An increase in plasma adiponectin multimeric complexes follows hypocaloric diet-induced weight loss in obese and overweight pre-menopausal women

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A B S T R A C T

Adiponectin is involved in the regulation of glucose and fatty acid metabolism, influences whole-body insulin sensitivity and protects arterial walls against the development of atherosclerosis. Plasma adiponectin is decreased in obese, insulin-resistant and Type 2 diabetic patients. Adiponectin circulates in plasma as high-, medium- and low-molecular-weight ('mass') forms (HMW, MMW and LMW respectively). The HMW form is believed to be closely associated with insulin sensitivity. The aim of the present study was to investigate whether diet-induced changes in body weight and insulin sensitivity were associated with changes in the quantity of adiponectin multimeric complexes. A total of 20 overweight or obese women (age, 39.4 ± 9.5 years; body mass index, 32.2 ± 6.4 kg/m^2) underwent 12 weeks of low caloric diet (600 kcal/day less than energy requirements; where 1 kcal ≈ 4.184 kJ). Plasma samples were drawn before and after the study for biochemical analysis and Western blot detection of adiponectin multimeric complexes. The hypocaloric diet resulted in a weight reduction (89.8 ± 16.4 kg compared with 83.1 ± 15.6 kg; $P < 0.001$) and an improvement in whole-body insulin sensitivity, as measured by HOMA (homoeostasis model assessment index; 1.9 ± 0.8 compared with 1.5 ± 0.7; $P = 0.013$). Increases in the quantities of the HMW, MMW and LMW forms by 5.5, 8.5 and 18.1 % respectively, were observed ($P < 0.05$ for all of the forms). Total plasma adiponectin was increased by 36 % with borderline significance ($P = 0.08$). No correlations between changes in adiponectin complexes and changes in indices of insulin sensitivity were observed. In conclusion, diet-induced weight loss improved insulin sensitivity as well as increased the amount of HMW, MMW and LMW adiponectin complexes in plasma.

INTRODUCTION

Obesity is known to be associated with a whole-body pro-inflammatory state and a number of metabolic disturbances included in the metabolic syndrome. In the search for the mechanisms that explain the link between obesity and metabolic syndrome, it has been suggested that endocrine substances produced by adipose tissue (adipocytokines) might play a role [1,2]. Adiponectin is a 30 kDa plasma protein secreted by mature adipocytes [3], representing 0.01% of total plasma proteins [4]. Plasma adiponectin levels have been shown to be reduced in patients with Type 2 diabetes [5], insulin-resistant subjects [6] and obese individuals [4], as well as in patients with coronary heart disease [5]. These findings suggest that adiponectin has insulin-sensitizing and anti-atherosclerotic effects [7]. In human plasma, adiponectin has been shown to circulate in distinct multimeric complexes forming trimeric LMW [low-molecular weight ('mass')], hexameric MMW [medium-molecular weight ('mass')] and oligomeric HMW [high-molecular weight ('mass')] complexes. However, some investigators, using different analytical methods, have distinguished only two adiponectin isoforms: LMW and HMW [6,8–12]. Adiponectin cell-surface receptors (AdipoR1 and AdipoR2) are expressed in muscle, liver and adipose tissue [13,14]. In myocytes and hepatocytes (mouse model), adiponectin stimulates phosphorylation and the activation of AMPK (AMP-activated protein kinase) [15], a key regulatory enzyme in glucose and lipid metabolism, inducing glucose uptake and fatty acid oxidation in muscle [15,16] and reducing hepatic gluconeogenesis [17]. Activation of different cellular transduction pathways appears to be specific for different multimeric complexes in different organs [18,19].

Previous investigations revealed that plasma HMW adiponectin levels are positively associated with insulin sensitivity indices [10,12,20,21] and plasma HDL-cholesterol (high-density lipoprotein-cholesterol), whereas a negative association with BMI (body mass index) and central body fat mass has been observed [10]. Mutations in the adiponectin gene associated with impaired formation of HMW complexes have been phenotypically connected with hypoadiponectinaemia and Type 2 diabetes in humans [18].

Weight loss achieved through caloric restriction (accompanied by physical activity in some studies) is an important and cost-effective measure in the treatment of obese individuals who are at a high risk of developing Type 2 diabetes and atherosclerosis [22–24]. In this context, the role of adiponectin and the distribution of its multimeric complexes have attracted interest because of their insulin-sensitizing and anti-atherosclerotic effects. Recently published studies of changes in adiponectin multimeric complexes following dietary intervention have yielded contradictory results; some showing no changes in their distribution [25], whereas others found increased quantities of HMW and MMW complexes [8]. These conflicting results might be partially attributable to small sample sizes and/or the inclusion of both men and women in the above-mentioned studies, as marked gender differences in total plasma adiponectin and in the distribution of multimeric complexes have been demonstrated [5].

The aim of the present study was to investigate the effect of a 12-week LCD (low-calorie diet) on plasma adiponectin multimeric complexes in relation to biochemical and anthropometrical parameters in a cohort of obese pre-menopausal women.

MATERIALS AND METHODS

Subjects

A total of 20 pre-menopausal women (age, 39.4 ± 9.5 years; body weight, 89.8 ± 16.4 kg; BMI, 32.2 ± 6.4 kg/m^2) participated in the study. Subjects were recruited by a referral from collaborating obesity units and through an advertisement in the local media. Obesity was defined as BMI $\geqslant 30$ kg/m^2, and 13 women were obese and seven women were overweight, with a BMI of 26–30 kg/m^2. Elevated plasma total cholesterol (> 5.0 mmol/l) was present in 16 women (range, 4.1–8.1 mmol/l), elevated plasma LDL-cholesterol (low-density lipoprotein cholesterol; > 3 mmol/l) was present in 17 women (range, 2.5–5.6 mmol/l), and hypertriglyceridaemia (> 1.7 mmol/l) was found in two women (range, 0.8–2.1 mmol/l). All of the participants had a blood pressure below 135/80 mmHg. Two women had impaired fasting glucose levels at baseline, but none of the participants were found to have diabetes. None of the women had any other chronic disease and all were medication free. Pregnancy was excluded at the beginning of the study. The subjects had a stable body weight for at least 3 months prior to the beginning of the study. Each participant gave her written informed consent before starting the study. All aspects of the study were performed in accordance with the Declaration of Helsinki and were approved by the Ethical Committee of the Third Faculty of Medicine, Charles University, Prague, Czech Republic.

Clinical protocol

The subjects were investigated at 08.00 hours after an overnight fast before and again at the end of the 12-week LCD (see the description of the dietary intervention below). Body weight, waist circumferenc and hip circumference were measured. Body composition was assessed using the multi-frequency bioimpedance method (Quad scan 4000; Bodystat). CVs (coefficients of variability) of fat mass and fat-free mass were 1.7 and 0.8% respectively. Blood samples for plasma analyses were collected from an indwelling polyethylene catheter inserted into the
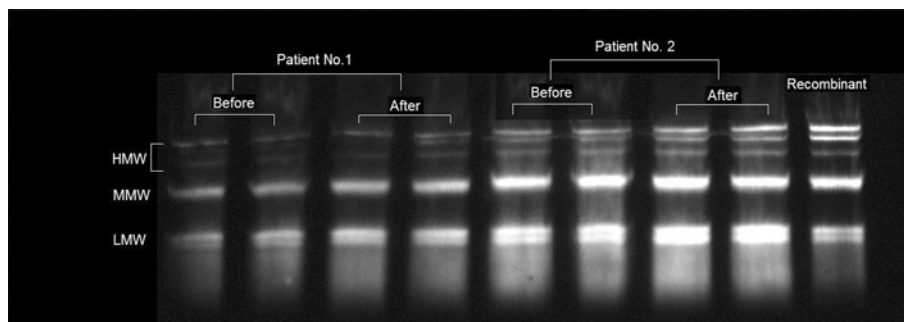
Figure 1   A representative example of the chemiluminescent detection of adiponectin multimeric complexes

Each sample was run in duplicate. Samples were drawn before and after the intervention and were run on the same gel. Recombinant human adiponectin served as a standard for the normalization of the individual band intensities of the patients' samples. The chemiluminescent signal was detected using the FujiFilm LAS 1000 detection system.

antecubital vein. After collection, blood was processed immediately in a refrigerated centrifuge. The plasma was stored at $-80\,^{\circ}$C until analysis.

Dietary intervention

The diet was designed to provide 600 kcal/day (where 1 kcal $\approx$ 4.184 kJ) less than individually calculated energy requirements, based on the subject's measured pre-treatment resting metabolic rate multiplied by 1.3 (a co-efficient of correction for physical activity level, assuming a sedentary lifestyle). Subjects were requested to abstain from alcohol consumption during the study. The diet was designed to provide 25–30 % energy derived from fat, 55–60 % energy from carbohydrates and 10–15 % energy from proteins. Dietary instructions were re-inforced and monitored weekly by dieticians throughout the intervention period. Subjects were instructed to follow their habitual patterns of physical activity during the study. A 3-day food record (two weekdays and one weekend day) was obtained from each participant and checked before the study and each week during the study. The dietary records were analysed using a country-specific food-nutrient database [NutriDan 1.2.; Mullerova D., Tychtl Z., Muller L. and Brazdova Z. (2002), produced in cooperation with the Danone Institute and distributed by DADI, Plzen, Czech Republic]. All of the subjects finished the study and, based on follow-up interviews with the study dietician, their compliance with the diet was very high.

Analytical methods

Plasma glucose was determined using the glucose-oxidase technique (Beckman Instruments). Plasma insulin concentration was measured using an Insulin RIA kit (Immunotech; CV, 2.8–4 %). Total plasma adiponectin concentration was measured using an Adiponectin Human ELISA kit (Biovendor Laboratory Medicine; CV, 4.1 %).

The HOMA (homoeostasis model assessment) index was calculated using the following equation: [fasting glucose (mmol/l) × fasting insulin (milli-international units/ml)]/22.5. Plasma LDL-cholesterol was calculated using the Friedewald equation: LDL-cholesterol = total cholesterol − HDL-cholesterol − (triacylglycerols/5).

Quantification of the adiponectin multimeric complexes

Samples (10 μl) of plasma, diluted (1:2) with Laemmli sample buffer (without 2-mercaptoethanol and SDS), were resolved using PAGE under non-reducing and non-denaturing conditions, as described previously [18,26]. Proteins were then transferred on to a nitrocellulose membrane, blocked for 1 h with 5 % (w/v) low-fat milk in PBS-T (PBS containing 0.5 % Tween 20) and incubated overnight with primary rabbit polyclonal anti-(human adiponectin) sera (Biovendor Laboratory Medicine) diluted 1:1000 in 1 % (w/v) low-fat milk in PBS-T. A second incubation (45 min) was carried out with secondary antibody [goat anti-(rabbit IgG) conjugated with horseradish peroxidase; Jackson ImmunoResearch] diluted 1:10 000 in 1 % (w/v) low-fat milk in PBS-T. Antibody binding was detected using a chemiluminescent substrate (Luminol; Sigma–Aldrich) [27] and was visualized using a FujiFilm LAS 1000 detection system. Band intensities were analysed using AIDA software. Plasma samples taken before and after the intervention were run on the same gel in duplicate (Figure 1). Signal intensities from the duplicate samples were averaged and used for statistical analysis. Native molecular-mass standards (Protein Markers for Native PAGE; Serva) and recombinant adiponectin (Adiponectin Human-HEK; Biovendor Laboratory Medicine) were also run on each gel. The individual signal intensity of each band was normalized using the intensity of the MMW form of the recombinant adiponectin protein. The standard was run using an identical concentration on all of the gels. These data were analysed using non-parametric tests and are

Table 1 Anthropometric and biochemical characteristics of subjects before and after a 12-week LCD

Values are means ± S.D., $n = 20$. mIU, milli-international units.

Characteristic	Before LCD	After LCD	P value
Body weight (kg)	89.8 ± 16.4	83.1 ± 15.6	< 0.001
BMI (kg/m^2)	32.2 ± 6.4	29.8 ± 6.2	< 0.001
Fat mass (kg)	37.8 ± 11.4	33.3 ± 11.7	< 0.001
Waist circumference (cm)	96.4 ± 13.3	88.8 ± 12.0	< 0.001
Hip circumference (cm)	118.6 ± 11.8	112.7 ± 12.0	< 0.001
WHR	0.81 ± 0.07	0.79 ± 1.0	0.004
Fasting glucose (mmol/l)	5.1 ± 0.4	5.0 ± 0.4	0.120
Fasting insulin (mIU/l)	8.2 ± 3.2	6.6 ± 2.9	0.014
Total cholesterol (mmol/l)	5.8 ± 1.0	5.3 ± 0.8	0.003
HDL-cholesterol (mmol/l)	1.7 ± 0.3	1.5 ± 0.2	< 0.001
LDL-cholesterol (mmol/l)	3.8 ± 0.8	3.6 ± 0.6	0.062
Triacylglycerols (mmol/l)	1.3 + 0.4	1.0 ± 0.3	0.002
HOMA index	1.9 ± 0.8	1.5 ± 0.7	0.013

Table 2 Adiponectin multimeric complexes and total plasma adiponectin before and after a 12-week LCD

Values are means ± S.D., $n = 20$. QL, quantity of light units normalized by reference to the QL of recombinant adiponectin MMW.

	Before LCD	After LCD	P value
Total adiponectin			
Determined by ELISA (μg/ml)	3.2 ± 1.7	4.4 ± 3.9	0.080
Determined by Western blotting (QL)	575.0 ± 215.1	655.5 ± 219.1	0.007
HMW (QL)	84.9 ± 37.1	89.6 ± 37.9	0.008
MMW (QL)	133.9 ± 57.3	145.1 ± 55.2	0.045
LMW (QL)	356.2 ± 138.6	420.8 ± 145.2	0.003
HMW/total ratio	0.14 ± 0.04	0.13 ± 0.04	0.009
HMW/LMW ratio	0.55 ± 0.25	0.52 ± 0.25	0.026
HMW/MMW ratio	0.65 ± 0.25	0.63 ± 0.24	0.461

shown in the Tables. CV of the Western blot analysis was 7.5%.

Although Western blot analyses are semi-quantitative in nature, comparisons of samples were possible when plasma samples derived before and after the intervention were run on the same gel. Therefore Western blots provide a useful tool in analysing all of the adiponectin multimeric complexes in human plasma, as has been demonstrated in other studies [25].

Statistical analysis

Statistical analysis was performed using SPSS 12.0 for Windows. The effect of weight loss was tested using the Wilcoxon sign rank test for paired observations for all of the variables studied. Univariate correlations were analysed using Spearman's correlation test. Values are means ± S.D. A level of $P \leqslant 0.05$ was considered statistically significant in all of the tests. The analyses of the differences of the means of all variables reached a power exceeding 80%, except for total adiponectin (ELISA), MMW and LMW forms and insulin, where the power was 44, 66, 58 and 41% respectively.

RESULTS

Anthropometric and biochemical parameters

Anthropometric and biochemical characteristics of the subjects before and after the diet intervention are summarized in Table 1. An LCD for 12 weeks resulted in a reduction in body weight, BMI and waist circumference by 7.4, 7.3 and 7.9% respectively. Fat mass diminished by 11.8% and was also accompanied by a 4% decrease in fat-free mass. The diet intervention improved the plasma lipid profile of the subjects; total cholesterol decreased by

8.5% and triacylglycerol decreased by 21% during the study. An 11% decrease in HDL-cholesterol was also observed.

Fasting plasma insulin levels and HOMA index of insulin resistance were lowered by 19.3 and 21.9% respectively, reflecting an improvement in whole-body insulin sensitivity. Fasting plasma glucose concentrations remained unchanged following the intervention.

Total plasma adiponectin and adiponectin multimeric complexes

Total plasma adiponectin levels, measured using ELISA, increased by 36% as a result of the LCD intervention; however, this difference was of borderline significance ($P = 0.08$). When the total adiponectin concentration was determined using Western blotting with chemiluminescence detection, a 13.5% increase ($P = 0.007$) was observed. The LCD induced increases in HMW, MMW and LMW plasma levels by 5.5, 18.1 and 8.5% respectively. Reductions in HMW/total adiponectin and HMW/LMW ratios were detected, whereas the HMW/MMW ratio remained unchanged. These results are summarized in Table 2.

Relationship between total plasma adiponectin and anthropometric/metabolic variables

Total plasma adiponectin levels were negatively associated with fasting insulin, HOMA index and HDL-cholesterol; however, these correlations were not significant before or after the study. No other associations between total plasma adiponectin and the biochemical, anthropometrical and insulin-sensitivity parameters analysed were observed before or after the

Table 3   Spearman's correlation coefficients between the variables before a 12-week LCD
*Significant difference. TPA-ELISA, total plasma adiponectin measured using ELISA.

Variable	HMW		MMW		LMW		TPA-ELISA	
	r	P value	r	P value	r	P value	r	P value
Weight	0.206	0.384	0.347	0.133	0.373	0.105	0.172	0.468
BMI	0.132	0.578	0.141	0.552	0.319	0.170	− 0.310	0.180
Fat mass	0.094	0.693	0.162	0.496	0.228	0.334	0.028	0.907
Fat-free mass	− 0.094	0.693	− 0.162	0.496	− 0.228	0.334	− 0.028	0.907
Waist circumference	− 0.043	0.857	− 0.072	0.762	0.177	0.455	0.101	0.672
Hip circumference	0.269	0.251	0.378	0.100	0.337	0.146	0.204	0.388
WHR	− 0.292	0.211	0.378	0.100	− 0.157	0.507	0.006	0.981
Triacylglycerols	− 0.125	0.600	− 0.335	0.149	− 0.221	0.349	− 0.139	0.560
HDL-cholesterol	0.269	0.265	0.527*	0.021	0.101	0.681	0.290	0.229
LDL-cholesterol	0.261	0.266	0.107	0.663	0.093	0.696	0.221	0.349
Fasting glucose	− 0.564*	0.010	− 0.447*	0.055	− 0.262	0.265	− 0.013	0.957
Fasting insulin	− 0.196	0.409	− 0.305	0.192	0.091	0.703	− 0.276	0.239
HOMA index	− 0.262	0.265	− 0.328	0.158	0.032	0.895	− 0.257	0.274

Table 4   Spearman's correlation coefficients between the variables after a 12-week LCD
*Significant difference. TPA-ELISA, total plasma adiponectin measured using ELISA.

Variable	HMW		MMW		LMW		TPA-ELISA	
	r	P value	r	P value	r	P value	r	P value
Weight	0.168	0.478	0.358	0.121	0.268	0.254	0.186	0.431
BMI	0.033	0.890	0.192	0.416	0.168	0.478	0.102	0.668
Fat mass	0.131	0.582	0.212	0.369	0.179	0.450	0.021	0.930
Fat-free mass	− 0.131	0.582	− 0.047	0.845	− 0.161	0.498	− 0.182	0.442
Waist circumference	− 0.153	0.519	0.042	0.860	0.017	0.945	0.222	0.346
Hip circumference	0.260	0.269	0.275	0.241	0.069	0.771	− 0.042	0.859
WHR	− 0.491*	0.028	− 0.371	0.107	− 0.147	0.535	0.198	0.403
Triacylglycerols	0.005	0.985	− 0.201	0.396	− 0.300	0.199	0.160	0.500
HDL-cholesterol	0.256	0.154	0.313	0.179	0.106	0.656	0.032	0.892
LDL-cholesterol	0.235	0.319	− 0.102	0.668	0.195	0.409	− 0.256	0.277
Fasting glucose	− 0.299	0.214	− 0.220	0.366	0.006	0.981	− 0.047	0.843
Fasting insulin	0.116	0.627	− 0.131	0.582	0.060	0.801	− 0.129	0.589
HOMA index	0.051	0.830	− 0.108	0.650	0.054	0.821	− 0.110	0.645

intervention period. These results are summarized in Tables 3 and 4.

Relationship between adiponectin multimeric complexes and anthropometric/metabolic variables

Among the multimeric complexes analysed, the HMW form was closely associated with fasting glucose level ($r = − 0.564$, $P = 0.010$) and the MMW form was closely associated with HDL-cholesterol ($r = 0.527$, $P = 0.021$) at the beginning of the study. An association between the MMW form and fasting glucose was of borderline significance ($r = − 0.447$, $P = 0.055$). The HMW form was negatively associated with WHR (waist/hip ratio; $r = − 0.491$, $P = 0.028$) at the end of the study. No other correlations between adiponectin multimeric complexes and biochemical and anthropometrical indices (lipid profile, BMI, waist circumference, fat mass and body weight) were found to be significant. No association between the HOMA index and any of the adiponectin multimeric complexes was detectable. These results are summarized in Tables 3 and 4.

Diet-induced changes in the HMW form were negatively associated with changes in the percentage of fat mass ($r = − 0.474$, $P = 0.035$). Changes in the MMW and LMW forms were not significantly associated with changes in the HOMA index or plasma insulin. No other correlations were observed between diet-induced changes in the total adiponectin, HMW, MMW and LMW

Table 5   Spearman's correlation coefficients between the diet-induced changes in the variables
*Significant difference. TPA-ELISA, total plasma adiponectin measured using ELISA.

Variable	HMW		MMW		LMW		TPA-ELISA	
	r	P value	r	P value	r	P value	r	P value
	0.076	0.750	— 0.226	0.337	0.202	0.392	0.181	0.444
Weight	0.005	0.982	— 0.270	0.249	0.160	0.502	0.177	0.456
BMI	— 0.474*	0.035	— 0.037	0.877	0.256	0.276	0.003	0.990
Fat mass	0.409	0.073	0.117	0.624	— 0.022	0.927	0.130	0.584
Fat-free mass	— 0.129	0.587	— 0.235	0.319	— 0.089	0.710	— 0.110	0.643
Waist circumference	0.215	0.362	— 0.054	0.822	0.334	0.150	0.165	0.486
Hip circumference	— 0.244	0.301	— 0.328	0.158	— 0.400	0.081	— 0.432	0.057
WHR	— 0.107	0.654	— 0.079	0.740	— 0.203	0.390	0.166	0.485
Triacylglycerols	— 0.284	0.226	0.023	0.922	— 0.260	0.269	0.049	0.838
HDL-cholesterol	— 0.151	0.525	0.113	0.636	— 0.106	0.656	0.080	0.736
LDL-cholesterol	— 0.313	0.179	— 0.074	0.758	— 0.275	0.240	— 0.126	0.596
Fasting glucose	0.120	0.613	— 0.105	0.659	— 0.027	0.910	— 0.005	0.985
Fasting insulin	0.017	0.942	— 0.325	0.162	— 0.184	0.437	— 0.013	0.957
HOMA index	— 0.050	0.835	— 0.343	0.139	— 0.254	0.280	— 0.008	0.975

forms and changes in anthropometrical or biochemical parameters. These results are summarized in Table 5.

DISCUSSION

In the present study, we have demonstrated for the first time that weight loss induced by an LCD in obese and overweight women is accompanied by an increase in plasma levels of all of the adiponectin multimeric complexes (HMW, MMW and LMW) studied. The most responsive form was shown to be the LMW form, with an 18.1 % increase following dietary intervention, followed by the MMW and HMW complexes (with increases of 8.5 and 5.5 % respectively). Whole-body insulin sensitivity, estimated using the HOMA index, improved following the diet. Reduction in anthropometrical parameters and improvement in lipid profile were also achieved.

To the best of our knowledge, three reports on weight-loss-induced changes in plasma distribution of adiponectin multimeric complexes have been published so far, showing either no changes in multimeric complexes distribution [25] or an increase in the HMW and MMW forms [8,28]. The major advantage of our present study is in the number and homogeneity of the subjects studied. Our sample consisted of 20 pre-menopausal obese and overweight women compared with 17 subjects (15 women and two men) in the study by Bobbert et al. [8], 12 subjects (eight women and four men) in the study by Abbasi et al. [25] and six subjects (three women and three men) in the study by Kobayashi et al. [28]. As gender differences in total adiponectin levels, as well as in the distribution of multimeric complexes, have been found, the results of the above-mentioned studies may be biased [5,18,29].

Our present finding of increased HMW and MMW forms after weight loss is in agreement with earlier studies [6,28]. However, we have demonstrated for the first time that the LMW form also increased after dietary intervention. In fact, the LMW form was the isoform with the highest increase. The degree of obesity and achieved weight loss in the subjects in our present study are comparable with those reported by Abbasi et al. [25] [(BMI, 32.7 ± 1.7 kg/m^2; average weight reduction, 7.4 kg) and Bobbert et al. [8] (BMI, 35.1 ± 1.2 kg/m^2; average weight reduction, 6.2 kg). The specific biological role and function of the LMW form relative to the other adiponectin multimeric complexes has not yet been established, so the interpretation of the increased LMW adiponectin remains open for discussion.

The HMW form has been suggested to be the most physiologically potent form of adiponectin and might be the form responsible for its beneficial insulin-sensitizing and anti-atherosclerotic effects [12,28]. It has been shown that the HMW/total adiponectin and HMW/LMW ratios are plausible indicators of TZD (thiazolidinedione)-induced changes in insulin sensitivity [12].

In the present study, we found an association between the HMW form and fasting glucose levels before the weight loss, which was compatible with the hypothesis of an important role of the HMW form in the regulation of insulin sensitivity under basal steady-state conditions. The HMW form increased by 5.5 % after weight loss, but no association with fasting glucose was observed at the end of the study. This small elevation in HMW adiponectin is probably of limited clinical significance, and other regulatory mechanisms possibly play more important roles in the control of fasting glucose following acute weight loss, i.e. changes in other plasma cytokines (interleukin-6, tumour necrosis factor and leptin) [30,31]

or a reduction in fat cell size and intrahepatic lipid content [32]. It has also been proposed that the caloric restriction can, by itself, improve glycaemic control, regardless of weight loss [33].

We observed a reduction in both HMW/total adiponectin and HMW/LMW ratios, but there was no change in the HMW/MMW ratio, reflecting the relatively large increase in the LMW form compared with HMW adiponectin. An association between the HMW form and whole-body insulin sensitivity has been suggested [12,25]. Nevertheless, no significant association between total adiponectin or its multimeric complexes and insulin sensitivity, as evaluated using a euglycaemic hyperinsulinaemic clamp, was found in the study by Bobbert et al. [8]. No associations between the HOMA index and any of the adiponectin oligomeric complexes, ratios or total plasma adiponectin were observed in our present study either at baseline or with respect to the diet-induced changes. On the basis of these findings and on those by Bobbert et al. [8], it can be hypothesized that the above-mentioned ratios and associations of HMW adiponectin with parameters of insulin sensitivity might be specific to TZD treatment and may play only a minor role in LCD-induced changes in insulin sensitivity. A recent study [34] showing that TZD treatment selectively stimulates the secretion of the HMW form in human adipocytes supports this hypothesis further.

The results concerning changes in total plasma adiponectin concentration after weight loss are inconsistent. No change in total plasma adiponectin during moderate weight loss was found in several studies [8,25,35], whereas an increase in plasma adiponectin following large weight reduction subsequent to bariatric surgery [36–38] or intensive lifestyle counselling [39] have been described by others. No change in plasma adiponectin or insulin sensitivity was demonstrated following liposuction [40]. Thus it might be suggested that adiponectin plays a minor role in the regulation of the changes in insulin sensitivity during the moderate weight loss induced either by diet or physical exercise [41]. Despite the absence of a significant association between changes in plasma adiponectin and insulin sensitivity, clinically beneficial effects of increased plasma adiponectin following weight loss might persist, since it also has marked anti-inflammatory and anti-atherosclerotic effects in humans [7,42], effects which are independent of its insulin-sensitizing action.

It has been shown previously [21,43–45] that total adiponectin levels are associated with plasma HDL-cholesterol. In our present study, a correlation between total adiponectin and HDL-cholesterol had only borderline significance ($r = 0.43$, $P = 0.07$). This might be explained by a substantially lower number of subjects participating in our present study (20 women) compared with larger samples in studies describing such an association (407 and 1174 subjects) [21,45].

BMI and total adiponectin were negatively associated in our present study at baseline ($r = -0.31$, $P = 0.18$); however, according to calculations of sample size, the minimal number of subjects required to obtain a statistically significant correlation would have been 73 women.

We observed a correlation between the MMW form and HDL-cholesterol; however, this association was not observed with other multimeric complexes or with total plasma adiponectin. Our observations are in contrast with recent findings [8,10], which have shown that HMW adiponectin is predominantly responsible for the correlation between total adiponectin and HDL-cholesterol, perhaps through its impact on hepatic metabolism [8]. It should be noted here that both the HMW and MMW forms are able to stimulate AMPK in primary culture hepatocytes [18] and might therefore have similar effects on hepatocytes.

The 11.8 % reduction in plasma HDL-cholesterol seen in our present study is in agreement with observations of several other studies, including a meta-analysis [46], and it might be partially explained in terms of the impaired activity of lipoprotein lipase [47] and changes in the macronutrient composition of the diet [48–50]. HDL-cholesterol decreases during an active weight-loss phase in contrast with a stabilized period, when HDL-cholesterol is increased following the reduction of body weight [46].

The increase in all three types of adiponectin multimeric complexes in the presence of a non-significant change in total plasma adiponectin levels measured using ELISA is due to intrinsic differences between the two methods. ELISA provides a quantitative determination of actual plasma concentration, whereas Western blotting yields semi-quantitative data in the form of arbitrary units (quantity of light). Moreover, the difference could be due to different binding capacities of the respective clones of antibodies used in the ELISA and Western blotting.

In conclusion, diet-induced weight loss associated with insulin-sensitizing effects promotes an increase in the amount of HMW, MMW and LMW adiponectin multimeric complexes in plasma. No direct relationships between the diet-induced changes in individual adiponectin multimeric complexes and those of insulin sensitivity were found. Further studies elucidating the physiological relevance and function of adiponectin multimeric complexes with respect to obesity and insulin resistance are warranted.

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REFERENCES

1   Berg, A. H. and Scherer, P. E. (2005) Adipose tissue, inflammation, and cardiovascular disease. Circ. Res. **96**, 939–949

2   Rondinone, C. M. (2006) Adipocyte-derived hormones, cytokines, and mediators. Endocrine **29**, 81–90

3   Scherer, P. E., Williams, S., Fogliano, M., Baldini, G. and Lodish, H. F. (1995) A novel serum protein similar to C1q, produced exclusively in adipocytes. J. Biol. Chem. **270**, 26746–26749

4   Arita, Y., Kihara, S., Ouchi, N. et al. (1999) Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. Biochem. Biophys. Res. Commun. **257**, 79–83

5   Hotta, K., Funahashi, T., Arita, Y. et al. (2000) Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. Arterioscler. Thromb. Vasc. Biol. **20**, 1595–1599

6   Abbasi, F., Chu, J. W., Lamendola, C. et al. (2004) Discrimination between obesity and insulin resistance in the relationship with adiponectin. Diabetes **53**, 585–590

7   Ouchi, N., Kihara, S., Arita, Y. et al. (2001) Adipocyte-derived plasma protein, adiponectin, suppresses lipid accumulation and class A scavenger receptor expression in human monocyte-derived macrophages. Circulation **103**, 1057–1063

8   Bobbert, T., Rochlitz, H., Wegewitz, U. et al. (2005) Changes of adiponectin oligomer composition by moderate weight reduction. Diabetes **54**, 2712–2719

9   Korner, A., Wabitsch, M., Seidel, B. et al. (2005) Adiponectin expression in humans is dependent on differentiation of adipocytes and down-regulated by humoral serum components of high molecular weight. Biochem. Biophys. Res. Commun. **337**, 540–550

10 Lara-Castro, C., Luo, N., Wallace, P., Klein, R. L. and Garvey, W. T. (2006) Adiponectin multimeric complexes and the metabolic syndrome trait cluster. Diabetes **55**, 249–259

11 Richards, A. A., Hickman, I. J., Wang, A. Y. et al. (2006) Olanzapine treatment is associated with reduced high molecular weight adiponectin in serum: a potential mechanism for olanzapine-induced insulin resistance in patients with schizophrenia. J. Clin. Psychopharmacol. **26**, 232–237

12 Pajvani, U. B., Hawkins, M., Combs, T. P. et al. (2004) Complex distribution, not absolute amount of adiponectin, correlates with thiazolidinedione-mediated improvement in insulin sensitivity. J. Biol. Chem. **279**, 12152–12162

13 Yamauchi, T., Kamon, J., Ito, Y. et al. (2003) Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. Nature **423**, 762–769

14 Fu, Y., Luo, N., Klein, R. L. and Garvey, W. T. (2005) Adiponectin promotes adipocyte differentiation, insulin sensitivity, and lipid accumulation. J. Lipid Res. **46**, 1369–1379

15 Yamauchi, T., Kamon, J., Minokoshi, Y. et al. (2002) Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. Nat. Med. **8**, 1288–1295

16 Winder, W. W. and Hardie, D. G. (1999) AMP-activated protein kinase, a metabolic master switch: possible roles in type 2 diabetes. Am. J. Physiol **277**, E1–E10

17 Lochhead, P. A., Salt, I. P., Walker, K. S., Hardie, D. G. and Sutherland, C. (2000) 5-Aminoimidazole-4-carboxamide riboside mimics the effects of insulin on the expression of the 2 key gluconeogenic genes PEPCK and glucose-6-phosphatase. Diabetes **49**, 896–903

18 Waki, H., Yamauchi, T., Kamon, J. et al. (2003) Impaired multimerization of human adiponectin mutants associated with diabetes. Molecular structure and multimer formation of adiponectin. J. Biol. Chem. **278**, 40352–40363

19 Tsao, T. S., Tomas, E., Murrey, H. E. et al. (2003) Role of disulfide bonds in Acrp30/adiponectin structure and signaling specificity. Different oligomers activate different signal transduction pathways. J. Biol. Chem. **278**, 50810–50817

20 Matsubara, M., Katayose, S. and Maruoka, S. (2003) Decreased plasma adiponectin concentrations in nondiabetic women with elevated homeostasis model assessment ratios. Eur. J. Endocrinol. **148**, 343–350

21 Tschritter, O., Fritsche, A., Thamer, C. et al. (2003) Plasma adiponectin concentrations predict insulin sensitivity of both glucose and lipid metabolism. Diabetes **52**, 239–243

22 Brook, R. D. (2006) Obesity, weight loss, and vascular function. Endocrine **29**, 21–25

23 Eddy, D. M., Schlessinger, L. and Kahn, R. (2005) Clinical outcomes and cost-effectiveness of strategies for managing people at high risk for diabetes. Ann. Intern. Med. **143**, 251–264

24 Knowler, W. C., Barrett-Connor, E., Fowler, S. E. et al. (2002) Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. N. Engl. J. Med. **346**, 393–403

25 Abbasi, F., Chang, S. A., Chu, J. W. et al. (2006) Improvements in insulin resistance with weight loss, in contrast to rosiglitazone, are not associated with changes in plasma adiponectin or adiponectin multimeric complexes. Am. J. Physiol. Regulatory Integrative Comp. Physiol. **290**, R139–R144

26 Laemmli, U. K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227**, 680–685

27 Leong, M. M. and Fox, G. R. (1990) Luminescent detection of immunodot and western blots. Methods Enzymol. **184**, 442–451

28 Kobayashi, H., Ouchi, N., Kihara, S. et al. (2004) Selective suppression of endothelial cell apoptosis by the high molecular weight form of adiponectin. Circ. Res. **94**, e27–e31

29 Yamamoto, Y., Hirose, H., Saito, I. et al. (2002) Correlation of the adipocyte-derived protein adiponectin with insulin resistance index and serum high-density lipoprotein-cholesterol, independent of body mass index, in the Japanese population. Clin. Sci. **103**, 137–142

30 Ryan, A. S. and Nicklas, B. J. (2004) Reductions in plasma cytokine levels with weight loss improve insulin sensitivity in overweight and obese postmenopausal women. Diabetes Care **27**, 1699–1705

31 Bastard, J. P., Jardel, C., Bruckert, E. et al. (2000) Elevated levels of interleukin 6 are reduced in serum and subcutaneous adipose tissue of obese women after weight loss. J. Clin. Endocrinol. Metab. **85**, 3338–3342

32 Larson-Meyer, D. E., Heilbronn, L. K., Redman, L. M. et al. (2006) Effect of calorie restriction with or without exercise on insulin sensitivity, β-cell function, fat cell size, and ectopic lipid in overweight subjects. Diabetes Care **29**, 1337–1344

33 Wing, R. R., Blair, E. H., Bononi, P., Marcus, M. D., Watanabe, R. and Bergman, R. N. (1994) Caloric restriction per se is a significant factor in improvements in glycemic control and insulin sensitivity during weight loss in obese NIDDM patients. Diabetes Care **17**, 30–36

34 Bodles, A., Banga, A., Rasouli, N., Ono, F., Kern, P. A. and Owens, R. J. (2006) Pioglitazone increases secretion of high molecular weight adiponectin from adipocytes. Am. J. Physiol. Endocrinol. Metab. **291**, E1100–E1105

35 Abbasi, F., Lamendola, C., McLaughlin, T., Hayden, J., Reaven, G. M. and Reaven, P. D. (2004) Plasma adiponectin concentrations do not increase in association with moderate weight loss in insulin-resistant, obese women. Metab., Clin. Exp. **53**, 280–283

36 Yang, W. S., Lee, W. J., Funahashi, T. et al. (2001) Weight reduction increases plasma levels of an adipose-derived anti-inflammatory protein, adiponectin. J. Clin. Endocrinol. Metab. **86**, 3815–3819

37 Serra, A., Granada, M. L., Romero, R. et al. (2006) The effect of bariatric surgery on adipocytokines, renal parameters and other cardiovascular risk factors in severe and very severe obesity: 1-year follow-up. Clin. Nutr. **25**, 400–408

38 Kotidis, E. V., Koliakos, G., Papavramidis, T. S. and Papavramidis, S. T. (2006) The effect of biliopancreatic diversion with pylorus-preserving sleeve gastrectomy and duodenal switch on fasting serum ghrelin, leptin and adiponectin levels: is there a hormonal contribution to the weight-reducing effect of this procedure? Obes. Surg. **16**, 554–559

39 Lazzer, S., Vermorel, M., Montaurier, C., Meyer, M. and Boirie, Y. (2005) Changes in adipocyte hormones and lipid oxidation associated with weight loss and regain in severely obese adolescents. Int. J. Obes. **29**, 1184–1191

40 Klein, S., Fontana, L., Young, V. L. et al. (2004) Absence of an effect of liposuction on insulin action and risk factors for coronary heart disease. N. Engl. J. Med. **350**, 2549–2557

41 Polak, J., Klimcakova, E., Moro, C. et al. (2006) Effect of aerobic training on plasma levels and subcutaneous abdominal adipose tissue gene expression of adiponectin, leptin, interleukin 6, and tumor necrosis factor α in obese women. Metab., Clin. Exp. **55**, 1375–1381

42 Okamoto, Y., Arita, Y., Nishida, M. et al. (2000) An adipocyte-derived plasma protein, adiponectin, adheres to injured vascular walls. Horm. Metab. Res. **32**, 47–50

43 Kazumi, T., Kawaguchi, A., Hirano, T. and Yoshino, G. (2004) Serum adiponectin is associated with high-density lipoprotein cholesterol, triglycerides, and low-density lipoprotein particle size in young healthy men. Metab., Clin. Exp. **53**, 589–593

44 Heliovaara, M. K., Strandberg, T. E., Karonen, S. L. and Ebeling, P. (2006) Association of serum adiponectin concentration to lipid and glucose metabolism in healthy humans. Horm. Metab. Res. **38**, 336–340

45 von Eynatten, M., Hamann, A., Twardella, D., Nawroth, P. P., Brenner, H. and Rothenbacher, D. (2006) Relationship of adiponectin with markers of systemic inflammation, atherogenic dyslipidemia, and heart failure in patients with coronary heart disease. Clin. Chem. **52**, 853–859

46 Dattilo, A. M. and Kris-Etherton, P. M. (1992) Effects of weight reduction on blood lipids and lipoproteins: a meta-analysis. Am. J. Clin. Nutr. **56**, 320–328

47 Taskinen, M. R. and Nikkila, E. A. (1979) Effects of caloric restriction on lipid metabolism in man: changes of tissue lipoprotein lipase activities and of serum lipoproteins. Atherosclerosis **32**, 289–299

48 Sacks, F. M. and Katan, M. (2002) Randomized clinical trials on the effects of dietary fat and carbohydrate on plasma lipoproteins and cardiovascular disease. Am. J. Med. **113** (Suppl. 9B), 13S–24S

49 Zambon, A., Sartore, G., Passera, D. et al. (1999) Effects of hypocaloric dietary treatment enriched in oleic acid on LDL and HDL subclass distribution in mildly obese women. J. Intern. Med. **246**, 191–201

50 Mensink, R. P., Zock, P. L., Kester, A. D. and Katan, M. B. (2003) Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. Am. J. Clin. Nutr. **77**, 1146–1155