

Effects of the traditional Mediterranean diet on adiponectin and leptin concentrations in men and premenopausal women: Do sex differences exist?

Short title: Sex differences, adipokines and Mediterranean diet

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## Abstract

**Background/Objective:** Most of the interventional studies have investigated the impact of the diet on adiponectin and leptin concentrations only in men or in women. Consequently, it is still unknown whether the consumption of a healthy diet influences in a sex-specific manner these adipocytokines. We examined sex differences in the effects of the Mediterranean diet (MedDiet) on adiponectin and leptin concentrations and determined whether changes in these adipocytokines are associated with changes in cardiovascular risk factors in both sexes.

**Subjects/Methods:** Participants were 38 men and 32 premenopausal women (24-53y) with slightly elevated LDL-C concentrations (3.4-4.9 mmol/l) or total cholesterol/HDL-C $\geq$ 5.0. Adiponectin, leptin and cardiovascular risk factors were measured before and after a 4-week fully-controlled isoenergetic MedDiet.

**Results:** Adiponectin concentration decreased in response to the MedDiet, but this decrease reached statistical significance only in men (P<0.001 for men and P=0.260 for women; sex-by-time interaction, P=0.072). Adjustments for body weight or waist circumference did not change results obtained. Changes in adiponectin were positively associated with concomitant variations in HDL-C in men (r=0.52, P=0.003) and with variations in apo A-1 and insulin sensitivity as calculated by both HOMA-IS and Cederholm indices in women (respectively r=0.44, P=0.021; r=0.79, P<0.001 and r=0.47, P=0.020). The MedDiet had no impact on leptin and leptin-to-adiponectin ratio in both sexes.

**Conclusions:** Results suggest sex difference in adiponectin response to the short-term consumption of the MedDiet, with only men experiencing a decrease. Also sex-specific patterns of associations between changes in adiponectin concentration and changes in cardiovascular risk factors were observed.

**Keywords:** Sex differences; Adiponectin; Leptin; Leptin-to-adiponectin ratio; Mediterranean diet

## Introduction

It is now widely known that adipose tissue, besides its capacity to store and release energy, is an important metabolically active endocrine organ producing and secreting several hormones, called adipocytokines. Of these adipose tissue-derived hormones, adiponectin and leptin have attracted much interest because of their significant impact on cardiovascular health. Adiponectin is known as having anti-inflammatory, antiatherogenic and insulin-sensitizing properties <sup>1</sup> and it has been previously demonstrated that a low concentration of this hormone is an independent predictor of the metabolic syndrome <sup>2</sup>, coronary heart disease <sup>3</sup> and type 2 diabetes <sup>4</sup>. In the case of leptin, mixed results have been obtained, some studies observed that a high leptin concentration is a predictor of coronary events and type 2 diabetes independently of adiposity <sup>5,6</sup> while others showed that the association between leptin concentrations and these major chronic diseases is largely explained by concomitant variations in body weight or BMI <sup>7,8</sup>. It has been recently suggested that the leptin-to-adiponectin ratio could be a better marker of cardiometabolic alterations and insulin resistance than adiponectin or leptin alone <sup>9,10</sup>.

Sex differences exist in circulating adiponectin and leptin concentrations, and significantly higher concentrations are found in women than in men, independently of body composition <sup>11</sup>. Moreover, some previous studies have found sex-related differences in the link between these adipose tissue-derived hormones and the development of type 2 diabetes. In fact, the association between leptin concentrations and type 2 diabetes has been shown to be stronger in men than in women <sup>5,6</sup> while adiponectin concentrations would be more closely associated with type 2 diabetes in women than in men <sup>12</sup>. Sex-related differences have also been found in the relationship between blood concentrations of these adipocytokines and markers of cardiovascular risk <sup>11,13</sup>.

Most of the interventional studies have investigated the impact of the diet on adiponectin and leptin concentrations only in men or in women. Consequently, it is still unknown whether the consumption of

a healthy diet influences in a sex-specific manner these adipocytokines. The traditional Mediterranean diet (MedDiet) is a healthy food pattern recommended for its beneficial effects on cardiovascular health and insulin sensitivity, even when consumed under isoenergetic conditions. In fact, a previous publication from our research team showed that the MedDiet improves lipid and lipoprotein profile and blood pressure in both men and women <sup>14</sup>. On the other hand, sex differences have been observed in the beneficial effects of the MedDiet on insulin homeostasis, with improvements found in men but not in women <sup>14</sup>. No study has yet investigated whether adiponectin and leptin respond differently to the MedDiet according to the sex and whether the beneficial effects of the MedDiet found in men and women can be partially explained by changes in the concentrations of these adipocytokines. Accordingly, the objective of this study was to investigate sex differences in the effects of a 4-week controlled isoenergetic MedDiet on adiponectin and leptin concentrations in premenopausal women and age-matched men, and to determine whether changes in adiponectin and leptin concentrations were associated with changes in cardiovascular risk factors in men and in women separately.

## Participants and Methods

### Study population

Participants were 38 men and 32 premenopausal women aged from 24 to 53 years characterized by a slightly elevated LDL-C concentration (between 3.4 and 4.9 mmol/l) or total cholesterol to HDL-C ratio  $\geq 5.0$ . Moreover, participants had at least one of the four following cardiovascular risk factors: 1) waist circumference  $> 94$  cm in men and  $> 80$  cm in women; 2) TG  $> 1.7$  mmol/l; 3) fasting glycemia between 6.1 and 6.9 mmol/l and 4) blood pressure concentrations  $\geq 130 / 85$  mm Hg. Participants with a significant weight change ( $> 2.5$  kg) in the three months before the study, a prior cardiovascular event and / or diagnosis of type 2 diabetes and individuals on medication that could affect dependent variables under study (namely lipid-lowering, hypoglycemic, insulin sensitizers and anti hypertensive medication) were excluded from this study. Smokers, pregnant women or those who used a systemic hormonal contraceptive were also excluded. The present study was conducted according to the guidelines laid down in the Declaration of Helsinki. The study protocol was approved by the Laval University Research Ethics Committee on human experimentation. Written informed consent was obtained from all subjects.

### Study design

Details about the study design have been reported in a previous publication from our research team <sup>14</sup>. Briefly, during a 4-week run-in period, participants had to comply with the health recommendations of Canada's Food Guide <sup>15</sup> as prescribed by a registered dietician. During this period, no food and drink was provided to participants. Therefore they had to comply with these health recommendations in a free-living context. Despite the non-controlled nature of this run-in period, evaluation of dietary intakes by a validated food frequency questionnaire (FFQ) <sup>16</sup> at the end of the run-in period showed that both men and women adhered to the recommended number of servings per day for all food groups included in the

Canada's Food Guide, except for grain products for which both men and women consumed fewer portions than the recommendation. Therefore, this run-in period permitted to minimize the intra- and inter-variability in dietary intakes and to ensure similar dietary habits between men and women prior to the controlled MedDiet phase, as indicated in a previous publication <sup>14</sup>. No change in body weight was found during the run-in period ( $+0.02 \pm 0.19$  kg in men and  $-0.01 \pm 0.16$  kg in women, respectively  $P=0.928$  and  $P=0.835$ ).

The run-in period was followed by a consumption of a fully-controlled MedDiet for 4 weeks. Participants were instructed to consume only the provided foods and drinks, which corresponded to 100% of their estimated energy needs. The 7-day cyclic menu was formulated to be concordant with the characteristics of the traditional MedDiet. Details about the composition of the MedDiet have been previously reported <sup>14</sup>. The habitual energy intake of each participant was established before the controlled MedDiet phase in order to maintain body weight during the entire controlled feeding phase. Accordingly, body weight was measured on each weekday of the controlled MedDiet phase immediately before lunch and foods and energy provided were revised if necessary for minimizing body weight fluctuations. Participants were instructed to maintain their usual physical activity. Women's feeding was shortened or prolonged if needed in order to be able to carry out all tests in the early follicular phase of their menstrual cycle (from the third to the ninth day of the menstrual cycle; mean duration of the feeding period in women  $28.8 \pm 4.3$  days) since fluctuations in female hormones may influence leptin concentration <sup>17</sup> and cardiovascular risk factors <sup>18,19</sup>.

### Laboratory measurements

Metabolic variables were measured after a 12-hour overnight fast, at the end of the run-in period, and immediately after the 4-week controlled MedDiet phase. Serum adiponectin and leptin concentrations

were measured using commercial enzyme-linked immunosorbent assay kits (respectively B-Bridge International, Mountain View, California, USA and EMD Millipore Corporation, St. Charles, Missouri, USA). Thereafter the leptin-to-adiponectin ratio was calculated. Serum was separated from blood cells by centrifugation at 3000 rpm for 10 minutes at 4°C and thereafter cholesterol and TG concentrations were determined with a Roche/Hitachi MODULAR analyzer (Roche Diagnostics) using Roche Diagnostics reagents. LDL-cholesterol was obtained by the equation of Friedewald et al.<sup>20</sup>. HDL-cholesterol concentrations were obtained using autoanalyser after precipitation of very low density lipoprotein and LDL in the infranantant with heparin and MnCl<sub>2</sub><sup>21</sup>. Apo A-1 and apo B concentrations were measured by using a Behring Nephelometer BN-100 (Behring Diagnostic, Westwood, MA, USA) with reagents and calibrators (Dade Behring, Mississauga, ON, Canada) provided by the manufacturer. A 180-min OGTT (75g of glucose) was also performed during which blood samples were collected into vacutainer tubes containing EDTA at -15, 0, 15, 30, 45, 60, 90, 120, 150, and 180 min. At each time, plasma glucose concentration was determined using the hexokinase-glucose-6-phosphate dehydrogenase method<sup>22</sup> and plasma insulin concentration was measured by radioimmunoassay<sup>23</sup>. Fasting values were computed as the mean of -15 and 0 min values. Incremental areas under the curve (iAUC) for glucose and insulin were obtained by calculating the total AUC with the trapezoidal method and then subtracting the area from the baseline concentration over the 180-min period<sup>24</sup>.

Hepatic insulin sensitivity was assessed by the homeostasis model assessment (HOMA-IS) index ( $1 / [\text{fasting glucose (mmol/l)} \times \text{fasting insulin (mIU/l)} / 22.5]$ ) and peripheral insulin sensitivity was assessed with the Cederholm index according to the following formula:  $[75\ 000 + (\text{fasting glucose} - \text{glucose 120-min post load}) \times 1.15 \times 180 \times 0.19 \times \text{body weight}] / [120 \times \log(\text{mean insulin}) \times \text{mean glucose}]$  in which glucose concentrations are expressed in mmol/l, insulin concentrations in mIU/l and body weight in kg<sup>25</sup>.

## Anthropometric and blood pressure measurements

Body weight, height and waist circumference measurements were performed using standardized methods<sup>26</sup> and systolic and diastolic blood pressure measurements were taken on the right arm using an automated blood pressure monitor as previously described<sup>14</sup>.

## Statistical analyses

Values are presented as means. For variables with a skewed distribution, a transformation was performed prior to statistical analysis. Sex differences in characteristics before the controlled MedDiet phase were assessed by the Student's t-test for unpaired data (SAS statistical package version 9.2; SAS Institute Inc., Cary, NC, USA). MIXED procedures for repeated measurements were used to assess time and sex-by-time interaction main effects on adiponectin and leptin concentrations as well as on the leptin-to-adiponectin ratio. Tukey-Kramer adjusted P-values were used to determine the precise location of differences when a significant main effect was detected by the MIXED analysis. In order to determine whether sex differences in adipocytokine response were due to differences in adiposity between men and women, body weight and waist circumference were subsequently added to the models. Statistical analyses were adjusted for baseline values (i.e. values after the run-in period and therefore those just before the controlled MedDiet). Associations between changes in adiponectin and changes in cardiovascular risk factors were assessed by Pearson's correlation analyses whereas associations between changes in leptin and leptin-to-adiponectin ratio and changes in cardiovascular risk factors were assessed by Spearman's correlation analyses due to the failure to comply with the normality assumption even with transformed values. Pearson's and Spearman's correlation analyses were adjusted for body weight loss during the controlled MedDiet phase.



Despite the fact that the controlled MedDiet phase aimed at being isoenergetic, a small body weight change occurred (1.2 kg or 1.3% of initial body weight in men and 0.5 kg or 0.7% body weight in women). Adjustment for this small body weight loss did not change results obtained (results not shown), suggesting that changes in adipocytokines in the present study were not due to change in body weight during the controlled MedDiet phase. We excluded one man from our analyses due to illness, which led to a significant reduction in food intake during several days at the end of the controlled MedDiet phase. Therefore, thirty-seven men and thirty-two women were included in the analyses. Moreover, one man was excluded from the analyses related to leptin due to extreme values (more than three standard deviations above the mean of the group of men). A  $P \leq 0.05$  (two-sided) was considered significant.

## Results

### Participant characteristics after the run-in period

As previously reported in another publication<sup>14</sup>, men and women had similar age and BMI (Table 1). Body weight and waist circumference measurements were higher in men than in women (respectively  $P < 0.001$  and  $P = 0.013$ ). Men displayed higher TG ( $P = 0.029$ ), systolic and diastolic blood pressure (respectively  $P = 0.004$  and  $P = 0.003$ ) and fasting glucose ( $P = 0.044$ ), and lower HDL-C concentration ( $P = 0.002$ ) than women. No sex difference was found for LDL-C concentration.

There was a strong tendency for higher fasting adiponectin concentrations in women than in men ( $P = 0.053$ ). Moreover, fasting leptin concentrations and fasting leptin-to-adiponectin ratio were higher in women than in men (both  $P < 0.001$ ).

### Adiponectin response to the MedDiet

The MedDiet resulted in a decrease in adiponectin concentration when data was pooled across both sexes (Time effect;  $P < 0.001$ ) (Table 2). However, a trend toward a sex-by-time interaction effect was observed for adiponectin change during the MedDiet ( $P = 0.072$ ), decreasing in men ( $P < 0.001$ ), whereas there was no significant change in women ( $P = 0.260$ ; Table 2). Inclusion of body weight or waist circumference in the model did not change results obtained (results not shown).

In men, no association was found between changes in adiponectin concentration and changes in variables related to the lipid profile, blood pressure and glucose/insulin homeostasis, except for HDL-C, for which a positive association was noted ( $r = 0.52$  (95% CI: 0.18, 0.70),  $P = 0.003$ ; Table 3). In women, a positive association between changes in adiponectin concentration and changes in apo A-1 concentration was observed ( $r = 0.44$  (95% CI: 0.07, 0.67),  $P = 0.021$ ; Table 3). Moreover, inverse associations were found between changes in adiponectin concentration and changes in fasting glucose

( $r=-0.48$  (95% CI: -0.69, -0.11),  $P=0.011$ ) and insulin ( $r=-0.75$  (95% CI: -0.81, -0.36),  $P<0.001$ ) concentrations and 120-min post load glucose concentration ( $r=-0.42$  (95% CI: -0.66, -0.05),  $P=0.025$ ; Table 3). Finally, a positive association between changes in adiponectin concentration and changes in insulin sensitivity, as calculated by both HOMA-IS ( $r=0.79$  (95% CI: 0.39, 0.82),  $P<0.001$ ) and Cederholm indices ( $r=0.47$  (95% CI: 0.07, 0.69),  $P=0.020$ ), was observed in women (Table 3).

#### Leptin response to the MedDiet

No significant change in leptin concentration was observed when data was pooled across both sexes (Time effect:  $P=0.167$ ; Table 2). Leptin response to the MedDiet was similar in men and women (Sex-by-time interaction:  $P=0.216$ ; Table 2). Inclusion of body weight or waist circumference in the model did not change results obtained (results not shown).

Positive associations were found between changes in leptin concentration and changes in body weight and BMI during the MedDiet, but they reached statistical significance only in men (respectively  $r=0.45$  (95% CI: 0.11, 0.66),  $P=0.009$  and  $r=0.47$  (95% CI: 0.13, 0.67),  $P=0.007$  in men and  $r=0.35$  (95% CI: -0.01, 0.62),  $P=0.057$  and  $r=0.35$  (95% CI: -0.01, 0.61),  $P=0.058$  in women; Table 3). After adjustments for the change in body weight, changes in leptin concentration were positively associated with changes in diastolic blood pressure ( $r=0.40$  (95% CI: 0.05, 0.64),  $P=0.025$ ) and fasting insulin concentration ( $r=0.51$  (95% CI: 0.16, 0.69),  $P=0.004$ ) and negatively associated with changes in insulin sensitivity as measured by the HOMA-IS index ( $r=-0.55$  (95% CI: -0.72, -0.20),  $P=0.002$ ) in men whereas no significant correlations were found in women ( $P>0.141$ ; Table 3).

#### Leptin-to-adiponectin ratio response to the MedDiet

No time ( $P=0.795$ ) nor sex-by-time interaction ( $P=0.661$ ) effect was found for the leptin-to-adiponectin ratio in response to the MedDiet (Table 2). Inclusion of body weight or waist circumference in the model did not change results obtained (results not shown).

In both men and women, change in this ratio was negatively associated with change in insulin sensitivity as calculated by the HOMA-IS index (respectively  $r=-0.39$  (95% CI: -0.63, -0.04),  $P=0.028$  and  $r=-0.46$  (95% CI: -0.68, -0.09),  $P=0.015$ ; Table 3).

## Discussion

The consumption of the MedDiet in isoenergetic conditions during a 4-week period resulted in a decrease in adiponectin concentration in men but not in women, with a trend toward a sex difference. Moreover, sex-specific patterns of associations between changes in adiponectin concentration and changes in cardiovascular risk factors have been highlighted. Leptin concentration and the leptin-to-adiponectin ratio did not change in response to the MedDiet in both men and women.

The MedDiet is now well recognized as a healthy food pattern with several beneficial effects on cardiovascular health, independent of weight loss<sup>14,27,28</sup>. Therefore, one surprising result from this study is that short-term consumption of the MedDiet in isoenergetic conditions reduced the concentration of adiponectin, an adipocytokine which has been demonstrated as having many antidiabetogenic features, such as increasing glucose uptake in muscles and reducing gluconeogenesis in the liver, and antiatherosclerotic properties<sup>1</sup>. This result contradicts those from previous studies on this issue. In fact, cross-sectional studies have suggested that individuals with a higher adherence to the MedDiet, as determined by a MedDiet score, have higher concentrations of adiponectin than individuals with lower scores in both healthy and diabetic individuals, even after adjustments for potential confounding variables such as age, body composition and traditional risk factors<sup>29,30</sup>. Besides our study, no fully controlled interventional study has investigated this issue yet. Only a few non-controlled interventional studies have documented the link between the adoption of the MedDiet and adiponectin, showing increased concentration of this adipocytokine<sup>31,32</sup>. However, a major methodological difference between our study and those previously conducted must be considered. In fact, these previous studies have investigated the impact of the MedDiet on adiponectin concentration with a concomitant weight loss<sup>31,32</sup>, which may have largely influenced the results obtained. Indeed, it has been repeatedly observed that a body weight loss corresponding to 5-10% of initial body weight leads to an increase in adiponectin concentration<sup>33</sup>.

These previous studies that have examined the impact of the MedDiet on adiponectin concentrations have been conducted either in men <sup>32</sup> or in women <sup>31</sup>. Although results from these studies suggest that the MedDiet led to an increase in adiponectin in both men and women, it has to be emphasized that men and women have never been directly compared within the same study in the context of a controlled feeding intervention. In the present study, we found a trend toward a sex difference, men having a significant decrease (-17.3%) while women experienced only a non-significant decrease (-10.7%) in adiponectin concentrations. These findings highlight the fact that other studies designed to document the effect of the MedDiet on adiponectin concentration in men and women are needed to investigate the underlying mechanisms of this sex difference. However, one possible hypothesis is that this decrease in adiponectin concentration and its sex-related response to the MedDiet may be due to adipose tissue remodeling and redistribution. In fact, small changes in body weight may not be an accurate representation of body fat change and remodeling, which are major determinants of the synthesis and secretion of adiponectin <sup>34</sup>. Accordingly differences in changes in body fat and its distribution between men and women could explain sex difference in adiponectin response to the MedDiet observed in the present study. We did not directly measure changes in adipose tissue and its distribution in response to the MedDiet, which impede us to confirm the possible link between change in body fat, especially visceral fat, and sex-specific changes in adiponectin concentration. However, waist circumference may be used as an indirect measure of visceral adiposity. Both men and women experienced no change in waist circumference during the controlled MedDiet (-0.3 cm or -0.3% of initial waist circumference in men, P=0.941 and -0.8 cm or -0.8% in women, P=0.310, P for sex-by-time interaction=0.378), suggesting that the decrease in adiponectin concentration and its sex-related response to the MedDiet are not mediated by changes in visceral adipose tissue.

Clinically, a decrease in adiponectin concentration might appear to be detrimental for cardiovascular health. However, it must be stressed that, in spite of this decrease in adiponectin concentration in men,

previously reported results from our group highlighted that this controlled isoenergetic MedDiet intervention led to improvements in many cardiovascular risk factors (lipid and lipoprotein profile and blood pressure in both men and women as well as insulin sensitivity in men were all improved)<sup>14</sup>, suggesting that, when considering the effects globally, the MedDiet is beneficial for cardiovascular health in both men and women. Moreover, leptin-to-adiponectin ratio has been suggested to be a better marker of cardiovascular disease and type 2 diabetes risk than adiponectin concentration alone<sup>9,10</sup>. In this study, this ratio was unchanged in response to the MedDiet in both sexes, suggesting that the decrease in adiponectin concentration found in response to the MedDiet in men may have, overall, a rather mild negative impact on cardiovascular health. However, our results clearly indicate that cardiovascular benefits attributable to the MedDiet in the absence of clinically meaningful weight loss are not explained by adiponectin or leptin changes in this subpopulation of men and women in the short-term.

The decrease in adiponectin concentration observed in men was associated with a concomitant reduction in HDL-C concentration. This association is in line with the literature, which suggests that the cardioprotective effect of adiponectin may be mediated by effects on the metabolism of HDL-C<sup>35</sup>. It must be emphasized that although the decrease in adiponectin concentration observed in men was associated with reduction in HDL-C concentration, previous results from this study suggest that this decrease in adiponectin concentration was not sufficient in magnitude to be detrimental for cardiovascular health and/or was counterbalanced by other metabolic changes<sup>14</sup>. In fact, there was no significant decrease in HDL-C in men in response to this fully-controlled feeding phase (-4.4%;  $P=0.129$ )<sup>14</sup>.

Our results suggest that short-term consumption of the MedDiet has no impact on leptin concentration in men as well as in women. However, it has been found that change in leptin concentration was associated with the small weight loss during the MedDiet phase in a significant manner in men and

with a strong tendency in women, which is consistent with the fact that changes in plasma leptin closely reflect changes in body weight, as previously outlined <sup>36</sup>.

A major strength of this study is that all foods and drinks were provided to participants during the MedDiet, ensuring an optimal control over energy intake and diet quality. Moreover, the fully-controlled nature of the nutritional phase permitted to document sex differences in adipocytokine responses to the MedDiet with a maximum of control over confounding variables. In addition, since previous studies have suggested that associations between these adipocytokines and cardiovascular risk factors may be sex-specific <sup>5,6,12,13</sup>, the inclusion of both men and premenopausal women allowed us to document for the first time sex-related differences in these associations in response to the MedDiet. On the other hand, one limitation of the present study is the absence of a control diet, which limits the conclusions on true treatment effects. However, even if the run-in period was not controlled, both men and women complied with the health recommendations of the Canada's Food Guide during this period. Another limitation is that this publication reports post-hoc analyses and that the original study was not directly powered for studying sex differences in adiponectin and leptin responses. Therefore other studies are needed to confirm the trend for a sex difference observed in the adiponectin response to the MedDiet.

In summary, these results suggest the existence of sex differences in adiponectin response to the short-term consumption of MedDiet in isoenergetic conditions, with only men experiencing a decrease in this adipocytokine. Moreover, sex-specific patterns of associations between change in adiponectin concentration in response to the MedDiet and changes in cardiovascular risk factors have been highlighted in the present study. These results underline the importance of including both men and women in further studies. Finally, other studies are needed to confirm this sex difference in adiponectin response and to investigate its underlying mechanisms, and whether this sex difference persists over the long term.



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## Conflict of interest

All authors declare no conflict of interest.

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Table 1. Characteristics of men and premenopausal women after the run-in period

	Men (n=37)		Women (n=32)		Sex difference
	Mean	SD	Mean	SD	P-value <sup>1</sup>
Age (years) <sup>2</sup>	42.6	7.3	41.2	7.3	0.428
Body weight (kg) <sup>2,3</sup>	91.8	14.0	78.0	14.7	<0.001
BMI (kg/m <sup>2</sup> ) <sup>2,3</sup>	29.1	3.2	29.6	5.4	0.969
Waist circumference (cm) <sup>2,3</sup>	102.6	10.7	96.4	10.5	0.013
TG (mmol/l) <sup>2,3</sup>	1.86	1.17	1.36	0.63	0.029
LDL-C (mmol/l) <sup>2</sup>	3.61	0.72	3.47	0.52	0.358
HDL-C (mmol/l) <sup>2,3</sup>	1.09	0.31	1.30	0.26	0.002
Total cholesterol/HDL-C (mmol/l)	5.30	1.04	4.26	0.78	<0.001
Systolic blood pressure (mm Hg) <sup>2</sup>	117.1	12.6	108.6	10.4	0.004
Diastolic blood pressure (mm Hg) <sup>2</sup>	80.3	9.0	73.5	9.0	0.003
Fasting glucose (mmol/l) <sup>2,3</sup>	5.89	0.37	5.68	0.63	0.044
Fasting adiponectin (ng/l) <sup>3</sup>	7.03	2.96	9.18	5.23	0.053
Fasting leptin (pg/l) <sup>3</sup>	9.65	5.78	25.90	13.89	<0.001

TG, triglyceride

These characteristics were measured after the run-in period, i.e. immediately before the controlled MedDiet phase.

<sup>1</sup> Differences between men and premenopausal women were assessed by Student's t-test for unpaired data.

<sup>2</sup> These characteristics have been reported in a previous publication (<sup>14</sup>).

<sup>3</sup> Analysis was performed on transformed values.

Table 2. Adiponectin, leptin and leptin-to-adiponectin ratio responses to the 4-week fully-controlled Mediterranean diet

	Men (n=37)					Women (n=32)					Time	Sex by time interaction
	Pre <sup>1</sup>	SEM	Post <sup>1</sup>	SEM	Change %	Pre <sup>1</sup>	SEM	Post <sup>1</sup>	SEM	Change %	P-value	
Fasting adiponectin (ng/l) <sup>2</sup>	7.03	0.49	5.82*	0.41	-17.3	9.18	0.92	8.20	0.73	-10.7	<0.001	0.072
Fasting leptin (pg/l) <sup>2,3</sup>	9.65	0.96	8.31	0.93	-13.8	25.90	2.46	25.30	2.34	-2.3	0.167	0.216
Fasting leptin-to-adiponectin ratio <sup>3</sup>	1.58	0.16	1.56	0.16	-1.3	3.92	0.59	3.91	0.57	-0.3	0.795	0.661

Inclusion of the overall body weight or waist circumference in the models did not change results obtained.

<sup>1</sup> “Pre” measurements were taken at the end of the run-in period (i.e. immediately before the 4-week controlled MedDiet phase) and “Post” measurements were taken at the end of the 4-week controlled MedDiet phase.

<sup>2</sup> Analysis was performed on transformed values.

<sup>3</sup> One man was excluded of analyses related to leptin due to extreme values.

\* A significant decrease was observed for fasting adiponectin concentration in men, P<0.001



Table 3. Correlation coefficients for the associations between changes in adipocytokines and changes in cardiovascular risk factors in men and premenopausal women in response to the fully-controlled Mediterranean diet <sup>1</sup>

	Men (n=37)			Women (n=32)		
	Adiponectin	Leptin <sup>2</sup>	Leptin/ Adiponectin <sup>2</sup>	Adiponectin	Leptin <sup>2</sup>	Leptin/ Adiponectin <sup>2</sup>
Body weight	0.11 (-0.22, 0.42)	0.45 (0.11, 0.66)**	0.33 (-0.01, 0.58)	-0.23 (-0.54, 0.13)	0.35 (-0.01, 0.62)	0.28 (-0.09, 0.57)
BMI	0.12 (-0.21, 0.43)	0.47 (0.13, 0.67)**	0.34 (-0.00, 0.59)	-0.25 (-0.55, 0.12)	0.35 (-0.01, 0.61)	0.31 (-0.05, 0.59)
Waist circumference	0.15 (-0.19, 0.46)	0.01 (-0.33, 0.34)	-0.18 (-0.49, 0.16)	0.08 (-0.28, 0.42)	0.11 (-0.26, 0.44)	-0.03 (-0.38, 0.32)
TG	-0.10 (-0.42, 0.23)	0.08 (-0.26, 0.40)	0.13 (-0.21, 0.45)	0.23 (-0.14, 0.54)	-0.19 (-0.50, 0.18)	-0.10 (-0.43, 0.27)
LDL-C	0.17 (-0.17, 0.47)	0.07 (-0.27, 0.40)	0.04 (-0.30, 0.37)	0.18 (-0.18, 0.50)	0.24 (-0.13, 0.54)	0.09 (-0.28, 0.43)
HDL-C	0.52 (0.18, 0.70)**	0.20 (-0.15, 0.50)	0.00 (-0.33, 0.33)	0.29 (-0.08, 0.58)	0.21 (-0.16, 0.52)	0.06 (-0.30, 0.41)
Apo B	0.17 (-0.17, 0.47)	0.03 (-0.30, 0.36)	-0.12 (-0.44, 0.22)	0.13 (-0.23, 0.47)	0.28 (-0.09, 0.57)	0.15 (-0.21, 0.48)
Apo A-1	0.31 (-0.03, 0.58)	0.11 (-0.23, 0.43)	0.02 (-0.32, 0.35)	0.44 (0.07, 0.67)*	-0.07 (-0.41, 0.29)	-0.18 (-0.50, 0.19)
Total cholesterol / HDL-C	-0.16 (-0.46, 0.18)	0.03 (-0.30, 0.36)	-0.05 (-0.38, 0.28)	0.09 (-0.27, 0.43)	0.00 (-0.35, 0.36)	-0.06 (-0.41, 0.30)
LDL-C / HDL-C	-0.30 (-0.57, 0.04)	-0.13 (-0.44, 0.21)	0.04 (-0.30, 0.37)	0.01 (-0.34, 0.37)	0.10 (-0.26, 0.44)	0.06 (-0.30, 0.41)
Systolic blood pressure <sup>3</sup>	0.04 (-0.29, 0.37)	0.03 (-0.31, 0.36)	-0.19 (-0.50, 0.16)	0.09 (-0.27, 0.43)	0.15 (-0.21, 0.48)	-0.10 (-0.44, 0.26)
Diastolic blood pressure <sup>3</sup>	0.28 (-0.06, 0.56)	0.40 (0.05, 0.64)*	0.07 (-0.27, 0.40)	-0.05 (-0.39, 0.31)	-0.21 (-0.52, 0.16)	-0.16 (-0.48, 0.21)
Glucose						
Fasting	0.15 (-0.19, 0.45)	0.06 (-0.28, 0.39)	0.02 (-0.32, 0.35)	-0.48 (-0.69, -0.11)*	0.14 (-0.23, 0.47)	0.36 (-0.01, 0.62)
120 min	0.15 (-0.19, 0.46)	-0.23 (-0.52, 0.12)	-0.27 (-0.55, 0.08)	-0.42 (-0.66, -0.05)*	0.02 (-0.33, 0.37)	0.13 (-0.23, 0.46)

iAUC <sup>4</sup>	-0.06 (-0.39, 0.29)	-0.35 (-0.61, 0.01)	-0.19 (-0.50, 0.17)	-0.23 (-0.55, 0.16)	0.11 (-0.28, 0.46)	0.08 (-0.30, 0.44)
Insulin						
Fasting	-0.11 (-0.42, 0.23)	0.51 (0.16, 0.69)**	0.23 (-0.12, 0.52)	-0.75 (-0.81, -0.36)***	-0.15 (-0.47, 0.22)	0.30 (-0.07, 0.58)
120 min	-0.04 (-0.37, 0.37)	0.02 (-0.32, 0.35)	-0.03 (-0.36, 0.31)	-0.24 (-0.54, 0.13)	0.02 (-0.34, 0.37)	0.30 (-0.07, 0.58)
iAUC <sup>4</sup>	-0.18 (-0.49, 0.17)	0.15 (-0.20, 0.47)	0.30 (-0.06, 0.58)	-0.21 (-0.54, 0.18)	-0.04 (-0.41, 0.34)	0.18 (-0.20, 0.52)
HOMA-IS index	0.03 (-0.30, 0.36)	-0.55 (-0.72, -0.20)**	-0.39 (-0.63, -0.04)*	0.79 (0.39, 0.82)***	0.12 (-0.25, 0.45)	-0.46 (-0.68, -0.09)*
Cederholm index <sup>4</sup>	0.10 (-0.24, 0.43)	0.14 (-0.22, 0.46)	-0.02 (-0.36, 0.33)	0.47 (0.07, 0.69)*	-0.03 (-0.40, 0.34)	-0.24 (-0.56, 0.15)

TG, triglyceride; Apo, apolipoprotein; iAUC, incremental area under the curve; HOMA-IS, homeostasis model assessment index for insulin sensitivity.

Analyses were adjusted for body weight change which occurred during the controlled MedDiet phase, except for results related to change in body weight and change in BMI.

<sup>1</sup> Results are presented as  $r$  (95% CI)

<sup>2</sup> One man was excluded of analyses related to leptin due to extreme values.

<sup>3</sup> Missing value for one man

<sup>4</sup> Due to incomplete data from the oral glucose tolerance test, n=35 in men and n=29 in women.

\* P<0.05, \*\* P<0.01, \*\*\* P<0.001, otherwise non-significant P>0.050