CLINICAL STUDY

Weight loss larger than 10% is needed for general improvement of levels of circulating adiponectin and markers of inflammation in obese subjects: a 3-year weight loss study

Erik L Madsen, Aila Rissanen², Jens M Bruun, Kristin Skogstrand³, Serena Tonstad¹, David M Hougaard³ and Bjørn Richelsen

Department of Endocrinology and Metabolism C, Aarhus University Hospital, Aarhus Sygehus, Tage Hansensgade 2, DK-8000 Aarhus C, Denmark,

¹Department of Preventive Cardiology, Ulleval Hospital, SFM N-0407 Oslo, Norway, ²Obesity Research Unit, Helsinki University Hospital, 00260 Helsinki, Finland and ³Department of Clinical Biochemistry, Statens Serum Institut, Artillerivej 5, DK-300 Copenhagen, Denmark

(Correspondence should be addressed to B Richelsen; Email: ovl06br@as.aaa.dk)

Abstract

Objective: To investigate the effects of: I) short- (8 weeks), II) long-term (3 years) weight loss, and III) the degree of weight loss on circulating levels of adiponectin, high sensitive-C reactive protein (hs-CRP), and fibrinogen in obese subjects. Moreover, to evaluate the effect of the lipase inhibitor, orlistat, on these parameters.

Design: Weight loss induced in 93 obese subjects (mean weight: 108.9 ± 15.8 kg) through 8-week very-low-energy diet (VLED, 800 kcal/day) followed by randomization to orlistat or placebo together with lifestyle intervention for further 3 years. Adiponectin and hs-CRP were measured at baseline, after 8 weeks of VLED and 6, 12, and 36 months after the VLED by flowmetric xMAP technology (Luminex Multi-Analyte Profiling System, Luminex Corp., Austin, TX, USA). Fibrinogen was measured in a coagulation assay.

Results: Weight loss after VLED treatment was 14.3 ± 4.5 kg and after 3 years 7.7 ± 8.7 kg. Orlistat-treated subjects regained 3.9 kg less than placebo-treated from the end of the VLED to 3 years ($P=0.01$). No differences were detected between the two groups regarding changes in adiponectin, hs-CRP, or fibrinogen. Accordingly, the groups were combined for further analyses. Serum adiponectin increased by 22% ($P<0.05$) after the VLED but returned to baseline after 3 years. Both short- and long-term weight losses needed to be in excess of 10% (~ 12 kg) in order to increase adiponectin levels significantly. Weight loss was associated with a significant decrease in hs-CRP. Fibrinogen decreased by 12% ($P<0.05$) after 3 years.

Conclusions: In obese subjects, weight loss was associated with an increase in serum adiponectin and a decrease in hs-CRP and plasma fibrinogen. Long-term weight loss (3 years) must exceed 10% to induce a combined significant improvement in these inflammatory markers.

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Introduction

Obesity is associated with a propensity towards the development of dyslipidemia, insulin resistance, hyperglycemia, hypertension, and a state of chronic low-grade inflammation, leading to an increased risk of cardiovascular morbidity and mortality (1–4). Human adipose tissue (AT) is not only a storage organ for excess energy but also an active endocrine organ known to produce and release a number of bioactive proteins known as adipokines, e.g., adiponectin, leptin, tumor necrosis factor (TNF)- α , interleukin (IL)-6, IL-8, and monocyte chemoattractant protein (MCP)-1, suggested to exert paracrine or endocrine effects (5–7). The obesity-associated low-grade inflammation may be caused by an increase in pro-inflammatory adipokines

such as IL-6, TNF- α , IL-8, and MCP-1 and a decrease in the anti-inflammatory adipokine adiponectin, contributing to the development of long-term complications such as insulin resistance, endothelial dysfunction, and atherosclerosis (8–10). From animal models, it is found that adiponectin may improve insulin sensitivity as well as decrease inflammation and atherogenesis (11–13). Plasma adiponectin has an inverse correlation with measures of body fat (4) and insulin sensitivity (13). Weight loss is associated with an increase in plasma adiponectin in some (14–16) but not all studies (17, 18). It remains unknown whether these opposing findings are due to differences in the degree of weight loss or the way weight loss is achieved, for example, short- versus long-term weight loss. Thus, the aim of the present study was to investigate the effect of both

short- (8 weeks of very-low-energy diet, VLED) and long-term weight loss (3 years) on plasma levels of anti- and pro-inflammatory markers (e.g., adiponectin, hs-CRP, fibrinogen) in abdominally obese subjects and to assess the level of weight loss needed to achieve a long-term improvement in these markers. Moreover, we investigated whether the anti-obesity drug, orlistat (a lipase inhibitor), had an independent effect on these parameters in a randomized controlled study.

Materials and methods

Study design

The present study is a sub-study of the Scandinavian multicenter study of obese subjects with the metabolic syndrome (SMOMS) which was performed to investigate whether treatment with the lipase inhibitor, orlistat, led to greater weight loss in the long term (3 years) as compared with placebo in a randomized placebo-controlled study (19). The present study consists of a subgroup of 93 subjects (49 in the orlistat group and 44 in the placebo group) who completed all visits of the SMOMS trial. Inclusion criteria were age between 18 and 65 years with a body mass index (BMI) of ≥ 30 – ≤ 45 kg/m² and a waist circumference ≥ 102 (male) or ≥ 92 cm (female) with at least one of the following risk factors: impaired fasting glucose (≥ 6.1 mmol/l), manifest type 2 diabetes (only diet treated), or dyslipidemia (high density lipoprotein (HDL) cholesterol ≤ 0.9 (male) or ≤ 1.1 mmol/l (female) and/or serum triglycerides ≥ 2.0 – < 10 mmol/l). Exclusion criteria were drug-treated diabetes mellitus, severe dyslipidemia (serum triglycerides ≥ 10 mmol/l), use of lipid-lowering agents, uncontrolled hypertension, or significant gastrointestinal problems. The 5% weight loss criterion during the VLED for continuation into the randomized treatment phase was fulfilled by 81% of the whole study population and 65% of the randomized subjects completed 36 months of treatment in the trial with no significant differences in dropout rates between the treatment groups (19). The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethical Committees in Denmark, Norway, and Finland. Written informed consent was obtained from all participants.

Lifestyle interventions and treatment

All subjects were initially asked to complete 8 weeks of VLED (600–800 kcal/day; Modifast (Novartis, Basel, Switzerland) or Nutrillett (Nycomed Pharma, Oslo, Norway)). Those subjects who were able to lose at least 5% of their initial body weight during the VLED phase were allowed to continue into the controlled double-blinded treatment phase of the study and were randomly assigned to either orlistat (120 mg three times daily) or

matching placebo capsules for further 3 years. Both treatment groups were supplemented with the same dietary and exercise counseling (19). In brief, the subjects were seen by dieticians ten times during the treatment period and advised to follow a diet with a reduced fat energy content in order to avoid weight regain and to avoid side effects of the lipase inhibitor. Energy intake and compliance to the diet were formally assessed at baseline and three times from after the VLED to 3 years. Average daily intake of total calories as well as fat energy content and cholesterol was significantly reduced from baseline to 3 years ($P < 0.05$; data not shown). The subjects were encouraged to increase their level of physical activity, and their physical fitness was assessed with the Urho Kaleva Kekkonen (UKK) institute walking test (20). The physical fitness increased significantly ($P < 0.05$) with no differences between the treatment groups in the original study (data not shown).

Blood sampling and anthropometrical measurements were done before and after the VLED period – and after 6 months, 1, and 3 years of treatment. The time course of the variables was evaluated over three periods: period I, from baseline to the end of the VLED; period II, from the end of the VLED to 3 years; and period III, from baseline to 3 years (see Table 1).

Measurements

Body weight, waist, and hip circumference were measured by standardized techniques. Plasma samples from all time points were analyzed together for fibrinogen (Clauss coagulation assay (21)), lipids, glucose, and insulin using standardized assays (NOVA Medical, Medilab A/S, Copenhagen, Denmark). Adiponectin and hs-CRP were analyzed together in serum by three-plexed sandwich immunoassays based on flow-metric xMAP technology (Luminex multi-analyte profiling system, Luminex Corp., Austin, TX, USA) performed and characterized essentially as described (22). In short, the serum samples were diluted 1:100 in buffer and set up in filter plate wells for duplicate determination. To each filter plate well we added 50 μ l diluted sample and 50 μ l suspension of capture antibody-conjugated beads, and 1500 beads per analyte. After 1½ h of incubation, the beads were washed twice and subsequently reacted for 1½ h with a mixture (50 μ l) of relevant biotinylated detection antibodies, each diluted 1:1000, before 50 μ l streptavidin–phycoerythrin, 20 μ g/ml was added to the wells, and the incubation was continued for an additional 30 min. The beads were finally washed twice and resuspended in 125 μ l buffer and analyzed on the Luminex 100 platform according to manufacturer's instructions. The intra- and inter-assay coefficients of variation (CV), assessed by repeated measurements on normal control plasma samples, for adiponectin were 4 and 23% respectively and for hs-CRP were 12 and 13% respectively. The working range for each analyte was

Table 1 Anthropometrical data, inflammatory markers, and lipids according to treatment group.

Variable	Treatment	Period III		
		Period I		Period II
		Baseline	After 8 weeks of VLED	3 years
Weight (kg)	Orlistat	109 (104.5–113.8)	95.8 (91.8–100.1)	99.9 (95–105.1)*
	Placebo	109.9 (105.1–115)	95.6 (91.3–100.1)	105 (99.4–110.9)
BMI (kg/m ²)	Orlistat	37.2 (35.9–38.5)	32.7 (31.5–33.9)	33.9 (32.4–35.4)*
	Placebo	37.7 (36.4–39.1)	32.8 (31.6–34.1)	35.8 (34.1–37.6)
Waist circumference (cm)	Orlistat	117.4 (114–121)	106.5 (103.3–109.9)	108.7 (104.7–112.8)*
	Placebo	117.7 (114–121.6)	105.9 (102.4–109.4)	112.2 (107.7–116.8)
Adiponectin (µg/ml)	Orlistat	0.38 (0.32–0.45)	0.49 (0.41–0.58)	0.43 (0.35–0.53)
	Placebo	0.44 (0.36–0.53)	0.5 (0.42–0.6)	0.42 (0.33–0.52)
Hs-CRP (µg/ml)	Orlistat	2.5 (2.3–2.6)	2.6 (2.3–2.8) [†]	2.4 (2.2–2.5)
	Placebo	2.3 (2.2–2.5)	2.2 (2–2.4)	2.2 (2–2.4)
Fibrinogen (g/l)	Orlistat	4.3 (3.9–4.7)	4.9 (4.6–5.2)	3.9 (3.7–4.1)
	Placebo	4.4 (4–4.8)	4.8 (4.5–5.1)	3.6 (3.4–3.8)
Total cholesterol (mmol/l)	Orlistat	6 (5.5–6.4)	4.9 (4.5–5.3)	5.6 (5.2–5.9)
	Placebo	5.8 (5.4–6.3)	4.5 (4.2–4.9)	5.4 (5.1–5.8)
HDL cholesterol (mmol/l)	Orlistat	1.15 (1.07–1.23)	1.09 (1.01–1.17)	1.14 (1.05–1.24)
	Placebo	1.16 (1.08–1.25)	1.08 (1–1.17)	1.17 (1.06–1.28)
LDL cholesterol (mmol/l)	Orlistat	3.8 (3.4–4.1)	3 (2.8–3.4)	3.4 (3.1–3.8)
	Placebo	3.5 (3.2–3.9)	2.7 (2.4–3)	3.2 (2.9–3.5)
Triglyceride (mmol/l)	Orlistat	2 (1.7–2.3)	1.5 (1.2–1.7)	1.8 (1.5–2.1)
	Placebo	2.2 (1.8–2.6)	1.5 (1.3–1.8)	1.9 (1.6–2.3)

The patients were randomized to orlistat and placebo after 8 weeks of VLED. * $P < 0.05$: differences between changes from the end of the VLED to 3 years (period II) according to treatment groups. [†] $P < 0.05$: differences between levels of treatment groups at noted time point. Values are geometric means (except hs-CRP: arithmetic mean) with 95% confidence intervals adjusted for gender, age, site, and baseline fasting plasma glucose category (normal versus elevated) $n = 66$ –68.

assessed from the precision profile and defined as the concentration range where the CV is below 20%. The range of concentrations investigated and approved for adiponectin was 1.95–1100 ng/ml and for hs-CRP was 0.2–5.12 µg/ml. As compared with conventional ELISA measurement, the level of adiponectin was lower when measured by the present Luminex method. In evaluation studies, we found by measuring random blood samples from the present study that the mean value of adiponectin was 0.77 ± 0.45 µg/ml measured by Luminex and 3.20 ± 1.49 µg/ml by ELISA (B-Bridge International, San Jose, CA, USA). Thus, the present adiponectin levels should be added with 1.77 and multiplied by 1.84 as compared with the levels obtained by ELISA. This may be due to the interference of heterophile antibodies which are well-recognized causes of interference in immunoassays (23). The heterophile antibodies can cause false-negative results if binding directly to the capture antibodies and thereby blocking the reactive site from binding the specific analyte (adiponectin). Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as $\text{HOMA-IR} = (\text{fasting p-glucose} \times \text{fasting p-insulin}) / 22.5$.

Statistical analysis

The dropout rates for the involved sites did not differ significantly by the treatment groups or gender. Data were checked for normal distribution and transformed by logarithm base 'e' if needed. Absolute values (log base e)

apart from hs-CRP which conformed better to normal distribution in untransformed values and changes were analyzed in a linear mixed model with unstructured covariance structure adjusting for gender, site, age, treatment, and baseline fasting plasma glucose category (normal versus elevated glucose ≥ 6.1 mmol/l) together with interaction for time. For analysis of log base e changes during periods I–III, a multiple linear regression analysis was used including adjustment for gender, age, site, baseline fasting plasma glucose category, and changes in weight. For analysis of relative changes of non-transformed data, values were indexed with each individual's baseline value set as 100%, and non-parametric statistics were applied: Friedman repeated measurements ANOVA on ranks with modification for multiple comparisons against control and the Mann–Whitney test were used when appropriate. Correlations were analyzed by the Spearman rho method for non-normally and by the Pearson method for normally distributed parameters. The statistical software used for the analysis was Sigma-Stat v. 3.1, Systat Software, Inc. and SPSS v. 14.0, SPSS, Chicago, IL, USA). Results are geometric means with 95% confidence intervals or medians (25th–75th percentiles) unless otherwise noted. The number of participants was 93, but for adiponectin and hs-CRP only samples from 68 subjects were eligible for analysis. Thus, the exact numbers of participants included in the various measurements are stated in the figures and tables. A P value < 0.05 was considered statistically significant.

Results

Baseline characteristics

Baseline characteristics are given in Table 1. The participants were obese with a mean BMI of 37 kg/m^2 and had elevated total cholesterol ($6.0 \pm 1.2 \text{ mmol/l}$), glucose ($6.6 \pm 1.6 \text{ mmol/l}$), insulin ($15.2 \pm 8.1 \text{ IU/l}$), and systolic and diastolic blood pressure ($150/96 \text{ mmHg}$). Mean age was 45.8 ± 8.2 years, and the proportion of males, was 54.4%. The two treatment groups did not differ significantly concerning age or proportion of males, anthropometrical variables or lipids (Table 1) nor concerning blood pressure ($148/94$ vs $151/97 \text{ mmHg}$) or HOMA-IR (4.2 ± 3.1 vs 5.1 ± 3.7).

Weight loss

VLED treatment for 8 weeks (period I) induced a mean weight loss of $14.3 \pm 4.5 \text{ kg}$ corresponding to 13.1% of initial body weight (Fig. 1). From the end of the VLED to the end of the 3-year trial (period II), the total weight regain was significantly smaller in the orlistat group compared with the placebo group (4.8 ± 8.5 vs $8.7 \pm 6.3 \text{ kg}$, $P=0.01$; Table 1 showing adjusted values; Fig. 1). However, when calculating the total weight loss from baseline to 3 years (period III), the weight loss was 2.6 kg (8.9 ± 8.3 vs $6.3 \pm 9.1 \text{ kg}$) larger in the orlistat group compared with the placebo group. This difference did not, however, reach statistical significance ($P=0.16$; for unadjusted values). The orlistat group continued losing weight up to 6 months after the VLED ($P<0.05$; Fig. 1), whereas the placebo group regained weight at all visits following the VLED. Also, regain of BMI and waist circumference from the end of the VLED to the end of the 3-year trial (period II) was significantly

reduced in the orlistat group ($P<0.05$) compared with placebo (Table 1). Regarding the overall outcome of weight changes from baseline to 3 years (period III) in the two groups together: 31% of the subjects lost more than 10% of their initial body weight, 29% of the subjects lost between 5 and 10%, another 29% lost from 0 to 5%, and 11% of the subjects gained weight compared with baseline levels. These proportions did not differ significantly ($P=0.29$, χ^2) between the two treatment groups (data not shown).

Effect of treatment and weight changes on adiponectin, hs-CRP and fibrinogen

Changes in adiponectin, hs-CRP and fibrinogen did not differ significantly between the treatment groups during period I (baseline to after the VLED), period II (from after the VLED to 3 years), nor in period III (from baseline to 3 years) analyzed through a mixed linear model adjusting for age, gender, site, and baseline category of normal versus elevated fasting plasma glucose also adjusting for interaction with time (Fig. 2). A non-significant tendency towards lower levels of fibrinogen in the placebo group was present at 3 years ($P=0.06$). A multiple linear regression for periods I–III also including weight change was made in order to check whether weight change was associated with changes in adiponectin, hs-CRP, and fibrinogen and also to check for whether the effects of weight change might depend upon treatment group. Two results emerged from this analysis: 1) weight change was significantly negatively associated with changes in adiponectin independent of treatment, gender, age, site, and fasting plasma glucose level during the study ($P<0.01$). Changes in hs-CRP were significantly ($P<0.05$) positively associated with changes in weight from baseline to 3 years (period III) but not during the VLED (period I; $P=0.06$) nor from the end of the VLED to 3 years (period II). Changes in fibrinogen are not associated with changes in weight during the study. 2) Treatment by itself adjusted for the mentioned factors was not significantly associated with changes in adiponectin, hs-CRP, and fibrinogen. As visualized in Fig. 2, changes in the various markers of inflammation did not differ between the two treatment groups during the study. These findings are in accordance with the results from the original cohort finding that no significant differences between metabolic changes in the two treatment groups were observed (19). In order to increase the power of the analysis, we pooled the results from the two treatment groups (orlistat and placebo) and further analysis of the effect of weight change was conducted on all subjects taken together.

Adiponectin increased significantly by 22% (-10 to $+50\%$) median (interquartile range; $P<0.05$) from baseline to the end of VLED (period I; Fig. 3). Six months after the VLED, adiponectin was still significantly

Figure 1 Weight changes during the study. After 8 weeks of VLED, the patients were randomized to either orlistat (●) or placebo (○) for a further 3 years. Data are mean $\pm$ S.E.M. $N=93$. * $P<0.05$ and ** $P<0.01$ for differences in weight regain between treatment groups from the end of the VLED.

Figure 2 Adiponectin, hs-CRP and fibrinogen according to treatment groups. Values are A: fibrinogen (g/L), B: hs-CRP ($\mu\text{g/ml}$), C: adiponectin ($\mu\text{g/ml}$). A and C represent geometric and B arithmetic means with 95% CI adjusted for gender, site, age, and normal versus elevated fasting plasma glucose according to treatment groups: orlistat (●) or placebo (○). * $P < 0.05$ for differences between levels. No significant differences between the treatment groups regarding changes from baseline: periods I and III or from the end of the VLED: period II; $n = 63$ –68.

elevated by 24% (0 to +55%; $P < 0.05$) compared with baseline levels. Thereafter, adiponectin decreased and at 3 years, no significant changes compared with baseline levels were detected in the whole group, despite a still significant body weight reduction (~ 7.5 kg; Fig. 1).

hs-CRP did not change significantly from baseline to the end of the VLED (period I) but was significantly reduced by 10% (+8 to –25%) 6 months after the VLED ($P < 0.05$) and by 18% (–2 to –36%) after 1 year ($P < 0.05$). However, after 3 years, only a non-significant reduction was found compared with baseline (period III; Fig. 3).

Figure 3 Weight-related changes in levels of inflammatory markers. Relative changes in adiponectin (●), hs-CRP (▽), fibrinogen (■), and weight (◇) during the study. Data are medians of relative changes compared with baseline values. $N = 63$ –68. * $P < 0.05$ as compared with baseline.

Compared with baseline, fibrinogen increased significantly by +10% (–1 to +27%) at the end of the VLED (period I; $P < 0.05$) but was found to be significantly reduced by –12% (–2 to –25%) after 3 years (period III; $P < 0.05$; Fig. 3).

Influence of the degree of weight loss on adiponectin, hs-CRP and fibrinogen

In order to investigate the significance of the degree of weight loss upon changes in adiponectin, a linear mixed model was established for changes in adiponectin from baseline to 3 years (period III) adjusted for treatment, gender, site, and normal versus elevated fasting plasma glucose using age and weight change as covariates. This model confirmed the significant ($P < 0.01$) negative relationship between changes in adiponectin and changes in weight. The analysis showed that a weight loss of 10% resulted in a significant 11% increase in adiponectin with 95% confidence intervals (0–23%) after adjustment. This relationship is illustrated by the scatterplot in Fig. 4 showing unadjusted changes in weight and adiponectin with 95% confidence intervals of the predicted mean indicating that a weight loss of $\sim 10\%$ or more is needed for a significant increase in adiponectin.

In order to further investigate these findings on unadjusted values, we divided the population into quartiles of relative weight loss from baseline to the end of the VLED (period I) and from baseline to 3 years (period III).

Adiponectin A VLED-induced weight loss larger than 12% corresponding to more than 13 kg weight loss resulted in significantly elevated levels of adiponectin of +22.3% (–10 to +49.5%) $P < 0.01$ (data not shown),

Figure 4 Scatterplot with relative changes in adiponectin (%) from baseline to 3 years (period III) plotted by changes in weight (%). Regression curve with 95% confidence interval of predicted mean together with reference line indicating baseline adiponectin levels (0% change). The values are unadjusted log (base e) differences transformed into relative changes explaining the non-linear scale; $n = 68$.

whereas weight losses below 12% only induced non-significant increases in adiponectin levels. From baseline to 3 years (period III), the relative increase in adiponectin (percentage of baseline values) was significantly higher ($P<0.01$) when comparing subjects losing 11.2–28.9% of baseline weight (highest quartile) compared with subjects with a weight change of -2.5 to $+14.5\%$ (lowest quartile). Moreover, only subjects from the highest weight loss quartile during period III (weight loss $>11\%$) achieved a significantly increased adiponectin level ($P<0.05$; Fig. 5), when comparing changes from baseline to 3 years.

Hs-CRP Relative levels of hs-CRP did not differ significantly between the quartiles of relative weight change after the VLED (period I) and even the subjects from the highest weight loss quartile did not have a significant reduction of hs-CRP compared with baseline.

Figure 5 Effect of the level of weight loss on changes in inflammatory markers. The population was divided according to the degree of weight loss from baseline to 3 years. Relative levels of adiponectin, hs-CRP, and fibrinogen are shown according to quartiles of relative weight changes. Data are presented as box plots where the upper and lower lines of the boxes represent the 25th and 75th percentiles and the middle line represents the median value. Whiskers represent 5th and 95th percentiles. $N=66-68$. * $P<0.05$ and ** $P<0.01$, comparison of first versus fourth weight change quartile. # $P<0.05$, comparison of baseline levels with the fourth weight change quartile.

After 3 years, the decrease in hs-CRP was significantly larger in subjects from the highest versus the lowest quartile of relative weight change ($P<0.05$, Fig. 5). After 3 years, weight losses larger than 11% were also associated with a significantly lower hs-CRP level than baseline levels ($P<0.05$, Fig. 5). A significantly lower level of hs-CRP ($P=0.015$) was also found in the subjects with the highest ($>10.8\%$) compared with those with the lowest relative waist circumference decrease (data not shown).

Fibrinogen Relative levels of fibrinogen did not differ significantly between quartiles of relative weight loss after the VLED (data not shown) nor after 3 years (Fig. 5)

Lipids, HbA1c and HOMA-IR The changes (%) in lipids, HbA1c, and HOMA-IR according to the highest and lowest quartiles of weight change (%) from baseline to 3 years (period III) are referred in Table 2. Subjects from the fourth quartile (-11.2 to -28.9% weight loss) had significantly larger decreases in HOMA-IR ($P<0.001$), HbA1c ($P<0.001$), and triglycerides ($P<0.01$) as well as larger increases in HDL cholesterol ($P<0.05$) compared with subjects from the first quartile ($+14.5$ to -2.4%), but no significant differences regarding changes in total and LDL cholesterol were detected.

Bivariate correlations

Changes in adiponectin after the VLED treatment (period I) correlated negatively with weight loss ($r=-0.27$, $P<0.05$) and positively with changes in HDL cholesterol ($r=0.23$, $P=0.05$) but was not correlated ($r=-0.03$) with changes in HOMA-IR. After 3 years, changes in adiponectin were correlated with weight loss ($r=-0.33$, $P<0.01$) and HDL cholesterol ($r=0.30$, $P<0.05$) in a similar way as shown already after 8 weeks. The changes in adiponectin were also negatively correlated with changes in insulin resistance ($r=-0.24$, $P=0.05$) after 3 years. But the changes in hs-CRP correlated positively both with weight loss ($r=0.24$, $P<0.05$) and with HOMA ($r=0.25$, $P<0.05$) after 3 years. Fibrinogen did not correlate to any variables in this study.

Discussion

A relatively large weight loss was induced by 8 weeks of VLED (period I) followed by a 3-year randomized phase (period II) with treatment with either orlistat or placebo during which the majority of the subjects regained weight. The treatment with orlistat did not result in a significantly larger overall weight loss compared with placebo when including the effect of the VLED (baseline to 3 years; period III). But orlistat treatment did diminish the weight gain from the end of the VLED to

Table 2 Relative changes in metabolic parameters (%) from baseline to 3 years according to weight loss quartiles (%).

	First quartile of weight loss (range: +14.5 to -2.5%; n=17) Mean (95% CI)	Fourth quartile of weight loss (range: -11.2 to -28.9%; n=17) Mean (95% CI)	All subjects (range: +14.5 to -28.9%; n=68) Mean (95% CI)
Δ-Weight	+1.3% (+3.6 to -1.0)	-17.4% (-14.3 to -20.3) [‡]	-7.8% (-5.8 to -9.7) [¶]
Δ-Total cholesterol	-2.2% (+11.8 to -14.4)	-8.9% (-4.5 to -13.1)	-7.5% (-2.9 to -11.8) [¶]
Δ-LDL cholesterol	-8.6% (+9.4 to -23.6)	-9.2% (-3.2 to -14.9)	-10.5% (-4.1 to -16.4) [¶]
Δ-HDL cholesterol	-4.7% (+1.8 to -10.8)	10.4% (24 to -1.7) [*]	1.6% (6.1 to -2.7)
Δ-Triglyceride	20.6% (+45.6 to -0.1)	-32.7% (-11.8 to -48.7) [‡]	-12% (-1.3 to -21.5) [§]
Δ-HbA1c	-8.1% (-5.9 to -10.3)	-17.7% (-13 to -22.2) [‡]	-13.1% (-11.3 to -14.9) [¶]
Δ-HOMA-IR	24.8% (+63.9 to -5.0)	-59% (-41.3 to -71.4) [‡]	-29.9% (-17.3 to -40.5) [¶]

* $P < 0.05$, [†] $P < 0.01$, and [‡] $P < 0.001$; fourth versus first quartile of weight loss (%), ANOVA with adjustment for multiple comparisons (Dunnett). [§] $P < 0.05$ unadjusted, [¶] $P < 0.01$, and [‡] $P < 0.001$ for changes from baseline to 3 years (period III) in all subjects. Linear mixed model for repeated measurements with adjustment for multiple comparisons (Sidak). Values are geometric means with 95% CI of relative changes (transformed from log changes (base e)); n=67–68.

3 years (period II) (19). However, this difference was not associated with any significant differences between changes in the inflammatory markers in the two groups. Accordingly, the results presented are the changes of the combined orlistat and placebo group.

Interestingly, we found that a mean weight loss of ~14 kg after 8 weeks of VLED resulted in a significant 22% increment in serum levels of the adipocyte-specific protein adiponectin. In contrast, a smaller weight loss (e.g. 5–7 kg) was unable to increase plasma adiponectin significantly neither in the short term (8 weeks) nor in the long term (3 years). We also found that relatively large weight losses (more than 10–11%) were necessary in order to obtain a continuous significant increase in plasma adiponectin levels. These results may in part explain some of the discrepancy between the various studies reporting no association between weight losses in the range of 6.5–8% and adiponectin increase (24, 25) compared with significant increases in plasma adiponectin seen with a weight loss of ~14–15% (16).

Adiponectin is of particular interest due to its inverse association with obesity and its stipulated insulin-sensitizing effects (13). Moreover, adiponectin may play a central role for the low-grade inflammation milieu found in obesity – being inversely associated with IL-6 and TNF- α (16, 26) – and through its ability to stimulate release of anti-inflammatory proteins such as IL-10 and IL-1 receptor antibodies (27). The visceral AT depot seems to be more proinflammatory than the s.c. as demonstrated by a higher release of proinflammatory adipokines (6), more macrophage infiltration (7, 28), and lower adiponectin expression (29). In cross-sectional studies, the visceral AT depot is suggested to be an important determinant of circulating adiponectin levels, indicating that reduced adiponectin might link visceral obesity with the insulin resistance/metabolic syndrome (30). However, our data indicate that a substantial relative weight reduction over 3 years is needed for lasting increases in adiponectin levels.

The validated markers of chronic low-grade inflammation hs-CRP and fibrinogen were also affected by weight loss with a significant reduction in hs-CRP up to 12 months after the VLED with a non-significant

tendency to remain reduced after 3 years. Parallel to our findings on adiponectin, we found that the long-term (3 years) weight loss needed to be larger than 10% in order to induce a significant reduction in hs-CRP. Several other studies have also found that weight loss in obese patients is associated with reductions in hs-CRP (26, 31–33). Some of these studies have a shorter follow-up period and it may therefore be difficult to distinguish between the effects of the hypocaloric state induced by diet from the weight loss *per se*. The data presented in this study indicate that a relatively large net weight loss is needed in order to be associated with a stable reduction in hs-CRP levels up to 3 years. Plasma fibrinogen increased transiently after the VLED but was followed by a long-term significant reduction. The exact mechanism for the VLED-induced increase in fibrinogen remains unknown but it has been shown that starvation and intracellular mobilization of fatty acids may represent a stress to, for example, the hepatic metabolism resulting in increased generation of acute phase reactants (34). A similar explanation can be proposed for the lack of decrease in hs-CRP levels following the VLED in the present study. This tendency was also noted in a study where the weight loss was induced by gastric restrictive surgery also showing increased levels of acute phase reactants (35).

In the present study, we have not investigated whether weight loss was differently associated with the various molecular forms of adiponectin (high, medium, and low molecular weight) but a recent study (36) has shown that total adiponectin increased by 36% after weight loss and the various molecular forms of adiponectin all increased to a similar degree.

We found no additional effects of orlistat treatment on plasma levels of adiponectin, hs-CRP, and fibrinogen, which is in accordance with our recent study where no extra effects of orlistat were seen on the metabolic profile (19). This fact may be related to the pronounced metabolic effects induced by the VLED phase (inducing a mean weight loss of 14 kg) that may mask the possibly more discrete effects of orlistat (23). Generalization of these findings, considering the possible intervention effect of the regime described, must take into

consideration that they are based on a selected population with the capability of losing at least 5% of their body mass during 8 weeks of VLED and complete 3 years of participation in a formalized study. But even though we did not find any additive effect of orlistat treatment following 8 weeks of VLED, this does not preclude possible effects of orlistat in other studies with different types of adjuvant weight loss support. In fact, other studies have found that orlistat treatment may induce independent reductions in the levels of triglycerides, hs-CRP, TNF- α , insulin-like growth factor-I and isoprostane (37, 38), or improvements in insulin sensitivity and free fatty acids at equivalent weight losses compared with placebo (39). However, these studies lasting 6–12 months may explain why decreasing long-term effects induced by lessened compliance or adaption to the drug might not be seen.

It is well known that weight losses in the range of 2.5–5% are able to improve factors of the metabolic syndrome such as insulin sensitivity (40). Although several studies have been published concerning weight loss and subsequent changes in inflammatory markers (15, 31, 41), it still remains unknown what the magnitude of weight loss should be in order to obtain long-term improvements in these inflammatory markers. But our present study indicates that higher weight losses are necessary to affect the inflammatory markers than are needed to affect insulin sensitivity.

In conclusion, we found a dose–effect relationship between the degree of weight loss and the improvement in plasma levels of adiponectin and hs-CRP. A net weight loss larger than 10% of the initial body weight seemed necessary for long-term combined improvement of adiponectin, hs-CRP and fibrinogen levels.

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