Available online at www.sciencedirect.com
ScienceDirect
Metabolism Clinical and Experimental 58 (2009) 946–953
Metabolism
Clinical and Experimental
www.metabolismjournal.com

Long-term weight loss decreases the nontraditional cardiovascular risk factors interleukin-18 and matrix metalloproteinase–9 in obese subjects

Erik L. Madsen[a], Jens M. Bruun[a], Kristin Skogstrand[b], David M. Hougaard[b], Tore Christiansen[a], Bjørn Richelsen[a,*]

[a]*Department of Endocrinology and Metabolism C, Aarhus University Hospital, Aarhus Sygehus, 8000 Aarhus C, Denmark*
[b]*Department of Clinical Biochemistry, Statens Serum Institut, 2300 Copenhagen S, Denmark*

Received 28 August 2008; accepted 13 February 2009

Abstract

The objective of the study was to investigate the effect of long-term (3.2 years) weight loss on serum levels of the nontraditional cardiovascular risk factors interleukin (IL)-18 and matrix metalloproteinase (MMP)–9. Moreover, we wanted to assess the significance of the magnitude of the weight loss and evaluate the potential effects of 36 months of treatment with the lipase inhibitor orlistat on these parameters. Sixty-eight abdominally obese subjects completed 8 weeks of very low energy diet (600-800 kcal/d) followed by 36 months of randomized treatment with either orlistat or placebo together with lifestyle intervention. Serum levels of IL-18, MMP-9, and leptin were measured by flowmetric xMAP technology (Luminex, Austin, TX). Changes in the levels of IL-18, MMP-9, and leptin were similar in the orlistat and the placebo group during this study. Thus, the 2 groups were combined for further analysis. A weight loss of 8.4 ± 8.8 kg from baseline to 3.2 years was associated with significant decreases in IL-18 ($P < .001$), MMP-9 ($P < .01$), and leptin ($P < .001$). Matrix metalloproteinase–9 was, however, significantly increased after 8 weeks of very low energy diet–induced weight loss ($P < .05$). The long-term changes in IL-18 were significantly associated with changes in body mass index independent of changes in blood pressure and lipids ($P < .05$). Levels and changes of IL-18 and MMP-9 were significantly positively associated at 3.2 years ($P < .01$). Long-term changes in leptin were significantly associated with changes in IL-18 ($P < .01$) at 3.2 years. Diet-induced long-term weight loss decreased IL-18 and MMP-9. The decrease in IL-18 was associated with changes in body mass index independent of changes in blood pressure and lipids, indicating that even a minor weight reduction (>5%) has beneficial effects on nontraditional cardiovascular risk markers. Orlistat treatment had no independent effects on IL-18, MMP-9, or leptin in the present study.

1. Introduction

Obesity is associated with dyslipidemia, hypertension, insulin resistance, type 2 diabetes mellitus, and an increased risk of cardiovascular morbidity and mortality [1-4]. Traditional cardiovascular risk markers such as high-sensitivity C-reactive protein (hs-CRP) and fibrinogen are elevated, and the vascular function assessed by endothelial function testing is impaired already in obese adolescents [5]. An early feature of abdominal obesity is the development of a chronic low-grade inflammation [5-7], suggested to originate from especially the visceral adipose tissue (AT), in which adipocytes [8] as well as infiltrating adipose tissue macrophages [ATM] [9] secrete a number of pro- and anti-inflammatory proteins, collectively referred to as *adipokines* [10].

Interleukin (IL)-18 is a proinflammatory cytokine belonging to the IL-1 family involved in a number of inflammation-related diseases including atherosclerosis [11]. Plasma IL-18 is increased in obesity [12-14]; is associated with insulin resistance [12,15]; and is in some studies associated with atherogenesis, atherosclerotic plaque instability, and cardiovascular death [16-19]. Interleukin-18 is expressed primarily in macrophages and dendritic cells [19] but also in the AT, particularly in the stromal vascular fraction [12,20,21]. Interleukin-18 has the ability to induce the secretion of matrix metalloproteinase (MMP)–9, a member of the family

* Corresponding author. Tel.: +45 8949 7696; fax: +45 8949 7659.
E-mail address: bjoerich@rm.dk (B. Richelsen).

0026-0495/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2009.02.031

of zinc-binding proteolytic enzymes involved in the remodeling of the extracellular matrix [22]. Matrix metalloproteinase–9 can also be found in the vulnerable region of atherosclerotic plaques, where it increases the risk of plaque rupture; and it is associated with increased risk of cardiovascular morbidity and mortality [23-25]. Matrix metalloproteinase–9 is increased in obesity and decreased by weight loss [26,27]. It is produced in the AT where it is involved in adipocyte differentiation and AT remodeling [28]. Matrix metalloproteinase–9 release from human endothelial cells and coronary artery smooth muscle cells is reported to be induced by leptin [29].

The aim of the present study was to assess the effects of a diet-induced weight loss in middle-aged obese subjects on the nontraditional cardiovascular risk markers IL-18 and MMP-9. Being a weight loss study, changes in the AT-specific protein leptin was assessed in parallel. In addition, the possible differences in the response to an intensive short-term (8 weeks) and long-term (3.2 years) intervention were investigated. Finally, we also investigated whether the antiobesity drug orlistat (a lipase inhibitor) had an independent effect on these parameters in a randomized controlled study.

2. Materials and methods

2.1. Study design

The data presented in the present study originated from a subset of participants in the Scandinavian Multicenter study of Obese subjects with the Metabolic Syndrome (SMOMS) [30] where blood samples were taken for cytokine/adipokine measurements in those of the original study centers that accepted to participate in this substudy. All participants who have taken these blood samples are included in the present substudy (68 of the originally 308 randomized participants). The SMOMS trial was a double-blinded, randomized intervention study investigating the antiobesity drug orlistat on weight loss maintenance/regain after an initial very low energy diet (VLED)–induced weight loss as compared with placebo [30]. In short, the study design was: age between 18 and 65 years, body mass index (BMI) between 30 and 45 kg/m^2, and waist circumference greater than 102 cm (men) or greater than 92 cm (women) with at least one of the following risk factors: impaired fasting plasma glucose and/or dyslipidemia as described in Richelsen et al [30]. The study consisted of 2 phases. Phase I involved 8 weeks of VLED (600-800 kcal/d) where subjects who lost greater than 5% of their initial body weight continued into phase II, which was a randomized double-blinded treatment phase with 36 months of treatment of 35 participants taking orlistat (120 mg TID) and 33 participants taking placebo in this substudy combined with a hypocaloric diet and regular consultations with a dietician as previously described [30,31]. 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.

2.2. Measurements

Blood samples were obtained at baseline, post-VLED, and after 3.2 years, respectively. Serum IL-18, MMP-9, and leptin levels were determined by a multiplex sandwich immunoassay that was performed as described using xMAP technology (Luminex, Austin, TX) and a Luminex 100 platform [32]. 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, 50 μL of diluted sample and 50 μL of a suspension of capture-antibody–conjugated beads, 1500 beads per analyte, were added. After 1 1/2 hours of incubation, the beads were washed twice and subsequently reacted for 1 1/2 hours with a mixture (50 μL) of relevant biotinylated detection antibodies, each diluted 1:1000, before 50 μL of streptavidin-phycoerythrin (20 μg/mL) was added to the wells; and the incubation was continued for an additional 30 minutes. The beads were finally washed twice, resuspended in 125 μL of buffer, and analyzed on the Luminex 100 platform according to manufacturer's instructions and as described by Skogstrand et al [32]. The IL-18 antibodies are mouse monoclonal D044-3 (capture) and D045-6 (detection) (MBL, Naka-Ku, Nagoya, Japan), the MMP-9 antibodies are mouse monoclonal MAB911 (capture) and goat polyclonal BAF911 (detection) (R&D Systems, Minneapolis, MN), and the leptin antibodies are mouse monoclonal MAB398 (capture) and goat polyclonal BAF398 (detection) (R&D Systems). The working range for each analyte was assessed from the precision profile and defined as the concentration range where the coefficient of variation was less than 20%. The range of protein concentrations investigated and approved was as follows: IL-18 (20-10 000 pg/mL), MMP-9 (1-500 ng/mL), and leptin (8-4000 pg/mL). To compare measurements obtained by the Luminex assay with conventional enzyme-linked immunosorbent assay (ELISA) technique, protein levels of IL-18, MMP-9 (R&D Systems), and leptin (Mediagnost, Reutlingen Germany) were reanalyzed in duplicate in 40 randomly chosen samples. The Luminex multiplex assays and the ELISA kits used different sets of antibodies that most probably recognize different epitopes, some of which may be more or less obscured by interacting serum proteins. Despite this, protein levels assessed by the ELISA and Luminex methods correlated significantly: IL-18 (Pearson $R = 0.75$, $P < .001$), MMP-9 ($R = 0.79$, $P < .001$), and leptin ($R = 0.78$, $P < .001$). Homeostasis model assessment as a measurement of insulin resistance (HOMA-IR) was calculated as (fasting plasma glucose $*$ fasting plasma insulin)/22.5. Lipids, glucose, and insulin were analyzed in plasma using standardized assays as previously described [30].

2.3. Statistics

Data were checked for normal distribution and log base e ([ln] − natural logarithm) transformed if needed. Differences between treatment groups were analyzed in a linear mixed model for repeated measurements with an unstructured covariance structure adjusted for age, sex, and site. For changes from baseline to 3.2 years, a multiple linear regression model was made regarding changes in ln IL-18 with adjustment for age, sex, site, and treatment. To explore the overall variation in IL-18, we established a linear mixed model for repeated measurements with unstructured covariance structure adjusted for age, sex, study site, and treatment groups with the following covariates: HOMA-IR, hemoglobin A_{1c} (HbA_{1c}), total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, triglyceride, diastolic and systolic blood pressure, and BMI. The *b* value with the 95% confidence interval (CI) is the estimated regression coefficient. Results are geometric means with 95% CIs unless otherwise noted. The level of significance was set at *P* less than .05 adjusted for multiple comparisons if noted (Sidak or Dunnet). The statistical software used for the analysis was SPSS version 14.0 (SPSS, Chicago, IL).

3. Results

3.1. Baseline characteristics

Baseline characteristics are shown in Table 1 (unadjusted values). The orlistat group did not differ significantly from the placebo group concerning anthropometric variables or lipids (Table 1), age (45.0 ± 8.4 vs 46.8 ± 8.0 years), proportion of women (51.1% vs 39.4%, χ^2, $P = .32$), systolic/diastolic blood pressure (148 ± 9/94 ± 11 vs 151 ± 14/97 ± 11 mm Hg), or HOMA-IR (4.1 ± 3.1 vs 5.1 ± 3.7).

3.2. Weight loss

Eight weeks of VLED treatment induced a mean reduction in BMI from 37.7 ± 3.7 to 33.0 ± 3.6 kg/m^2 ($P < .001$), corresponding to a decrease of 12.5% ± 3.2% ($P < .05$) of initial body weight. Waist circumference was reduced by 9.9% ± 4.2% ($P < .05$). After 3.2 years, mean BMI remained significantly reduced compared with baseline (34.9 ± 4.6 kg/m^2, $P < .001$), corresponding to a mean weight loss of 7.4% ± 7.8% ($P < .05$). Weight loss from baseline to 3.2 years did not differ significantly between the orlistat and the placebo group (9.9 ± 8.2 vs 6.8 ± 9.3 kg, $P = .16$). However, weight regain from the end of the VLED to the end of the study (3.2 years) was significantly reduced in the orlistat group compared with the placebo group (3.4 ± 8.5 vs 8.1 ± 6.5 kg, $P < .05$, Table 1). In parallel, regain of BMI and waist circumference was also significantly reduced in the orlistat group as compared with placebo from the end of the VLED to 3.2 years ($P < .05$, Table 1).

3.3. IL-18, MMP-9, and leptin

Neither changes over time nor levels of IL-18, MMP-9, and leptin (adjusted for sex, age, and study site) differed significantly between the treatment groups (Table 1). Thus, the 2 groups were combined for further analyses. Moreover,

Table 1
Anthropometric data, cardiovascular risk markers, and lipids according to treatment group

Variable	Baseline	Post-VLED	3.2 y	Treatment	Treatment group differences
Weight (kg)	110.8 ± 16.8	97.5 ± 15	100.9 ± 17.7	Orlistat	($P < .05$) for period II
	113.1 ± 16.1	98.1 ± 12.8	106.2 ± 14.6	Placebo	
BMI (kg/m^2)	37.5 ± 3.4	33 ± 3.3	34.1 ± 4.2	Orlistat	($P < .05$) for period II
	37.9 ± 4.1	33 ± 3.9	35.7 ± 5	Placebo	
Waist circumference (cm)	118.4 ± 11.6	107.4 ± 9.7	109.7 ± 12.6	Orlistat	($P < .05$) for period II
	119.5 ± 11	107.8 ± 9.8	114.1 ± 12	Placebo	
IL-18 (pg/mL)	124.7 (99-157.1)	112.9 (91-140)	89.1 (70.5-112.6)	Orlistat	
	106.8 (87.5-130.4)	93.3 (76.2-114.2)	86 (72.6-102)	Placebo	
MMP-9 (ng/mL)	128.7 (101.4-163.4)	159.1 (122.8-206.2)	106.7 (82.1-138.6)	Orlistat	
	122.6 (92.7-162.2)	151.5 (113.5-202.4)	81.3 (62.9-105)	Placebo	
Leptin (pg/mL)	1217.4 (964.7-1536.3)	470.2 (336.8-656.5)	724.3 (532.7-984.7)	Orlistat	
	989 (759.9-1287.3)	352.9 (243.3-512)	658 (470.5-920.2)	Placebo	
Total cholesterol (mmol/L)	6.0 ± 1.2	5.0 ± 1.1	5.5 ± 1.0	Orlistat	
	5.9 ± 1.2	4.7 ± 1.0	5.4 ± 0.9	Placebo	
HDL cholesterol (mmol/L)	1.16 ± 0.28	1.1 ± 0.3	1.2 ± 0.3	Orlistat	
	1.16 ± 0.22	1.1 ± 0.2	1.2 ± 0.3	Placebo	
LDL cholesterol (mmol/L)	3.9 ± 1.1	3.2 ± 0.9	3.5 ± 0.9	Orlistat	
	3.6 ± 1.0	2.8 ± 0.8	3.2 ± 0.9	Placebo	
Triglyceride (mmol/L)	2.2 ± 0.8	1.6 ± 0.7	2.0 ± 0.9	Orlistat	
	2.5 ± 1.4	1.7 ± 0.8	2.2 ± 1.1	Placebo	

The patients were randomized to orlistat or placebo after 8 weeks of VLED (orlistat, n = 35; placebo, n = 33). There were no significant differences between the 2 treatment groups concerning absolute levels, but there were differences between changes in treatment groups regarding period II: from the end of the VLED to 3.2 years ($P < .05$) as shown. Values are unadjusted arithmetic means ± SD except those for IL-18, MMP-9, and leptin, which are unadjusted geometric means with 95% CIs.

we investigated the possible influence of sex and found no differences in changes in IL-18, MMP-9, and leptin between women and men (data not shown). During the VLED, IL-18 decreased by 11% in the combined group ($P = .017$, Fig. 1). From baseline to 3.2 years, IL-18 decreased significantly by 24% even when adjusted for multiple comparisons ($P < .001$, Fig. 1). Matrix metalloproteinase–9 increased significantly by 24% during the VLED-induced acute weight loss ($P < .05$) but decreased significantly from baseline to 3.2 years ($P < .01$, Fig. 1). Leptin decreased significantly both during the VLED and from baseline to 3.2 years ($P < .001$, Fig. 1).

3.4. Relationships between degree of weight loss and changes in IL-18, MMP-9, and leptin

As shown in Fig. 2, the subjects were grouped into tertiles of changes in BMI from baseline to the end of the VLED and from baseline to 3.2 years. The changes in IL-18 induced by the 8 weeks of VLED did not differ between the tertiles of BMI decrease. The decrease in IL-18 from baseline to 3.2 years was significantly higher in those with the highest (third tertile) compared with those with the lowest (first tertile) decrease in BMI (−9.8% to −28.2% vs −3.5% to +14.6%, Fig. 2). The linear trend of change in IL-18 across the tertiles of BMI change was also significant ($P < .01$). Equivalent trends could be observed regarding changes in waist circumference and weight (data not shown). The increase in MMP-9 from baseline to the end of the VLED was significantly higher ($P < .05$) in subjects with a larger decrease in BMI ranging from (~−14% to −19%) compared with subjects with a lesser decrease in BMI (~−6% to −11%); and the linear trend for change in MMP-9 across

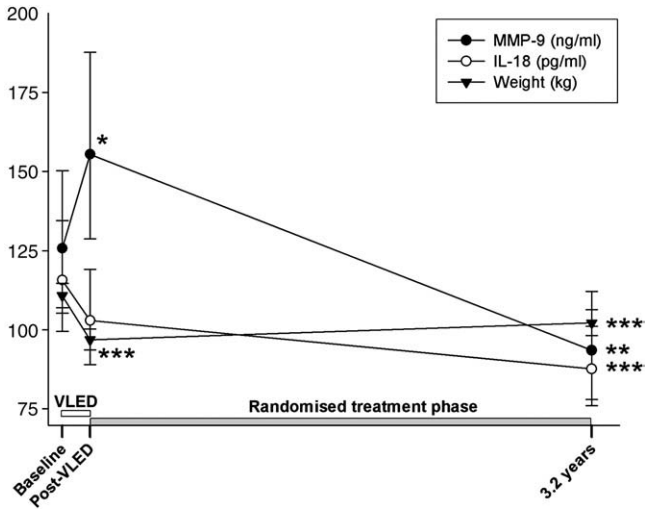

Fig. 1. Interleukin-18, MMP-9, and body weight during the 3.2-year intervention. Data from the orlistat group and the placebo group are combined. Values are geometric means with 95% CIs of unadjusted values: MMP-9 (nanograms per milliliter) (solid dots), IL-18 (picograms per milliliter) (open dots), and weight (kilograms) (solid triangles). *P less than .05, **P less than .01, and ***P less than .001 for changes compared with baseline values. Linear mixed model for repeated measurements (with adjustment for multiple comparisons, Sidak). N = 68.

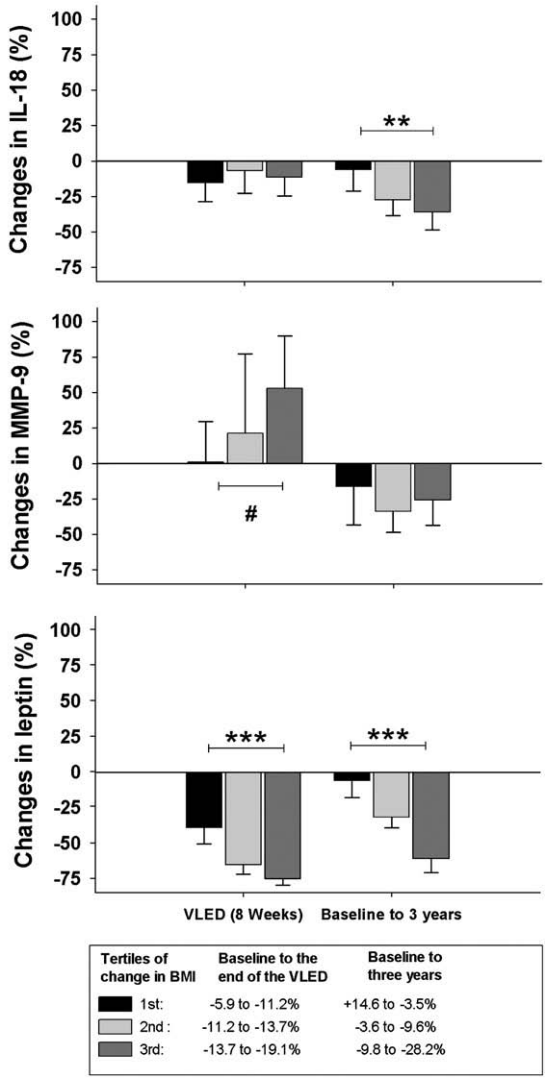

Fig. 2. Changes in IL-18, MMP-9, and leptin according to changes in BMI. Values are geometric means with 95% CI of IL-18, MMP-9, and leptin according to tertiles of change in BMI from baseline to the end of the 8-week VLED and from baseline to 3.2 years (antilog of ln differences transformed into percentage changes). **P less than .01 and ***P less than .001 for third vs first tertile (with adjustment for multiple comparisons, Dunnet). #P less than .05 for third vs first tertile unadjusted for multiple comparisons. N = 68.

these tertiles was also significant ($P < .05$), whereas long-term changes in MMP-9 from baseline to 3.2 years did not differ between the tertiles. The decrease in leptin was significantly higher both during the VLED ($P < .001$) and from baseline to 3.2 years ($P < .001$) when comparing subjects from the third tertile vs subjects from the first tertile of change in BMI (Fig. 2).

3.5. Correlations

At baseline, IL-18 correlated positively with diastolic blood pressure ($r = 0.27$, $P < .05$, Table 2). After 3.2 years, IL-18 correlated positively with MMP-9 ($r = 0.38$, $P < .01$), body weight ($r = 0.32$, $P < .01$), waist circumference ($r =$

Table 2
Correlations for IL-18

Levels or changes (Δ) of Pearson correlations (*r*)	IL-18 (levels)		Δ IL-18 (changes)	
	Baseline	3.2 y	Baseline to post-VLED (8 wk)	Baseline to 3.2 y
MMP-9	0.06	0.38†	0.18	0.40†
Leptin	0.14	0.15	0.17	0.58†
BMI	0.08	0.14	−0.05	0.32†
Weight	0.16	0.32†	−0.04	0.32†
Waist circumference	0.08	0.33†	0.05	0.30*
Waist-hip ratio	0.12	0.26*	0.17	0.09
Cholesterol	0.15	0.05	0.05	0.17
LDL	0.18	−0.02	0.05	0.13
Triglyceride	0.05	0.28*	−0.04	0.14
HDL	0.02	−0.25*	−0.21	−0.02
Systolic blood pressure	0.05	0.12	0.09	0.27*
Diastolic blood pressure	0.27*	0.06	0.16	0.15
Glucose	0.12	0.09	−0.03	0.29*
HOMA-IR	0.20	0.17	0.05	0.28*
HbA_{1c}	0.13	0.17	0.15	0.11
Insulin	0.19	0.18	0.06	0.24

Pearson correlations (*r*) for levels and changes of IL-18 based on the unadjusted log (base e) values of IL-18. N = 68.

* Correlation is significant at the .05 level (2-tailed).

† Correlation is significant at the .01 level (2-tailed).

0.33, $P < .01$), and triglyceride levels ($r = 0.28$, $P < .05$) and negatively with HDL cholesterol ($r = -0.25$, $P < .05$) (Table 2). Changes in IL-18 from baseline to 3.2 years correlated positively with changes in leptin ($r = 0.58$, $P < .01$), MMP-9 ($r = 0.40$, $P < .01$), body weight, BMI and waist circumference ($r = 0.30$-0.32, $P < .01$), systolic blood pressure, glucose, and HOMA-IR ($r = 0.27$-0.29, $P < .05$) (Table 2).

Changes in MMP-9 from baseline to the end of the VLED correlated negatively with changes in BMI ($r = -0.28$, $P < .05$), body weight ($r = -0.28$, $P < .05$), and waist circumference ($r = -0.34$, $P < .01$) (data not shown).

3.6. Multiple linear regression of changes in IL-18

Multiple linear regression showed that changes in IL-18 from baseline to 3.2 years were independently associated with changes in BMI ($B = 1.457$; 95% CI, 0.045-2.870; $P < .05$) even after adjustment for age, sex, site, treatment group, changes in blood pressure, triglyceride, total cholesterol, LDL cholesterol, and HDL cholesterol (log base e values) (model $R^2 = 0.33$, $P = .03$). The unadjusted bivariate correlation between changes in IL-18 and HOMA-IR was significant ($r = 0.28$, $P < .05$) but not after adjustment for lipids, blood pressure, and fixed factors ($P = .18$). Analyzing changes in MMP-9 from baseline to 3.2 years with an identical model did not show significant associations with changes in BMI. Inclusion of IL-18 demonstrated a significant positive association between long-term changes in MMP-9 and IL-18 even after adjustment for lipids, blood pressure, BMI, and fixed factors ($B = 0.686$ [0.262-1.110], $P < .01$).

To obtain a more detailed analysis of the factors correlated with the variation in IL-18, we established a linear mixed model for repeated measurements with an unstructured covariance structure incorporating all visits of the study using log base e values. This model incorporated the variation in BMI, HbA_{1c}, HOMA-IR, and systolic and diastolic blood pressure together with triglycerides, total cholesterol, LDL cholesterol, and HDL cholesterol and was adjusted for sex, age, treatment group, and study site. Using this model, it was shown that the overall variation in IL-18 during the study was independently positively associated with BMI ($B = 1.09$; 95% CI, 0.40-1.78; $P = .002$), HbA_{1c} (0.65 [0.03-1.28], $P = .040$), and diastolic blood pressure (0.58 [0.03-1.28], $P = ,042$), whereas HDL had a nonsignificant inverse association with IL-18 (−0.43 [−0.88 to +0.02], $P = .063$). All coefficients are independent of each other and adjusted for the other mentioned fixed factors and covariates (including HOMA-IR). No significant factors emerged when analyzing the variation in MMP-9 in the same manner as for IL-18.

4. Discussion

The present study is, to our knowledge, the first to report that weight loss up to 3.2 years induced by a hypocaloric diet and lifestyle intervention significantly reduces circulating levels of both IL-18 and MMP-9, and that the long-term changes in IL-18 are significantly associated with changes in BMI even after adjustment for lipids, blood pressure, sex, and age. Traditional cardiovascular risk markers such as hs-CRP, fibrinogen, and IL-6 are increased in obese subjects and are associated with the development of atherosclerosis and cardiovascular disease [33-36]. In this study, we have focused upon IL-18 and MMP-9, 2 newer nontraditional cardiovascular risk factors [16,25] involved in atherogenesis and plaque instability [17,18,24]. Interleukin-18 has been shown to take part in the chronic low-grade inflammation that may be a pathogenetic factor behind the metabolic syndrome and type 2 diabetes mellitus [37-39]. Thus, the finding that a lifestyle-induced weight loss greater than 5% maintained for up to 3.2 years was associated with significant reductions in the levels of both IL-18 and MMP-9 may have important implications for reducing the risk of development of the metabolic syndrome, diabetes, and cardiovascular diseases.

In the present study, we found no effects of long-term orlistat treatment on plasma levels of IL-18, MMP-9, or leptin. This may be related to the study design where the pronounced weight loss of approximately 14 kg induced by the VLED may have obscured the possibly more discrete effects of long-term orlistat treatment. This is in accordance with the original SMOMS study where orlistat treatment was without effects on cardiovascular risk factors, although orlistat reduced the weight regain by 2.5 kg at 3.2 years [30]. Other groups have shown that orlistat lowers free fatty acids

[40] and hs-CRP [41]. Although we did not find any effects of orlistat treatment on the examined variables, we retained the possible effects of the treatment groups in the analyses of the long-term changes and overall variation to adjust for a potential confounding of the results.

Circulating levels of IL-18 have been associated with various components of the metabolic syndrome [37] such as positive associations with insulin resistance, triglycerides, blood pressure, and visceral adiposity [42-46] and negative association with HDL cholesterol [42,47]. In agreement with this, we found that IL-18 was positively correlated with diastolic blood pressure and triglycerides and negatively correlated with HDL. Recently, a cross-sectional study reported significant associations between IL-18 and insulin/HOMA-IR, which however disappeared after adjustment for BMI or waist circumference [43]. We did not find any cross-sectional association with HOMA-IR but found an association between changes in HOMA-IR and IL-18, which however disappeared after additional adjustment for changes in lipids, blood pressure, and other factors. In the present study, we found a significant and independent association between changes in BMI (or waist circumference) and IL-18. This is in agreement with some studies [13,48] but opposed to other (short-term) diet or exercise studies and bariatric surgical interventions, in which circulating IL-18 has been reported to be more closely associated with insulin sensitivity than with measures of obesity [12,15]. Another study shows nearly similar associations between changes in IL-18 and changes in BMI or HOMA-IR, but stronger associations with other anthropometric variables such as changes in waist-hip ratio [49]. Thus, diverse evidence exists regarding the mechanisms that affect levels of and changes in circulating IL-18. Subjects in short-term weight loss or exercise studies as well as studies of bariatric surgery with a follow-up period of 1 year will still be in a hypocaloric state, which could result in a more dominating association between IL-18 and HOMA-IR than with anthropometric changes per se. Finally, gastric bypass surgery induces significant changes in levels of glucagon-like peptide–1, which through its beneficial effects on glucose homeostasis could be hypothesized to exert a weight loss–independent effect on IL-18 levels [50]. The discrepancies in the studies outlined above support a multifactorial view upon the regulation of IL-18 as reported in the present study. Here BMI, HbA_{1c}, and diastolic blood pressure were all independently associated with the variation in IL-18 even after adjustment for sex, age, HOMA-IR, and lipids. The association with long-term changes in BMI might be due to the integrative nature of weight loss as a marker/inducer of improvements in glucose and lipid metabolism as well as inflammation with a significant amount of intercorrelation between these factors, but the association with the reduction in BMI also underlines the positive effects of long-term weight loss upon inflammation and nontraditional cardiovascular risk markers.

There was a rather high correlation found between changes in leptin and IL-18 during the weight loss/weight loss maintenance intervention ($r = 0.58$). The precise reason for this is unknown; but it has previously been shown that leptin may affect inflammation and immune function and, moreover, may regulate the production of IL-18 [51]. The latter might be an explanation for the correlation between changes in leptin and IL-18 observed in the present study.

It has been shown that IL-18 may induce the secretion of MMP-9 in peripheral blood mononuclear cells, which is consistent with our finding that IL-18 is positively associated both with MMP-9 cross-sectionally (at 3.2 years) and with the changes during weight loss from baseline to 3.2 years. This association remains significant even after adjustment for metabolic variables; but this association says, however, nothing about the causality. Matrix metalloproteinase–9 has in several studies been associated with the development of a cardiovascular event [23,25]. The present study found that long-term weight loss was associated with a reduction in circulating levels of MMP-9. This may indicate that weight reduction greater than 5% sustained for up to 3.2 years may be beneficial and potentially reduce the risk of cardiovascular events. In contrast to this, a short-term intensive weight loss after the VLED was found to increase the level of MMP-9. This increase correlated inversely with changes in BMI and other measures of obesity and could hypothetically result from MMP-9 involvement in AT remodeling [28] during an intensive weight loss. Moreover, there are indications that rapid and pronounced weight loss (eg, induced by VLED) may induce a transient inflammatory response in the AT [52] that might also enhance the production of MMP-9 [53]. Given the association between plaque rupture and MMP-9, transiently elevated levels of MMP-9 during rapid and intensive weight loss regimens could potentially have adverse effects in susceptible individuals. The possibly negative health effects of rapid and pronounced weight loss need to be investigated in more details in future studies.

In conclusion, long-term weight loss in abdominally obese subjects was found to be associated with a significant reduction in circulating levels of IL-18 and MMP-9, suggesting that a modest weight loss in this high-risk population may be beneficial and potentially reduces the risk of cardiovascular disease.

Acknowledgment

The skilled technical assistance of Pia Hornbek and Lenette Pedersen is highly appreciated.

The original SMOMS study was sponsored by Roche. Moreover, we appreciate the support from Aarhus University, the Danish Research Council, and the Danish Diabetic Association.

References

[1] Chan JC, Cheung JC, Stehouwer CD, et al. The central roles of obesity-associated dyslipidaemia, endothelial activation and cytokines in the

metabolic syndrome—an analysis by structural equation modelling. Int J Obes Relat Metab Disord 2002;26:994-1008.
[2] Third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III) final report. Circulation 2002;106:3413-21.
[3] Yusuf S, Hawken S, Ounpuu S, et al. Obesity and the risk of myocardial infarction in 27,000 participants from 52 countries: a case-control study. Lancet 2005;366:1640-9.
[4] Weyer C, Funahashi T, Tanaka S, et al. Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. J Clin Endocrinol Metab 2001;86:1930-5.
[5] Woo KS, Chook P, Yu CW, et al. Overweight in children is associated with arterial endothelial dysfunction and intima-media thickening. Int J Obes Relat Metab Disord 2004;28:852-7.
[6] Yudkin JS, Kumari M, Humphries SE, et al. Inflammation, obesity, stress and coronary heart disease: is interleukin-6 the link? Atherosclerosis 2000;148:209-14.
[7] Caballero AE. Endothelial dysfunction in obesity and insulin resistance: a road to diabetes and heart disease. Obes Res 2003;11: 1278-89.
[8] Skurk T, berti-Huber C, Herder C, et al. Relationship between adipocyte size and adipokine expression and secretion. J Clin Endocrinol Metab 2007;92:1023-33.
[9] Bruun JM, Lihn AS, Pedersen SB, et al. Monocyte chemoattractant protein–1 release is higher in visceral than subcutaneous human adipose tissue (AT): implication of macrophages resident in the AT. J Clin Endocrinol Metab 2005;90:2282-9.
[10] Fain JN, Madan AK, Hiler ML, et al. Comparison of the release of adipokines by adipose tissue, adipose tissue matrix, and adipocytes from visceral and subcutaneous abdominal adipose tissues of obese humans. Endocrinology 2004;145:2273-82.
[11] Gillespie MT, Horwood NJ. Interleukin-18: perspectives on the newest interleukin. Cytokine Growth Factor Rev 1998;9:109-16.
[12] Bruun JM, Stallknecht B, Helge JW, et al. Interleukin-18 in plasma and adipose tissue: effects of obesity, insulin resistance, and weight loss. Eur J Endocrinol 2007;157:465-71.
[13] Esposito K, Pontillo A, Ciotola M, et al. Weight loss reduces interleukin-18 levels in obese women. J Clin Endocrinol Metab 2002; 87:3864-6.
[14] Schernthaner GH, Kopp HP, Kriwanek S, et al. Effect of massive weight loss induced by bariatric surgery on serum levels of interleukin-18 and monocyte-chemoattractant-protein–1 in morbid obesity. Obes Surg 2006;16:709-15.
[15] Leick L, Lindegaard B, Stensvold D, et al. Adipose tissue interleukin-18 mRNA and plasma interleukin-18: effect of obesity and exercise. Obes Res 2007;15:356-63.
[16] Blankenberg S, Tiret L, Bickel C, et al. Interleukin-18 is a strong predictor of cardiovascular death in stable and unstable angina. Circulation 2002;106:24-30.
[17] Mallat Z, Corbaz A, Scoazec A, et al. Interleukin-18/interleukin-18 binding protein signaling modulates atherosclerotic lesion development and stability. Circ Res 2001;89:41e-445e.
[18] Mallat Z, Corbaz A, Scoazec A, et al. Expression of interleukin-18 in human atherosclerotic plaques and relation to plaque instability. Circulation 2001;104:1598-603.
[19] Dinarello CA. Interleukin 1 and interleukin 18 as mediators of inflammation and the aging process. Am J Clin Nutr 2006;83: 447S-55S.
[20] Fain JN, Tichansky DS, Madan AK. Most of the interleukin 1 receptor antagonist, cathepsin S, macrophage migration inhibitory factor, nerve growth factor, and interleukin 18 release by explants of human adipose tissue is by the non-fat cells, not by the adipocytes. Metabolism 2006; 55:1113-21.
[21] Clement K, Viguerie N, Poitou C, et al. Weight loss regulates inflammation-related genes in white adipose tissue of obese subjects. FASEB J 2004;18:1657-69.
[22] Nold M, Goede A, Eberhardt W, et al. IL-18 initiates release of matrix metalloproteinase–9 from peripheral blood mononuclear cells without affecting tissue inhibitor of matrix metalloproteinases–1: suppression by TNF alpha blockage and modulation by IL-10. Naunyn Schmiedebergs Arch Pharmacol 2003;367:68-75.
[23] Tayebjee MH, Nadar S, Blann AD, et al. Matrix metalloproteinase–9 and tissue inhibitor of metalloproteinase–1 in hypertension and their relationship to cardiovascular risk and treatment: a substudy of the Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT). Am J Hypertens 2004;17:764-9.
[24] Galis ZS, Sukhova GK, Lark MW, et al. Increased expression of matrix metalloproteinases and matrix degrading activity in vulnerable regions of human atherosclerotic plaques. J Clin Invest 1994;94:2493-503.
[25] Blankenberg S, Rupprecht HJ, Poirier O, et al. Plasma concentrations and genetic variation of matrix metalloproteinase 9 and prognosis of patients with cardiovascular disease. Circulation 2003;107:1579-85.
[26] Glowinska-Olszewska B, Urban M. Elevated matrix metalloproteinase 9 and tissue inhibitor of metalloproteinase 1 in obese children and adolescents. Metabolism 2007;56:799-805.
[27] Laimer M, Kaser S, Kranebitter M, et al. Effect of pronounced weight loss on the nontraditional cardiovascular risk marker matrix metalloproteinase–9 in middle-aged morbidly obese women. Int J Obes Relat Metab Disord 2005;29:498-501.
[28] Bouloumie A, Sengenes C, Portolan G, et al. Adipocyte produces matrix metalloproteinases 2 and 9: involvement in adipose differentiation. Diabetes 2001;50:2080-6.
[29] Park HY, Kwon HM, Lim HJ, et al. Potential role of leptin in angiogenesis: leptin induces endothelial cell proliferation and expression of matrix metalloproteinases in vivo and in vitro. Exp Mol Med 2001;33:95-102.
[30] Richelsen B, Tonstad S, Rossner S, et al. Effect of orlistat on weight regain and cardiovascular risk factors following a very-low-energy diet in abdominally obese patients: a 3-year randomized, placebo-controlled study. Diabetes Care 2007;30:27-32.
[31] Madsen EL, Rissanen A, Bruun JM, et al. 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. Eur J Endocrinol 2008;158:179-87.
[32] Skogstrand K, Thorsen P, Norgaard-Pedersen B, et al. Simultaneous measurement of 25 inflammatory markers and neurotrophins in neonatal dried blood spots by immunoassay with xMAP technology. Clin Chem 2005;51:1854-66.
[33] Wilson PWF, D'Agostino RB, Sullivan L, et al. Overweight and obesity as determinants of cardiovascular risk: the Framingham experience. Arch Intern Med 2002;162:1867-72.
[34] Tzoulaki I, Murray GD, Lee AJ, et al. Relative value of inflammatory, hemostatic, and rheological factors for incident myocardial infarction and stroke: the Edinburgh artery study. Circulation 2007;115:2119-27.
[35] Kapiotis S, Holzer G, Schaller G, et al. A proinflammatory state is detectable in obese children and is accompanied by functional and morphological vascular changes. Arterioscler Thromb Vasc Biol 2006; 26:2541-6.
[36] Haffner SM. Abdominal adiposity and cardiometabolic risk: do we have all the answers? Am J Med 2007;120(Suppl 1):S10-6.
[37] Hung J, McQuillan BM, Chapman CML, et al. Elevated interleukin-18 levels are associated with the metabolic syndrome independent of obesity and insulin resistance. Arterioscler Thromb Vasc Biol 2005;25: 1268-73.
[38] Thorand B, Kolb H, Baumert J, et al. Elevated levels of interleukin-18 predict the development of type 2 diabetes: results from the MONICA/KORA Augsburg study, 1984-2002. Diabetes 2005;54:2932-8.
[39] Araki S, Haneda M, Koya D, et al. Predictive impact of elevated serum level of IL-18 for early renal dysfunction in type 2 diabetes: an observational follow-up study. Diabetologia 2007;50:867-73.
[40] Kelley DE, Kuller LH, McKolanis TM, et al. Effects of moderate weight loss and orlistat on insulin resistance, regional adiposity, and fatty acids in type 2 diabetes. Diabetes Care 2004;27:33-40.

[41] Bougoulia M, Triantos A, Koliakos G. Effect of weight loss with or without orlistat treatment on adipocytokines, inflammation, and oxidative markers in obese women. Hormones (Athens) 2006;5: 259-69.

[42] Evans J, Collins M, Jennings C, et al. The association of interleukin-18 genotype and serum levels with metabolic risk factors for cardiovascular disease. Eur J Endocrinol 2007;157: 633-40.

[43] Herder C, Schneitler S, Rathmann W, et al. Low-grade inflammation, obesity and insulin resistance in adolescents. J Clin Endocrinol Metab 2007;92:4569-74.

[44] Kim HJ, Kang ES, Kim DJ, et al. Effects of rosiglitazone and metformin on inflammatory markers and adipokines: decrease in interleukin-18 is an independent factor for the improvement of homeostasis model assessment-beta in type 2 diabetes mellitus. Clin Endocrinol (Oxf) 2007;66:282-9.

[45] Herder C, Baumert J, Kolb H, et al. Circulating levels of interleukin-18 independent of body fat and fat-free mass: results from the MONICA/ KORA study. Diabetes Care 2006;29:174-5.

[46] Vilarrasa N, Vendrell J, Maravall J, et al. IL-18: relationship with anthropometry, body composition parameters, leptin and arterial hypertension. Horm Metab Res 2006;38:507-12.

[47] Chapman CML, McQuillan BM, Beilby JP, et al. Interleukin-18 levels are not associated with subclinical carotid atherosclerosis in a community population: the Perth Carotid Ultrasound Disease Assessment Study (CUDAS). Atherosclerosis 2006;189:414-9.

[48] Esposito K, Pontillo A, Di Palo C, et al. Effect of weight loss and lifestyle changes on vascular inflammatory markers in obese women: a randomized trial. JAMA 2003;289:1799-804.

[49] Marfella R, Esposito K, Siniscalchi M, et al. Effect of weight loss on cardiac synchronization and proinflammatory cytokines in premenopausal obese women. Diabetes Care 2004;27:47-52.

[50] Korner J, Bessler M, Inabnet W, et al. Exaggerated glucagon-like peptide–1 and blunted glucose-dependent insulinotropic peptide secretion are associated with Roux-en-Y gastric bypass but not adjustable gastric banding. Surg Obes Relat Dis 2007;3:597-601.

[51] Faggioni R, Jones-Carson J, Reed DA, et al. Leptin-deficient (ob/ob) mice are protected from T cell–mediated hepatotoxicity: role of tumor necrosis factor alpha and IL-18. Proc Natl Acad Sci U S A 2000;97: 2367-72.

[52] Salas-Salvadó J, Bulló M, García-Lorda P, et al. Subcutaneous adipose tissue cytokine production is not responsible for the restoration of systemic inflammation markers during weight loss. Int J Obes (Lond) 2006;30:1714-20.

[53] Warner RL, Bhagavathula N, Nerusu KC, et al. Matrix metalloproteinases in acute inflammation: induction of MMP-3 and MMP-9 in fibroblasts and epithelial cells following exposure to pro-inflammatory mediators in vitro. Exp Mol Pathol 2004;76:189-95.