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Abstract:

Adiponectin is involved in the regulation of glucose and fatty acid metabolism. Adiponectin 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 form (HMW, MMW and LMW). The HMW is believed to be closely associated with insulin sensitivity. The aim of the study was to investigate whether diet-induced changes in body weight and insulin sensitivity were associated with changes in adiponectin multimeric complexes quantity.

20 overweight or obese women (age 39.4±9.5 years, BMI 32.2±6.4 kg/m^2) underwent 12 weeks´ low caloric diet (600kcal/d less than energy requirements). 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 vs 83.1±15.6 kg, $p<0.001$) and an improvement of whole body insulin sensitivity as measured by the Homeostasis Model Assessment Index (HOMA) (1.9±0.8 vs 1.5±0.7, $p = 0.013$). Increases in HMW, MMW and LMW quantities by 5.5%, 8.5% and 18.1%, respectively, were observed ($p<0.05$ for all 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 type 2 diabetic patients [5], insulin-resistant subjects [6], obese individuals [4], as well as in coronary heart disease patients [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 low molecular weight (LMW), hexameric medium molecular weight (MMW) and oligomeric high molecular weight complexes (HMW). However, some investigators, using different analytical methods, have distinguished only two adiponectin isoforms: LMW and HMW [6;8-12]. Adiponectin cell surface receptors (AdipoR1, AdipoR2) are expressed in muscle, liver and adipose tissue [13;14]. In myocytes and hepatocytes (mouse model), adiponectin stimulates phosphorylation and the activation of 5'-AMP-activated protein kinase (AMPK) [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 seems 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, while negative association with BMI 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 hypoadiponectinemia 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 patients 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], while others found increased quantity 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. Marked gender differences in total plasma adiponectin and in the distribution of multimeric complexes have been demonstrated [5].

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

Methods:

Subjects

Twenty premenopausal 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 a collaborating obesity units and through an advertisement in the local media. Obesity was defined as BMI ≥ 30 kg/m^2, 13 women were obese and 7 women were overweight with BMI 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 (> 3 mmol/l) was present in 17 women (range 2.5 - 5.6 mmol/l), and hypertriglyceridemia (> 1.7 mmol/l) was found in 2 women (range 0.8 - 2.1 mmol/l). All the participants had blood pressure below 135/80 mmHg. Two women had impaired fasting glucose levels at baseline, 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 stable body weight for at least three 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 8 a.m. 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 and hip circumference were measured. Body composition was assessed using the multi-frequency bioimpedance method (Bodystat, Quad scan 4000, Isle of Man, British Isles). Coefficients of variation (CV) 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 antecubital vein. After collection, blood was processed immediately in a refrigerated centrifuge. The plasma was stored at –80 °C until analysis.

Dietary intervention

The diet was designed to provide 600 kcal/d less than individually-calculated energy requirements, based on the subject's measured pre-treatment resting metabolic rate multiplied by 1.3 (a coefficient 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% of energy derived from fat, 55-60% of energy from

carbohydrates and 10-15% of energy from proteins. Dietary instructions were reinforced 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., Brazdova Z., 2002, produced in a cooperation with Danone Institute, distributed by DADI s.r.o., Plzen, Czech republic). All 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, Fullerton, CA). Plasma insulin concentration was measured using an Insulin RIA kit (Immunotech, Prague, Czech Republic) (coefficient of variability 2.8 - 4%). Total plasma adiponectin concentration was measured using an Adiponectin Human ELISA kit (Biovendor Laboratory Medicine Inc., Modrice, Czech Republic) (coefficient of variability 4.1%).

Adiponectin multimeric complexes quantification

Samples (10µl) of plasma diluted (1:2) with Laemmli sample buffer (without β-mercaptoethanol and SDS) were resolved using PAGE (polyacrylamide gel electrophoresis) under non-reducing, non-denaturing conditions, as previously described in other studies [18;26]. Proteins were then western-blotted to a nitrocellulose membrane, blocked for 1h with 5% low-fat milk in a phosphate-buffered saline with 0.5% Tween (PBS-T) and incubated overnight with primary human adiponectin antibody diluted 1:1000 in 1% low-fat milk in PBS-T (Anti-Human Adiponectin Rabbit Polyclonal Antibody, Biovendor Laboratory Medicine Inc., Modrice, Czech Republic). A second incubation (45 min) was caried out with secondary antibody (Goat Anti-Rabbit IgG conjugated with horseradish peroxidase, Jackson ImmunoResearch Europe Ltd., Cambridge, UK) diluted 1:10000 in 1% low-fat milk in PBS-T. Band detection using a chemiluminescent substrate (Luminol, Sigma-Aldrich, Prague, Czech Republic) [27] was done on a FujiFilm LAS 1000 detection system (Fuji Photo Film Co., Ltd., Tokyo, Japan). Band intensities were analyzed using AIDA Software. Plasma samples taken before and after the intervention were run on the same gel in duplicates. Signal intensities from the duplicate samples were averaged and used for statistical analysis. Native molecular weight standards (Protein Markers for Native PAGE, Serva, Heidelberg, Germany)

and recombinant adiponectin (Adiponectin Human-HEK, Biovendor Laboratory Medicine Inc., Modrice, Czech Republic) 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 the identical concentration on all the gels. These data were analyzed using non-parametric tests and are presented in the tables. Coefficient of variability of the Western blot analysis was 7.5%. We are aware that all Western blot analyses have a semi-quantitative character. However, before vs. after comparisons are meaningful, assuming plasma samples derived before and after the intervention are run on the same gel, consequently Western blots provide a useful analytical tool for analyses of all adiponectin multimeric complexes in human plasma, as has been demonstrated by other studies [25].

Statistical analysis

Statistical analysis was performed using SPSS 12.0 for Windows (SPSS Inc., Chicago, IL). The effect of weight loss was tested using the Wilcoxon Sign Rank Test for paired observations for all studied variables. Univariate correlations were analyzed using the Spearman's correlation test. The HOMA index (Homeostasis Model Assessment index) was computed following the equation: ((fasting glucose $_{(mmol/l)}$ * fasting insulin $_{(uIU/ml)}$)/ 22.5). Plasma LDL cholesterol was calculated using the Friedewald equation: $LDL_{cholesterol} = (Total_{cholesterol} - HDL_{cholesterol} - (triglycerides/5))$. Data are presented as mean values ± SD. A level of $p \leq 0.05$ was considered statistically significant in all 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. Twelve weeks of LCD resulted in a reduction of body weight, BMI and waist circumference by 7.4%, 7.3% and 7.9%, respectively. Fat mass diminished by 11.8% and was laso 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 triglycerides 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 blot with chemiluminescence detection, a 13.5% increase ($p = 0.007$) in total adiponectin was observed. The LCD induced elevations 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 , while the HMW/MMW ratio remained unchanged. Data are summarized in Table 2.

Relationship between the 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 the total plasma adiponectin and the analyzed biochemical, anthropometrical and insulin sensitivity parameters were observed before or after the intervention period. Data are summarized in Tables 3 and 4.

Relationships between adiponectin multimeric complexes and anthropometric/metabolic variables.

Among the analyzed multimeric complexes, the HMW form was closely associated with fasting glucose level ($r = -0.564$, $p = 0.010$) and the MMW form 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 the 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, body weight) were found to be significant. No association between HOMA index and any of the adiponectin multimeric complexes was detectable. Data 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, LMW forms and changes in anthropometrical or biochemical parameters. Data are summarized in Table 5.

Discussion

We have demonstrated for the first time that weight loss induced by a low caloric diet is accompanied by an increase in plasma levels of all studied adiponectin multimeric complexes (HMW, MMW and LMW) in obese and overweight women. The most responsive form was shown to be the LMW form, with an 18.1% increase following dietary intervention, followed by 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 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 form [8;28]. The major advantage of our study is in the number and homogeneity of the studied subjects. Our sample consisted of 20 premenopausal obese and overweight women, compared with 17 subjects (15 women and 2men) in the Bobbert et al. study [8], 12 subjects (8 women and 4 men) in the Abbasi et al. study [25] and 6 subjects (3 women and 3 men) in the Kobayashi et al. study [28]. Since 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 might be biased [5;18;29].

Our finding of increased HMW and MMW forms after weight loss is in agreement with earlier studies [6;28]. However we 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 subjects of our study are comparable to those reported by Abbasi et al. (BMI 32.7 ± 1.7 kg/m^2, average weight reduction = 7.4 kg) and Bobbert et al. (BMI 35.1 ± 1.2 kg/m^2, average weight reduction = 6.2 kg). The specific biological role and the 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 physiologically most 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 ratios of HMW/total adiponectin and HMW/LMW are plausible indicators of thiazolidinedione (TZD)-induced changes in insulin sensitivity [12].

We found an association between the HMW form and fasting glucose levels before the weight loss, which were compatible with the hypothesis of an important role for the HMW form in

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 possibly other regulatory mechanisms play more important roles in the control of fasting glucose following acute weight loss, i.e. changes in other plasma cytokines (interleukin-6, tumor necrosis factor, 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 glycemic 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 to 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 euglycemic hyperinsulinemic clamp, was found in the Bobbert et al. study [8]. No associations between the HOMA index and any of the adiponectin oligomeric complexes or ratios or total plasma adiponectin were observed in our study, either at baseline or with respect to the diet-induced changes. Based on these findings and on those published in the Bobbert et al. study [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 play only a minor role in LCD-induced changes in insulin sensitivity. A recent paper showing that TZD treatment selectively stimulates secretion of the HMW form in human adipocytes further supports this hypothesis [34].

The results concerning changes in total plasma adiponectin concentration after weight loss are inconsistent. No change in total plasma adiponectin during a moderate weight loss was found in several studies [8;25;35], while an increase in plasma adiponectin following large weight reductions subsequent to bariatric surgery [36-38] or intensive lifestyle counseling [39] were 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 insulin sensitivity changes 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 previously shown that total adiponectin levels are associated with plasma HDL cholesterol [21;43-45]. In our study a correlation between total adiponectin and HDL cholesterol had only borderline significance ($r = 0.43$, $p = 0.07$). This might be explained by substantially lower number of subjects participating in our study (20 women) comapred to larger samples in studies describing such an association (407 and 1174 subjects) [21;45]. BMI and total adiponectin were negatively associated in our study at the 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 showed that HMW adiponectin is predominantly responsible for the correlation between total adiponectin and HDL cholesterol - perhaps through its impact on hepatic metabolism [8]. It is to be noted here that both, the HMW and MMW forms are able to stimulate AMPK in primary culture hepatocytes [18] and might thus have similar effects on hepatocytes.

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

The increase in all three types of adiponectin multimeric complexes in a presence of a non-significant change in total plasma adiponectin levels measured using ELISA assay is due to intrinsic differencies between the two methods. ELISA provides a quantitive determination of actual plasma concentration, while Western blot yields semi-quantitative data in a 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 ELISA and Western blots.

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

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Table 1. Anthropometric and biochemical characteristics of subjects before and after a 12-week low calorie diet (LCD)

	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
Waist/Hip Ratio	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
Triglycerides (mmol/l)	1.3+0.4	1.0±0.3	0.002
HOMA	1.9±0.8	1.5±0.7	0.013

Data presented as Mean±SD, N = 20
HOMA (Homeostasis Model Assessment Index)

Table 2. Adiponectin multimeric complexes and total plasma adiponectin before and after a 12-week low calorie diet (LCD)

	Before LCD	After LCD	p-value
Total adiponectin ELISA (μg/ml)	3.2±1.7	4.4±3.9	0.080
Total adiponectin Western blot (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

Data presented as Mean±SD, N = 20
HMW (high molecular weight form), MMW (medium molecular weight form), LMW (low molecular weight form), QL (quantity of light units normalised by reference QL of recombinant adiponectin MMW), ELISA (Enzyme-linked immunosorbent assay)

Table 3.Spearman`s correlation coefficients between variables before the 12-week low calorie diet (LCD)

Before LCD		Weight	BMI	Fat mass	Fat free mass	Waist circumference	Hip circumference	WHR	Triglycerides	HDL cholesterol	LDL cholesterol	Fasting glucose	Fasting insulin	HOMA
HMW	Correl.	.206	.132	.094	-.094	-.043	.269	-.292	-.125	.269	.261	-.564*	-.196	-.262
	Sig.	*.384*	*.578*	*.693*	*.693*	*.857*	*.251*	*.211*	*.600*	*.265*	*.266*	*.010*	*.409*	*.265*
MMW	Correl.	.347	.141	.162	-.162	-.072	.378	.378	-.335	.527*	.107	-.447*	-.305	-.328
	Sig.	*.133*	*.552*	*.496*	*.496*	*.762*	*.100*	*.100*	*.149*	*.021*	*.663*	*.055*	*.192*	*.158*
LMW	Correl.	.373	.319	.228	-.228	.177	.337	-.157	-.221	.101	.093	-.262	.091	.032
	Sig.	*.105*	*.170*	*.334*	*.334*	*.455*	*.146*	*.507*	*.349*	*.681*	*.696*	*.265*	*.703*	*.895*
TPA-ELISA	Correl.	.172	-.310	.028	-.028	.101	.204	.006	-.139	.290	.221	-.013	-.276	-.257
	Sig.	*.468*	*.180*	*.907*	*.907*	*.672*	*.388*	*.981*	*.560*	*.229*	*.349*	*.957*	*.239*	*.274*

HMW (high molecular weight form of adiponectin), MMW (medium molecular weight form of adiponectin), LMW (low molecular weight form of adiponectin), TPA-ELISA (total plasma adiponectin measured using ELISA), BMI (body mass index), WHR (waist-hip ratio), HOMA (Homeostasis Model Assessment Index)

Table 4.Spearman`s correlation coefficients between variables after 12-week low calorie diet (LCD)

After LCD		Weight	BMI	Fat mass	Fat free mass	Waist circumference	Hip circumference	WHR	Triglycerides	HDL cholesterol	LDL cholesterol	Fasting glucose	Fasting insulin	HOMA
HMW	Correl.	.168	.033	.131	-.131	-.153	.260	-.491*	.005	.256	.235	-.299	.116	.051
	Sig.	*.478*	*.890*	*.582*	*.582*	*.519*	*.269*	*.028*	*.985*	*.154*	*.319*	*.214*	*.627*	*.830*
MMW	Correl.	.358	.192	.212	-.047	.042	.275	-.371	-.201	.313	-.102	-.220	-.131	-.108
	Sig.	*.121*	*.416*	*.369*	*.845*	*.860*	*.241*	*.107*	*.396*	*.179*	*.668*	*.366*	*.582*	*.650*
LMW	Correl.	.268	.168	.179	-.161	.017	.069	-.147	-.300	.106	.195	.006	.060	.054
	Sig.	*.254*	*.478*	*.450*	*.498*	*.945*	*.771*	*.535*	*.199*	*.656*	*.409*	*.981*	*.801*	*.821*
TPA-ELISA	Correl.	.186	.102	.021	-.182	.222	-.042	.198	.160	.032	-.256	-.047	-.129	-.110
	Sig.	*.431*	*.668*	*.930*	*.442*	*.346*	*.859*	*.403*	*.500*	*.892*	*.277*	*.843*	*.589*	*.645*

HMW (high molecular weight form of adiponectin), MMW (medium molecular weight form of adiponectin), LMW (low molecular weight form of adiponectin), TPA-ELISA (total plasma adiponectin measured using ELISA), BMI (body mass index), WHR (waist-hip ratio), HOMA (Homeostasis Model Assessment Index)

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Table 5.Spearman`s correlation coefficients between diet-induced changes of variables

Correlations of diet-induced changes		Weight	BMI	Fat mass	Fat free mass	Waist circumference	Hip circumference	WHR	Triglycerides	Total cholesterol	HDL cholesterol	LDL cholesterol	Fasting glucose	Fasting insulin	HOMA
HMW	**Correl.**	,076	,005	-,474*	,409	-,129	,215	-,244	-,107	-,284	-,151	-,313	,120	,017	-,050
	Sig.	,750	,982	,035	,073	,587	,362	,301	,654	,226	,525	,179	,613	,942	,835
MMW	**Correl.**	-,226	-,270	-,037	,117	-,235	-,054	-,328	-,079	,023	,113	-,074	-,105	-,325	-,343
	Sig.	,337	,249	,877	,624	,319	,822	,158	,740	,922	,636	,758	,659	,162	,139
LMW	**Correl.**	,202	,160	,256	-,022	-,089	,334	-,400	-,203	-,260	-,106	-,275	-,027	-,184	-,254
	Sig.	,392	,502	,276	,927	,710	,150	,081	,390	,269	,656	,240	,910	,437	,280
TPA-ELISA	**Correl.**	,181	,177	,003	,130	-,110	,165	-,432	,166	,049	,080	-,126	-,005	-,013	-,008
	Sig.	,444	,456	,990	,584	,643	,486	,057	,485	,838	,736	,596	,985	,957	,975

HMW (high molecular weight form of adiponectin), MMW (medium molecular weight form of adiponectin), LMW (low molecular weight form of adiponectin), TPA-ELISA (total plasma adiponectin measured using ELISA), BMI (body mass index), WHR (waist-hip ratio), HOMA (Homeostasis Model Assessment Index)

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Figure 1. An example of a chemoluminescent detection of adiponectin multimeric complexes.

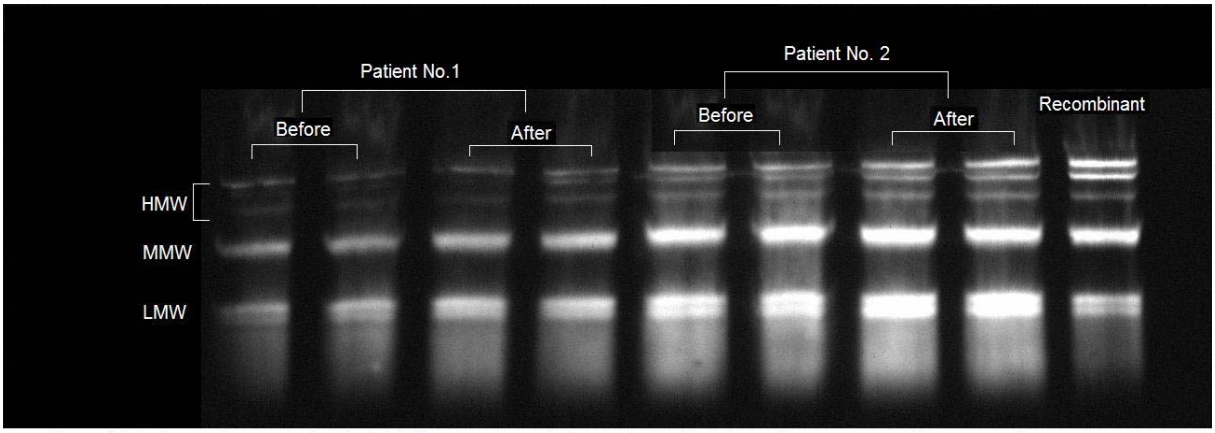

Figure 1. Each sample was run in a doublet. Samples drawn before and after the intervention were run on the same gel. Recombinant human adiponectin served as a standard for normalisation of patients` individual band intensities. Chemiluminescent signal was detected using FujiFilm LAS 1000 detection system.