment other than hormone replacement, and use of certain drugs altering body weight or plasma lipids, such as appetite suppressants, resins, retinoids and fish oil supplements. The study protocol conformed to the Declaration of Helsinki. The ethics committees of all study centres and the Swedish Medical Product Agency approved the study. All participants gave written informed consent.

Methods

This was a 54-week, double-blind, randomized, placebo-controlled study conducted at 33 Swedish primary care centres. After screening, eligible patients entered a 2-week lead-in period during which they received single-blind placebo and were encouraged to follow a mildly hypocaloric diet based on national dietary recommendations, designed to provide \sim 30% of energy intake as fat. Energy content was calculated from estimated basal metabolic rate multiplied by 1.3 to estimate total daily energy expenditure. From this, 600 kcal day⁻¹ was subtracted to give a mildly hypocaloric diet. The minimum calorie intake was 1200 kcal day⁻¹.

Table 1 Demographic and metabolic characteristics of treatment groups at screening [mean \pm SD (range) or n(%)]. BP = blood pressure

	Orlistat (n = 190)	Placebo (n = 186)
Male/female	66/124	71/115
Age (years)	53.7 \pm 9.4 (27–74)	53.2 \pm 9.9 (28–75)
Weight (kg)	96.1 \pm 13.7 (65–134)	95.9 \pm 13.5 (65–130)
BMI (kg/m ²)	33.2 \pm 3.0 (27.5–38.7)	33.2 \pm 3.1 (27.2–40.4)
Total cholesterol (mmol/l)	6.15 \pm 1.21 (3.7–10.2)	6.06 \pm 1.19 (3.7–9.9)
LDL cholesterol (mmol/l)	3.75 \pm 1.38 (0.0–7.5)	3.66 \pm 1.41 (0.0–7.7)
Fasting glucose (mmol/l)	6.62 \pm 2.53 (4.0–17.5)	6.35 \pm 1.96 (3.9–18.1)
HbA1c (%)	5.7 \pm 1.2 (4.3–12.2)	5.5 \pm 0.9 (4.3–10.5)
Systolic BP (mmHg)	146 \pm 19 (99–217)	145 \pm 17 (108–190)
Diastolic BP (mmHg)	87 \pm 10 (60–110)	88 \pm 10 (60–118)
Antihypertensive drugs	84 (44)	74 (40)
Lipid-lowering drugs	36 (18)	27 (15)
Antidiabetic drugs	40 (21)	39 (21)

After the 2-week lead-in period, patients were randomized to receive orlistat (Xenical[®], Roche) 120 mg or placebo three times daily together with the mildly hypocaloric diet. After 6 months, the energy content was reduced by a further 300 kcal day⁻¹ to account for expected reduction in energy requirements as a result of weight loss. Patients visited the clinic regularly for dietary counselling. If treatment compliance fell below 60%, the subject was withdrawn from the study. Changes in medication for cardiovascular risk factors were assessed.

Plasma (p-)samples for analysis of TNF- α , IL-6 and 8-epi-PGF_{2 α} were taken before and after 12 months of treatment.

P-TNF- α and p-IL-6 Analyses

p-TNF- α and p-IL-6 were measured by ELISA using commercially available test kits (PharMingen, San Diego, CA, USA) according to procedures described by the manufacturer.

8-epi-PGF_{2 α} Analysis

p-8-epi-PGF_{2 α} was measured by enzyme immunoassay using commercially available test kits (Cayman Chemical Company, Ann Arbor, MI, USA) according to procedures described by the manufacturer.

Statistics

Differences between orlistat and placebo groups were analysed by the Wilcoxon rank sum test. For comparisons within groups, absolute changes in the study variables were analysed using the signed Wilcoxon rank sum test. *p*-values < 0.05 were considered significant. For comparisons between groups, relative changes were analysed, calculated as the individual difference for each subject between baseline and 12 months, divided by the baseline value and multiplied by 1000. Correlations were analysed using Spearman's correlation co-efficient. Because of the multiple correlations tested, only *p*-values = 0.01 were considered significant for correlations. All analyses were performed using SAS software, version 8.2e.

Results

All 376 subjects

Weight reduction occurred in both orlistat and placebo groups [5.9 \pm 5.5% (5.6 \pm 5.2 kg) vs. 4.6 \pm 5.4%

(4.3 \pm 5.9 kg) of initial body weight; *p* < 0.05]. This, as well as results concerning waist/hip ratio, blood pressure, s-lipid profile, fasting s-glucose, HbA1c and reduction of antidiabetic medication has been presented elsewhere [23]. Body mass index did not correlate with TNF- α , IL-6 or 8-epi-PGF_{2 α} at baseline. Weight reduction was associated with decreasing (*p* < 0.001) levels of TNF- α and IL-6 in both orlistat and placebo groups (table 2 and figure 1). After 12 months, TNF- α was lower (*p* < 0.05) in the orlistat compared to the placebo group (table 2). In the orlistat group, the change in TNF- α correlated with the change in s-glucose (*r* = 0.22; *p* = 0.01), and the change in 8-epi-PGF_{2 α} correlated with changes in s-cholesterol (*r* = 0.27; *p* < 0.001) and s-LDL-cholesterol (*r* = 0.28; *p* < 0.001). No such correlations were seen in the placebo group. There were no correlations in any of the groups between the amount of weight reduction and changes in TNF- α , IL-6 or 8-epi-PGF_{2 α} (data not shown).

Subjects With a Weight Reduction \geq 10%

Sixty-three subjects had lost \geq 10% of their weight after 12 months. Tumour necrosis factor α was then lower (*p* < 0.01) in the orlistat compared to the placebo group (table 2). Tumour necrosis factor decreased (*p* < 0.001) in both groups, but the relative decrease in TNF was larger (*p* < 0.01) in the orlistat than in the placebo group. IL-6 decreased significantly (*p* < 0.01) in the placebo group, whereas no changes occurred in the orlistat group.

Diabetic Subjects

Among the 98 diabetic subjects, TNF decreased significantly (*p* < 0.001) in both groups, whereas IL-6 decreased significantly (*p* < 0.01) only in the orlistat group.

Subjects with Arterial Hypertension

Among the 280 subjects with arterial hypertension, both TNF- α and IL-6 decreased significantly (*p* < 0.001) in both groups.

Discussion

In this study of obese subjects with risk factors for cardiovascular disease, we demonstrate that the reduction of BMI achieved with both orlistat and placebo was accompanied by decreasing levels of the leucocyte-derived inflammatory mediators TNF- α and IL-6, which

Table 2 Absolute values (pg/ml, median and range). O, orlistat, P, placebo

	Whole material		$\geq 10\%$ Weight reduction		Diabetes mellitus		Hypertension	
	O (n = 190)	P (n = 186)	O (n = 36)	P (n = 27)	O (n = 54)	P (n = 44)	O (n = 143)	P (n = 137)
0 months								
TNF- α	13.6 (0.1–89.8)	14.1 (0.3–96.1)	17.9 (3.0–46.5)	13.8 (0.4–41.6)	13.6 (1.1–46.7)	14.9 (2.1–96.1)	13.1 (0.1–89.8)	13.6 (0.3–96.1)
IL-6	5 (0.007–460)	4.8 (0.001–404)	5.4 (0.007–22.1)	5.4 (0.001–40.8)	4.7 (0.4–46.6)	4.8 (2.0–52.3)	5.4 (0.007–460)	4.6 (0.001–404)
8-epiPGF _{2α}	215 (64–3991)	210 (28–9774)	227* (133–2545)	187 (70–410)	228 (64–3226)	204 (28–9774)	216 (73–3991)	214 (28–9774)
12 months								
TNF- α	3.8*, § (0.001–130)	5.9§ (0.001–37.5)	3.1†, § (0.001–14.4)	6.7§ (1.0–35.9)	3.7§ (0.001–69.2)	5.4§ (0.001–28.6)	3.9§ (0.001–130)	5.5§ (0.001–35.9)
IL-6	3.2§ (0.001–335)	3.5§ (0.001–142)	4.1 (0.2–246)	3.8† (0.1–19.8)	3.1† (0.001–15.1)	3.7 (0.001–142)	3.0§ (0.001–335)	3.4§ (0.001–19.8)
8-epiPGF _{2α}	218 (30–3446)	228 (0.01–5104)	249 (30–1297)	225 (39–5104)	197 (50–3446)	215 (73–3269)	219 (30–3446)	201 (0.01–5104)

*p < 0.05 compared with placebo group.

†p < 0.01 compared with placebo group.

‡p < 0.01 compared with same group at diagnosis.

§p < 0.001 compared with same group at diagnosis.

are known to be elevated in cardiovascular disease [5]. On the other hand, the BMI reduction was not associated with any decrease in levels of the oxidative stress marker isoprostane 8-epi-PGF_{2 α} [18].

Decreasing levels of TNF- α and IL-6 have previously been reported during weight reduction with non-pharmacological methods in a smaller exclusively female population without cardiovascular risk factors [13], and IL-6 levels also decrease during short time follow-up of weight reduction in adolescents [24] and obese women [25]. Furthermore, TNF- α expression in adipose tissue has been shown to decrease during weight reduction [26]. As orlistat treatment is associated with significantly larger weight reductions than placebo [23,27], even larger effects upon TNF- α and IL-6 might have been expected during orlistat treatment than during weight reduction with non-pharmacological methods only. It is therefore interesting to note that TNF- α was lower in the whole orlistat treated group after 12 months, and that among subjects losing more than 10% of their initial weight, the relative decrease in TNF- α was larger (p < 0.01) in the orlistat than in the placebo group. Apart from this, only small differences concerning TNF- α , IL-6 and 8-epi-PGF_{2 α} were seen between orlistat and placebo groups in our study, however. Whether these differences in cytokine levels between study groups were due to specific effects of orlistat upon cytokines, or to the differences in weight reduction between the two study groups [23] cannot be safely concluded. The fact that we, like previous investigators [28], found no significant

correlations between the degree of weight reduction and the degree of decrease in TNF- α primarily supports the hypothesis of cytokine-specific effects of orlistat as explanation for the differences between TNF- α in the orlistat compared with the placebo group after 12 months. However, there are no data available from cell culture or animal models to support such a hypothesis. Although it is unclear whether the greater reduction in TNF- α is clinically relevant, our results indicate that adverse effects of orlistat upon inflammatory markers are highly unlikely.

In mainly non-diabetic obese women [25], but not in this study, plasma concentrations of IL-6 correlated with BMI and percent body fat. Furthermore, decreases in IL-6 have been reported to correlate with decreases in BMI during short time weight reduction in children and adolescents [24]. Although we demonstrate decreasing IL-6 during weight reduction, we did not find any correlations between the degrees of decrease in BMI and IL-6 during our longer follow-up of a larger patient material at higher age, and with cardiovascular risk factors. Previous diet therapy for obesity as well as the presence of, or treatment for, the different cardiovascular risk factors in our subjects might perhaps have influenced cytokine levels and interfered with their relationships with BMI.

Among diabetic subjects, TNF- α decreased in both orlistat and placebo groups. Circulating TNF- α has been proposed to mediate insulin resistance in the obese [10,29], and decreasing TNF- α levels might therefore contribute to improving insulin sensitivity after

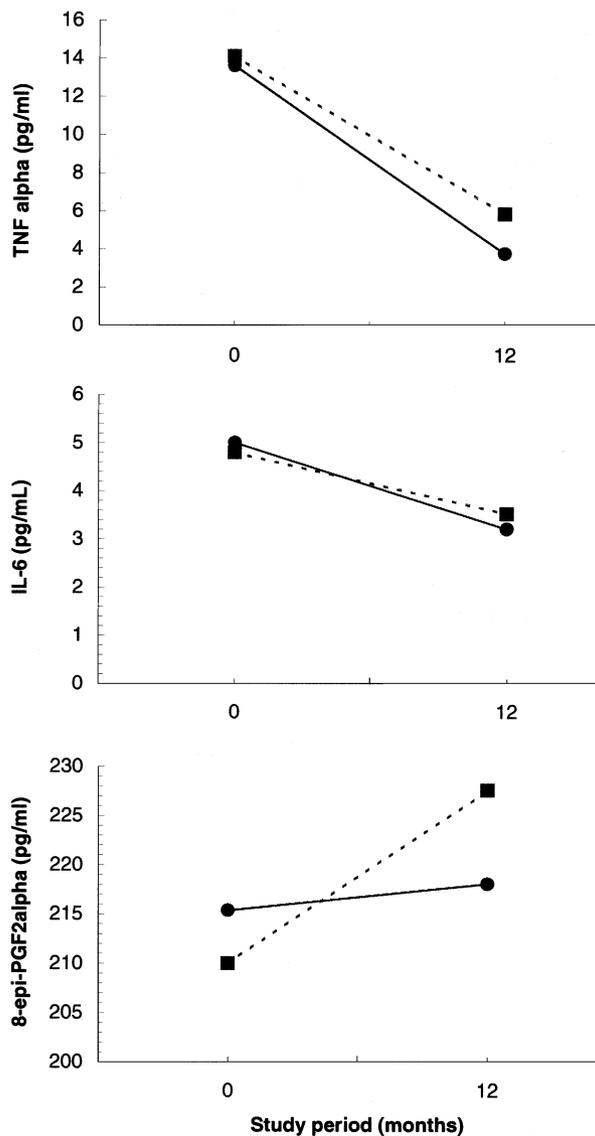


Fig. 1. Decreasing ($p < 0.001$) TNF- α and IL-6 but unchanged 8-epi-PGF $_{2\alpha}$ in orlistat and placebo groups at 0 and 12 months. After 12 months, TNF- α is lower ($p < 0.05$) in the orlistat than in the placebo group. Values are median, ●, orlistat ■, placebo.

weight loss [10,28]. Such mechanisms might also explain the relations between decreases in TNF- α and s-glucose in orlistat treated subjects in our study. Relationships between these results and the decreasing proportion of patients using antidiabetic medication during the study [23] cannot be excluded.

We failed to show decreases in 8-epi-PGF $_{2\alpha}$ levels during weight reduction in our study. Isoprostane-lowering effects such as those observed in obese rats after antioxidant vitamin E supplementation [20] does apparently not occur during weight reduction in obese humans with cardiovascular risk factors. As for IL-6, 8-epi-PGF $_{2\alpha}$ levels in our study might perhaps be influenced by the presence of other cardiovascular risk

factors [14,16,17], or the concomitant pharmacological treatment [14] which might influence isoprostane levels. The correlations between changes in 8-epi-PGF $_{2\alpha}$ and changes in S-LDL- and total cholesterol in our study are interesting, however, as 8-epi-PGF $_{2\alpha}$ levels are increased in hypercholesterolemia [16]. A change in one of these two variables seems to be related to a change in the same direction in the other variable. Our results do not justify speculations upon whether decreasing 8-epi-PGF $_{2\alpha}$ levels might contribute to beneficial effects of cholesterol lowering upon cardiovascular disease [30], however. Such a hypothesis needs to be tested by 8-epi-PGF $_{2\alpha}$ analysis in subjects treated with potent cholesterol-lowering agents.

In summary, weight reduction was associated with decreasing levels of both TNF- α and IL-6 in obese subjects with risk factors for cardiovascular disease. Furthermore, after 12 months of treatment, TNF- α levels were lower in orlistat than in placebo treated subjects. As CRP, endothelial reactivity or *in vivo* blood flow were not assessed in our study, it remains to be elucidated whether our results translate into reduced incidence of cardiovascular disease, as suggested by beneficial effects of reduction of cytokine levels upon vascular responses to L-arginine in healthy subjects [13].

Acknowledgements

The authors thank Barbro Palmqvist, MLT, for skilful technical assistance, and Fredrik Hansson, HH-statistik, Lund, Sweden, for expert statistical calculations and advice. This study was supported by the Ernhold Lundström Foundation, the Hulda Ahlmroth Foundation, Lund University Research Funds, Research Funds of Malmö University Hospital, the Foundation for Research upon Diabetes and Vascular Disease, and Roche AB, Stockholm, Sweden.

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