Original Article

Changes of Adiponectin Oligomer Composition by Moderate Weight Reduction

Thomas Bobbert,[1,2] Helmut Rochlitz,[1,2] Uta Wegewitz,[1,2] Suzan Akpulat,[1,2] Knut Mai,[1,2] Martin O. Weickert,[1,2] Matthias Möhlig,[1,2] Andreas F.H. Pfeiffer,[1,2] and Joachim Spranger[1,2]

Adiponectin affects lipid metabolism and insulin sensitivity. However, adiponectin circulates in three different oligomers that may also have distinct biological functions. We aimed to analyze the role of these oligomers in obesity and lipid metabolism after weight reduction. A total of 17 obese volunteers (15 women and 2 men) participated in a weight reduction program. Individuals were characterized before and after 6 months of a balanced diet. Adiponectin was determined by enzyme-linked immunosorbent assay, and oligomers were detected by nondenaturating Western blot. BMI decreased (35.1 ± 1.2 to 32.8 ± 1.1 kg/m^2, $P < 0.001$), which was associated with an improved metabolite profile. Total adiponectin increased from 5.3 ± 0.5 to 6.1 ± 0.6 μg/ml ($P = 0.076$). High (HMW) and medium molecular weight (MMW) adiponectin oligomers significantly increased during weight reduction (HMW: 0.37 ± 0.07 to 0.4 ± 0.08 μg/ml, $P = 0.042$; MMW: 2.3 ± 0.2 to 2.9 ± 0.3 μg/ml, $P = 0.007$), while low molecular weight (LMW) did not significantly change. Body weight inversely correlated with HMW ($r = -0.695$, $P = 0.002$) and positively with LMW ($r = 0.579$, $P = 0.015$). Interestingly, HDL cholesterol and HMW were strongly correlated ($r = 0.665$, $P = 0.007$). Indeed, HMW and free fatty acids before weight reduction predicted ~60% of HDL changes during intervention. In conclusion, weight reduction results in a relative increase of HMW/MMW adiponectin and a reduction of LMW adiponectin. Total adiponectin and especially HMW adiponectin are related to circulating HDL cholesterol. *Diabetes* 54: 2712–2719, 2005

Obesity is associated with dyslipidemia, hypertension, type 2 diabetes, and atherosclerotic cardiovascular disease (1,2). Adipose tissue was believed to be a fat-storage organ, but it is now acknowledged to be an active participant in energy homeostasis and other physiological functions. Adipose tissue is known to express and secrete a variety of novel adipocytokines that have been implicated in the development of insulin resistance and atherosclerosis (3,4). Dysregulation of adipocytokine production is directly involved in the pathophysiology of the metabolic syndrome, and normalization or elevation of plasma concentrations of some adipocytokines reverses the phenotype of the metabolic syndrome (5,6). Adipocytes secrete a variety of polypeptides, such as leptin, resistin, and adiponectin. Adiponectin, the gene product of the adipose tissue's most abundant gene transcript (7), may be a link between obesity and the development of insulin resistance. Especially in the regulation of the glucose and lipid metabolism, adiponectin has been shown to play an important role (8–10). In contrast to other adipose-derived proteins, plasma levels of adiponectin have been found to be decreased in a number of impaired metabolic states, including obesity (11), dyslipidemia (12), type 2 diabetes, or insulin resistance (9,13,14). Previous studies (14,15) have demonstrated that reduced circulating adiponectin levels are partially reversible by weight reduction in obese and in insulin-resistant subjects. Apart from the link to obesity or diabetes, further parameters like age, sex hormones, or glucocorticoids are likely to play a role in the regulation of adiponectin levels (16,17).

Adiponectin is composed of a carboxyl-terminal globular domain and an amino-terminal collagenous domain (18,19). Adiponectin belongs to the soluble collagen superfamily and has structural homology with collagen VIII and X, complement factor C1q (13), and the tumor necrosis factor family (7,18). This kind of structure is known to form characteristic multimers (20,21). Gel filtration and velocity gradient sedimentation studies revealed adiponectin circulating in serum to form several different molecular weight species; the largest species was more than several hundred kilodaltons in size (8,10,11). Scherer et al. (8) described that adiponectin from 3T3-L1 adipocytes forms trimers, hexamers, and larger multimers. Tsao et al. (22) and Arita et al. (11) analyzed multimer formation of

From the [1]Department of Clinical Nutrition, German Institute of Human Nutrition Potsdam-Rehbruecke, Nuthetal, Germany; and the [2]Department of Endocrinology, Diabetes and Nutrition, Charité-University Medical School Berlin, Campus Benjamin Franklin, Berlin, Germany.

Address correspondence and reprint requests to Joachim Spranger, MD, Department of Clinical Nutrition, German Institute of Human Nutrition Potsdam-Rehbruecke, Arthur-Scheunert-Allee 114-116, 14558 Nuthetal, Germany. E-mail: spranger@mail.dife.de.

Received for publication 21 April 2005 and accepted in revised form 23 May 2005.

FFA, free fatty acid; HMW, high molecular weight; LMW, low molecular weight; MMW, medium molecular weight; PPAR, peroxisome proliferator–activated receptor.

adiponectin in serum by gel filtration chromatography and showed adiponectin to be separable into three species. Waki et al. (23) showed a new method of evaluating the multimer formation of adiponectin. With an SDS-PAGE under nonreducing and nonheat-denaturing conditions, they separated multimers of adiponectin from various sources into three species: low molecular weight (LMW) trimers, middle molecular weight (MMW) multimers, and high molecular weight (HMW) multimers. The biological activities of these different multimers are discussed controversially. HMW multimer levels appear to be higher in women compared with men (23). Further on, adiponectin exerts multiple metabolic actions at multiple tissue sites. The isolated globular domain of adiponectin stimulates fatty acid oxidation in skeletal muscle, whereas full-length adiponectin synergizes with insulin to inhibit hepatic glucose production (13,24,25). In mice, disruption of the adiponectin locus leading to its ablation resulted in impaired fatty acid clearance, increased tumor necrosis factor-α levels, and aggravated insulin resistance in animals fed a high-fat diet (26,27). The HMW and hexameric adiponectin can activate the transcription factor nuclear factor-κB in undifferentiated or differentiated C2C12 cells, but trimeric adiponectin or the isolated globular domain of adiponectin cannot. The isolated globular domain of adiponectin, but not full-length adiponectin hexamer, enhances muscle fatty acid oxidation by inactivating acetyl-CoA carboxylase following stimulation of AMP-activated protein kinase (28,29). Waki et al. (23) have reported that the HMW isoform, specifically, promotes AMP-activated protein kinase in hepatocytes. In contrast, Tsao et al. (30) recently reported that only trimers activate AMPK in muscle, whereas hexamers and HMW forms activated nuclear factor-κB. Differences in the tissue-specific expression patterns of two adiponectin receptors may contribute to these divergent activities (31). There are first hints that weight reduction influences not only total adiponectin but also adiponectin oligomers. In six individuals, significantly increased levels of HMW adiponectin and decreased levels of the hexamer and trimer structure of adiponectin were found after 3 months of weight reduction (32).

The precise interplay between adiponectin oligomers and anthropometry, lipid, and glucose metabolism in humans has not yet been investigated. We therefore investigated within this study the relationship between anthropometry, metabolism, and adiponectin oligomers before and after moderate weight loss.

RESEARCH DESIGN AND METHODS

A total of 17 volunteers (15 women and 2 men) participated in a weight reduction program for 6 months. Mean age was 51.8 ± 3.1 years. Mean starting weight was 96.2 ± 3.1 kg and BMI was 35.1 ± 1.2 kg/m^2. All participants were screened for serious health problems and the intake of medication and were excluded if vascular diseases or hepatic or renal diseases were found. Individuals with insulin-dependent diabetes were excluded. One woman who had impaired fasting glucose was treated by diet. Two patients with type 2 diabetes (one man and one women) were treated with oral antidiabetes medication. One patient was treated with repaglinide and metformin, and the other patient was treated with sulfonylureas. No thiazolidinediones were used. Further characteristics of the participants are provided in Table 1. The experimental protocol was approved by the institutional review board, and all subjects gave written informed consent.

Dietary intervention. All volunteers documented their eating behavior for 3 days before intervention. Basal metabolic rate was measured with indirect calorimetry, which was 1,537 ± 60 kcal in average. Based on eating protocols, an individual consultation was performed, with the recommendation of a daily calorie intake of 400–600 kcal less than the total energy expenditure. The diet was composed according to the guidelines of the German Association of Nutrition, with the following distribution of macronutrients: carbohydrates 50%, fat 30%, and protein 20% of the daily energy intake. Meetings for all volunteers took place once a week over the 6 months. In the first 90 min at the first 10 dates, nutrition consultants accomplished group workshops with practical cooking exercises. At nine dates, the workshop was done by a psychologist with relaxation exercises. One workshop was done by a medical doctor with medical hints and advice. At all dates, moderate exercise with gymnastics or aqua fitness was performed during the last 60 min.

Measurement and laboratory parameters. The following measurements and laboratory parameters were determined in all participants before the dietary intervention and at the end of 6 months. Anthropometry was performed as previously described (33,34). Bioelectric impedance measurement was performed on resting participants. After attaching the electrodes to the right hand and the right food, three measurements were conducted and the mean value was calculated. Basal metabolic rate (MVmax29; Sensor Medics) was determined after a 20-min rest. During steady state, basal metabolic rate was measured for ~5–10 min.

Hyperinsulinemic-euglycemic clamp was performed for at least 2 h using 40 mIU · m^{-2} · min^{-1} human insulin (Actrapid; Novo Nordisk, Bagsvaard, Denmark) and a variable infusion of 10% glucose (Serag Wiessner, Naila, Germany). The priming dose of insulin was calculated as previously described (35). Capillary glucose concentration was monitored every 5 min and was maintained between 4.0 and 4.9 mmol/l. Blood samples were collected before the test and at least 2 h after starting the clamp during steady-state conditions. All infusions were administered into an antecubital vein, while blood samples for analysis were drawn from the antecubital vein at the contralateral arm. Blood samples were taken at baseline and during steady state of the euglycemic-hyperinsulinemic clamp. Blood potassium concentrations were controlled before and during clamp procedure to avoid insulin-induced hypokalemia. Potassium substitution was not required within this study.

TABLE 1
Baseline characteristics of the participants

	Before weight reduction	After weight reduction	*P* value
Age (years)	51.8 ± 2.8		
Sex (female/male)	15/2		
Weight (kg)	96.2 ± 3.1	90.0 ± 2.6	<0.001
BMI (kg/m^2)	35.1 ± 1.2	32.8 ± 1.1	<0.001
Waist-to-hip ratio	0.94 ± 0.02	0.92 ± 0.02	0.442
BIA BCM	31.7 ± 0.6	33.6 ± 0.9	0.010
BIA FM	39.6 ± 1.8	34.3 ± 2.0	<0.001
BIA FA	39.5 ± 0.8	37.3 ± 1.0	0.005
RQ	0.87 ± 0.01	0.86 ± 0.01	0.497
Basal metabolic rate (kcal)	1,537 ± 60	1,391 ± 43	<0.001
Adiponectin (μg/ml)	5.3 ± 0.5	6.1 ± 0.6	0.076
HMW (μg/ml)	0.37 ± 0.07	0.49 ± 0.08	0.042
MMW (μg/ml)	2.3 ± 0.2	2.9 ± 0.3	0.007
LMW (μg/ml)	2.6 ± 0.1	2.7 ± 0.2	0.609
HMW (%)	6.7 ± 0.9	7.9 ± 0.7	0.100
MMW (%)	43.1 ± 1.4	46.1 ± 1.5	0.070
LMW (%)	50.0 ± 1.8	45.8 ± 1.7	0.055
Total cholesterol (mmol/l)	5.7 ± 0.2	5.9 ± 0.2	0.305
LDL cholesterol (mmol/l)	3,7 ± 0,1	3,8 ± 0,1	0.693
HDL cholesterol (mmol/l)	1.28 ± 0.06	1.36 ± 0.06	0.029
Triglycerides (mmol/l)	1.6 ± 0.1	1.6 ± 0.2	0.699
FFAs (mmol/l)	0.74 ± 0.05	0.63 ± 0.06	0.121
M value	2.9 ± 0.3	3.2 ± 0.2	0.320
Glucose (mmol/l)	5.5 ± 0.1	5.6 ± 0.2	0.802
A1C (%)	5.4 ± 0.1	5.4 ± 0.1	1.000
C-reactive protein (mg/l)	4.5 ± 0.8	4.5 ± 0.8	0.991

Data are means ± SE. BCM, body cell mass; BIA, bioelectric impedance measurement; FA, fat allotment; FM, fat mass; RQ, respiratory quotient.

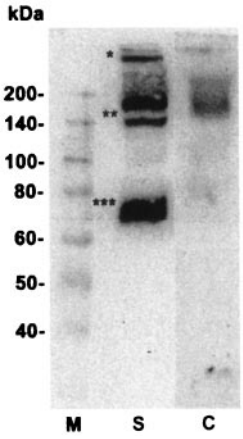

FIG. 1. Adiponectin oligomers under nonreducing and nonheat-denaturing conditions. Specificity of reaction is shown by competition (M, marker; S, sample; C, competition). Specific adiponectin oligomers were found at ~70 kDa (***), which represents the LMW trimer, two bands at ~140 and ~180 kDa (**), which represent the MMW hexamers, and the HMW complex at >300 kDa (*), as described previously (23).

Laboratory parameters. After sampling in ETDA or serum tubes, blood was immediately chilled on ice and centrifuged, and aliquots were immediately frozen at −20°C until assayed. Blood samples were analyzed for glucose, insulin, C-reactive protein, free fatty acids (FFAs), cholesterol, LDL and HDL cholesterol, and triglycerides with COBAS MIRA from Roche (Lörrach, Germany) (intra-assay coefficient of variation [CV]: glucose, 5.5%; insulin, 6.0%; C-reactive protein, 10.6%; FFAs, 10.5%; cholesterol, 5.1%; HDL cholesterol, 5.4%; and triglycerides, 5.1%). Adiponectin concentrations were measured by immunosorbent assay (ELISA; Biovendor, Nasheville, TN) (intra-assay CV, 14.7%; interassay CV, 7.3%).

Determination of adiponectin oligomers

SDS-PAGE and immunoblotting. SDS-PAGE was performed according to the standard Laemmli's method (36). Sample buffer for nonreducing, nonheat-denaturating conditions contained 3% SDS; 50 mmol/l Tris-HCl, pH 6.8; and 20% glycerol. For immunoblotting, proteins were separated by SDS-PAGE and transferred to nitrocellulose membranes. The membranes were washed with Tris-buffered saline with Tween (Tris-buffered saline, 0.1% Triton X-100) and then incubated with a polyclonal antibody (1:500; R&D Systems, Minneapolis, MN) for 1 h at room temperature. After rigorous washing with Tris-buffered saline with Tween (3 × 10 min), the membranes were incubated with horseradish peroxidase–conjugated polyclonal goat anti-rabbit immunoglobulins (1:4,000; DakoCytomation, Glostrup, Denmark) for 30 min at room temperature and then washed thoroughly. Bands were detected using a horseradish peroxidase Western blot detection system (Cell Signaling Technology, Beverly, MA). Specificity of bands was shown by a competition. Therefore, the primary antibody was preincubated overnight with excess of antigen (R&D Systems), and the protocol was otherwise unchanged. Results of competition are shown in Fig. 1.

Densitometry analysis of adiponectin oligomers was done with AIDA image analysis software. After adjusting for background activity, density of specific adiponectin oligomers bands were measured. Relative distribution of adiponectin oligomers were calculated by dividing band density through total density. Percentage of adiponectin oligomers were multiplied with total adiponectin levels to calculate absolute oligomer values.

Statistics. Statistical calculations were performed with SPSS 11.5 (SPSS, Chicago, IL).

All values are given as means ± SE. Paired *t* test was applied if parameters were normally distributed; otherwise, Wilcoxon test was used. Correlations between variables were investigated by Pearsons coefficient of correlation. Multivariate linear regression was calculated to analyze the factors independently affecting specific variants. An α-error <5% was considered statistically significant.

RESULTS

After 6 months of a low-caloric diet, body weight and BMI decreased significantly from 96.2 ± 3.1 to 90.0 ± 2.6 kg ($P < 0.001$) and from 35.1 ± 1.2 to 32.8 ± 1.1 kg/m^2 ($P < 0.001$) (Table 1), respectively. Parameters of body composition, like bioelectric impedance measurement or basal metabolic rate, changed significantly (Table 1). Total adiponectin increased from 5.3 ± 0.5 to 6.1 ± 0.6 μg/ml, which closely failed to be significant ($P = 0.076$). While relative proportion of HMW and MMW increased, the relative amount of LMW decreased correspondingly (HMW: 6.7 ± 0.9 to 7.9 ± 0.7%, $P = 0.1$; MMW: 43.1 ± 1.4 to 46.1 ± 1.5%, $P = 0.070$; and LMW: 50.0 ± 1.8 to 45.8 ± 1.7%, $P = 0.055$). Comparably, absolute levels of HMW, MMW, and LMW changed substantially (HMW: 0.37 ± 0.07 to 0.49 ± 0.08 μg/ml, $P = 0.042$; MMW: 2.3 ± 0.2 to 2.9 ± 0.3 μg/ml, $P = 0.007$; and LMW: 2.6 ± 0.1 to 2.7 ± 0.2 μg/ml, $P = 0.609$).

As expected, parameters of fat and glucose metabolism were only slightly changed by the presented moderate weight reduction here. Only HDL cholesterol increased significantly from 1.28 ± 0.06 to 1.36 ± 0.06 mmol/l ($P = 0.029$). FFAs were also altered (0.74 ± 0.05 to 0.63 ± 0.06 mmol/l, $P = 0.121$), which also closely failed significance. Total cholesterol (5.7 ± 0.2 to 5.9 ± 0.2 mmol/l, $P = 0.305$), LDL cholesterol (3.7 ± 0.1 to 3.8 ± 0.1 mmol/l, $P = 0.693$), and triglycerides (1.6 ± 0.1 to 1.6 ± 0.2 mmol/l, $P = 0.699$) remained nearly unchanged. Also, parameters for glucose metabolism were almost unaltered, like fasting glucose (5.5 ± 0.1 to 5.6 ± 0.2 mmol/l, $P = 0.802$), HbA_{1c} (A1C) (5.4 ± 0.1 to 5.4 ± 0.1%, $P = 1.0$), and *M* value (2.9 ± 0.3 to 3.2 ± 0.2, $P = 0.320$).

No significant correlation was found between total adiponectin and body weight before and after weight reduction, but there was a trend for an inverse relationship between these two parameters (Fig. 2*A*). Adiponectin oligomers in percent showed no correlation to body weight before weight reduction (Fig. 2*B*). However, after weight reduction, the negative correlation between HMW in percent and body weight was highly significant ($r = -0.695$, $P = 0.002$). In contrast, LMW in percent positively correlated with body weight ($r = 0.579$, $P = 0.015$). Although MMW in percent and body weight negatively correlated, this correlation failed to be statistically significant ($r = -0.352$, $P = 0.165$).

We also questioned whether the anthropometric changes induced by weight reduction (in percent of the basal value) correlated with the relative changes of oligomers (again in percent of basal values). Indeed, the correlations of MMW and LMW in percent with percentage changes of weight were considerable but closely failed significance (HMW: $r = -0.17$, $P = 0.951$; MMW: $r = -0.444$, $P = 0.098$; and LMW: $r = 0.454$, $P = 0.089$). The absolute levels of adiponectin oligomers showed no correlation to body weight both before or after weight reduction (Fig. 2*C*).

Next, we analyzed the relationship between adiponectin oligomers and obesity-associated metabolic changes. Surprisingly, no correlation was found between insulin sensitivity (*M* value) and total adiponectin before ($r = 0.365$, $P = 0.181$) and after ($r = 0.020$, $P = 0.944$) weight reduction. Also, no correlation between insulin sensitivity and relative amounts of adiponectin oligomers could be observed before (HMW: $r = 0.145$, $P = 0.591$; MMW: $r = -0.147$, $P = 0.587$; and LMW: $r = 0.071$, $P = 0.795$) and after (HMW: $r = 0.033$, $P = 0.904$; MMW: $r = -0.189$, $P = 0.484$; and LMW: $r = 0.128$, $P = 0.637$) weight reduction.

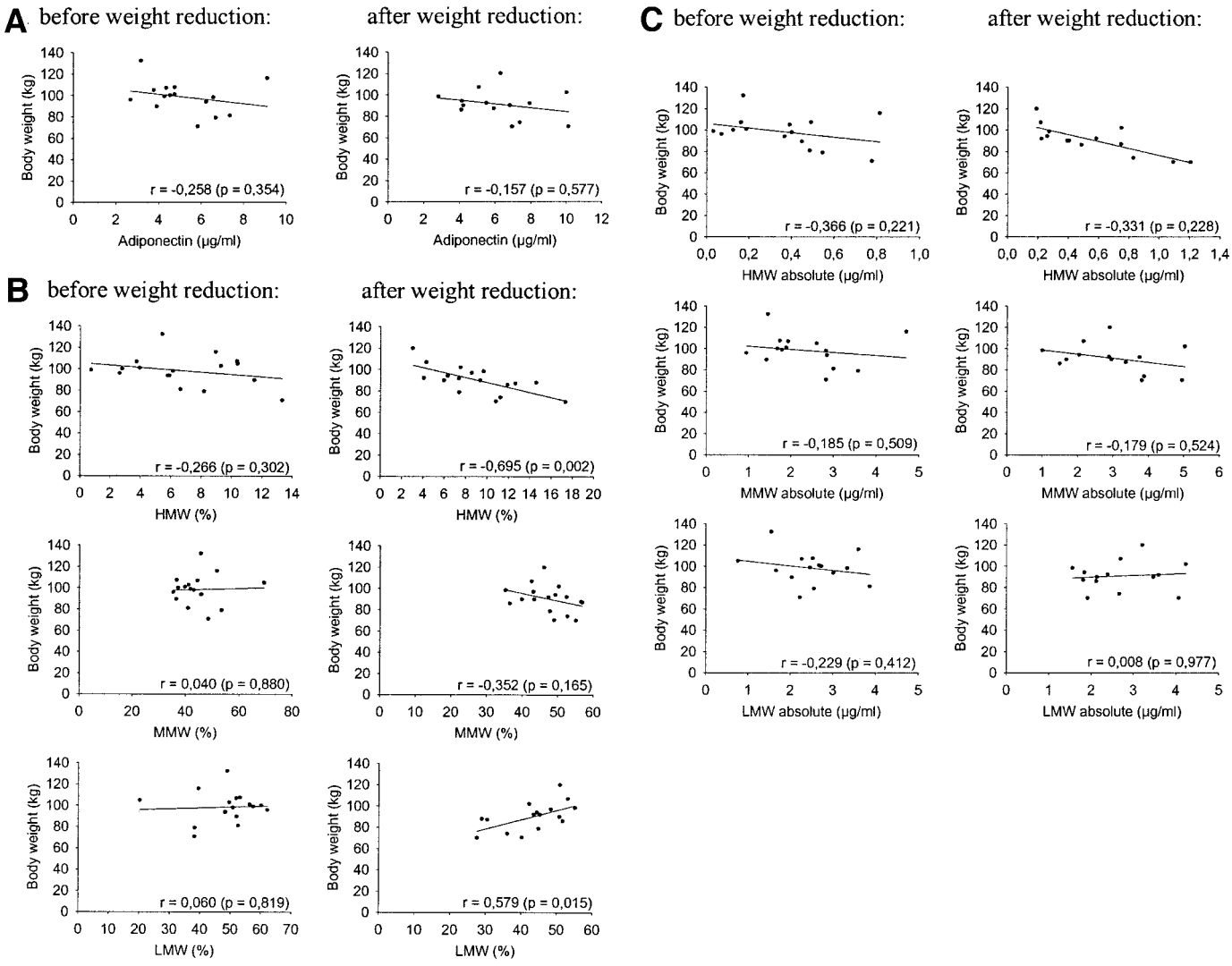

FIG. 2. Correlation between total adiponectin (*A*) and body weight, relative amount of adiponectin oligomers and body weight (*B*), and absolute concentrations of adiponectin oligomers and body weight (*C*).

Further on, no correlation between absolute adiponectin oligomers and insulin sensitivity was found before (HMW: $r = 0.252$, $P = 0.365$; MMW: $r = 0.229$, $P = 0.411$; and LMW: $r = 0.432$, $P = 0.107$) and after (HMW: $r = 0.052$, $P = 0.854$; MMW: $r = 0.013$, $P = 0.962$; and LMW: $r = 0.013$, $P = 0.963$) weight reduction.

In contrast, a positive correlation between total adiponectin and HDL cholesterol was observed before weight reduction ($r = 0.346$, $P = 0.206$), which was even stronger after weight reduction ($r = 0.61$, $P = 0.016$). Adiponectin oligomers in percent showed a correlation pattern to HDL cholesterol, which was comparable to that of body weight (Fig. 3*B*). HMW and MMW adiponectin in percent positively correlated with HDL cholesterol, whereas LMW in percent negatively correlated with HDL. All correlations failed to be statistically significant before weight reduction, while the correlations were again stronger after weight reduction, and the correlations from HDL cholesterol to HMW and LMW were especially of borderline significance ($r = 0.479$, $P = 0.052$ and $r = -0.445$, $P = 0.073$, respectively).

Absolute adiponectin oligomers positively correlated before and after weight reduction with HDL cholesterol (Fig. 3*C*). After weight reduction, the correlations between HDL cholesterol and absolute HMW ($r = 0.665$, $P = 0.007$), MMW ($r = 0.604$, $P = 0.017$), and LMW ($r = 0.536$, $P = 0.039$) adiponectin were statistically significant.

Including only women into the analyses yielded comparable results as including all individuals. If women were divided into pre-, peri-, and postmenopausal groups, trends of results were comparable to those in the whole group, although results usually failed to be significant due to the low number of individuals in the respective groups. We also found no significant differences in the mean values of adiponectin oligomers between pre-, peri-, and postmenopausal women, but again, groups were too small to investigate this question in detail. In addition, all calculations were done without those individuals with impaired glucose metabolism. Again, results did not considerably change.

In consideration of the effects of the weight reduction on the parameters of fat metabolism, we calculated a multivariate linear regression model aiming to predict changes in HDL by weight reduction. Our correlation data suggested that the effects on HDL cholesterol seemed to be affected quite strongly by HMW adiponectin rather than

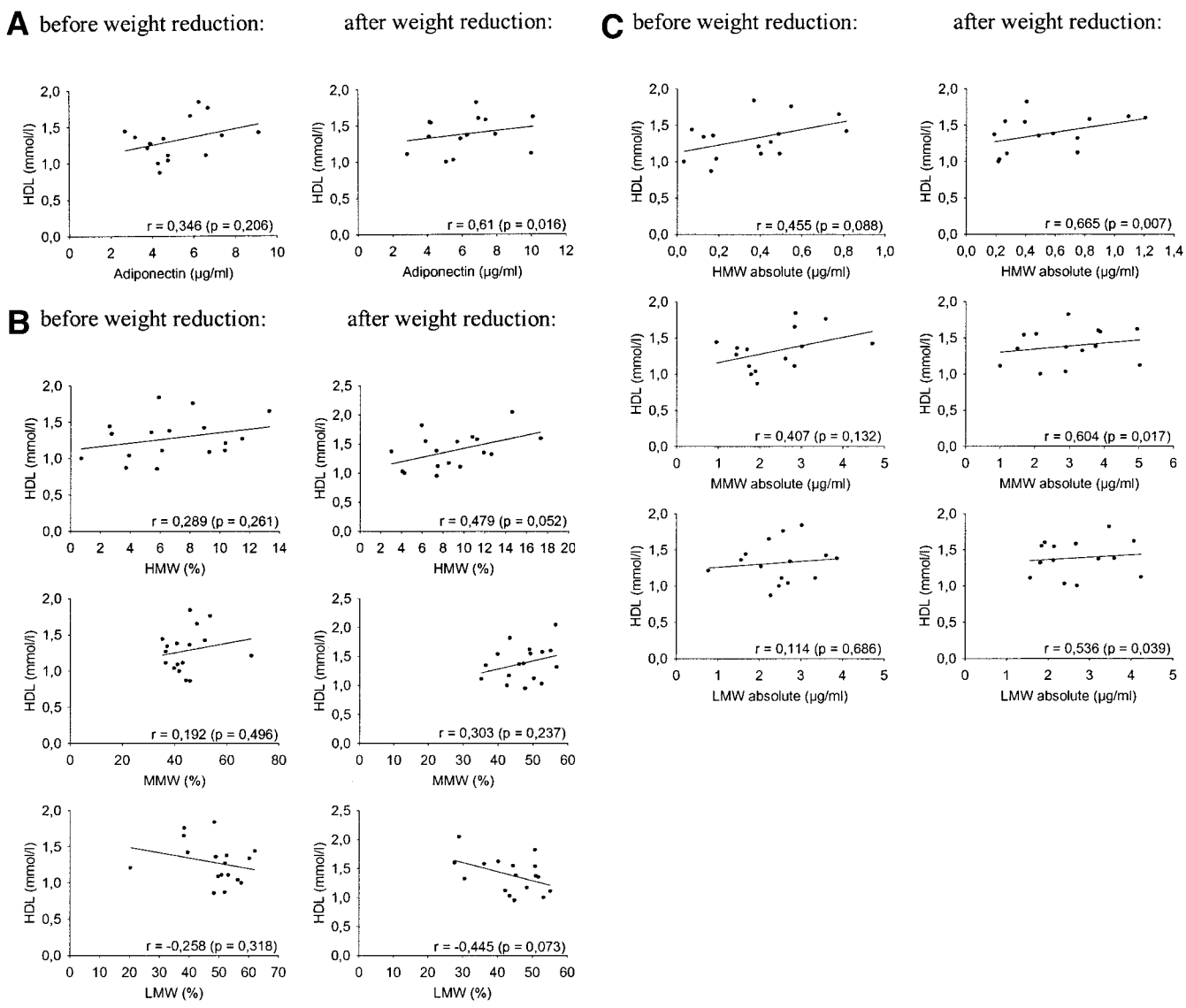

FIG. 3. Correlation between total adiponectin (*A*) and HDL, relative amount of adiponectin oligomers and HDL (*B*), and absolute concentrations of adiponectin oligomers and HDL (*C*).

TABLE 2
Linear regression was calculated after inclusion of potential covariates

Parameter	Correlation	Standardized β	Correlation × standardized β × 100	*P* value
A				
HMW absolute	−0.614	−0.548	33.64	0.012
FFA	−0.552	−0.475	26.22	0.024
Total			59.86	0.004
B				
Total adiponectin	−0.603	−0.479	28.88	0.043
FFAs	−0.313	−0.404	12.65	0.081
Total			41.53	0.014

Nonsignficant covariantes were subsequently excluded, leaving HMW absolute (A) and FFA values or total adiponectin and FFA values (B), both before weight reduction, as contributors within the most efficient model.

total adiponectin. Indeed, we found that absolute HMW oligomers and FFAs (both before weight reduction) had a considerable impact on subsequent changes of HDL cholesterol (Table 2 and Fig. 4). Absolute HMW and FFAs before weight reduction explained ~60% of the variability of subsequent changes of HDL cholesterol. The precise relationship is depicted in Fig. 4, showing that lower levels of absolute HMW and FFAs before weight reduction were associated with higher HDL changes. A multivariate linear regression was also calculated with total adiponectin instead of absolute HMW adiponectin (Table 2). With total adiponectin and FFAs, only ~40% of the variability of HDL cholesterol changes could be explained.

DISCUSSION

This study investigated the effects of moderate weight reduction by a lifestyle intervention on adiponectin oligomer composition and its relation to glucose and fat metabolism. While HMW and MMW adiponectin increased, a decreased amount of LMW adiponectin was found after

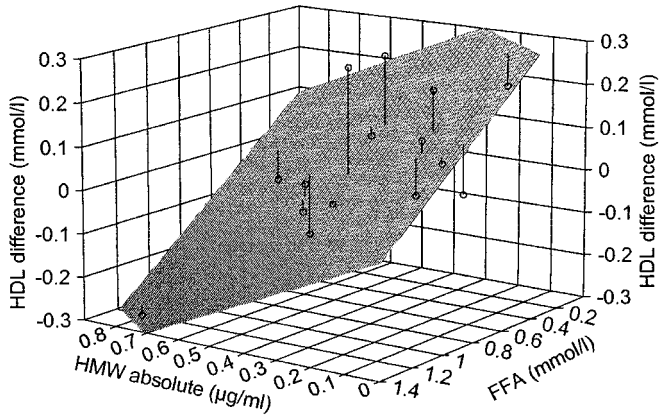

FIG. 4. Linear regression showing that HDL changes can be predicted by HMW and FFA values before weight reduction. For further characteristics of the regression analysis see Table 2. For better orientation, vertical lines show the differences from the calculated area to the real values.

weight reduction. Total adiponectin and especially HMW adiponectin strongly correlated with HDL cholesterol, while correlations with other markers of glucose or fat metabolism were weak. In a multivariate analysis, HMW adiponectin independently predicted ~30% of HDL cholesterol variation during weight reduction.

Total adiponectin levels were elevated after moderate weight reduction, although changes were small, especially compared with those found in individuals after surgical intervention and subsequent pronounced weight loss. This is in agreement with other studies, showing that moderate weight loss results in relatively small changes of circulating adiponectin levels (37). Until now, only one study with six patients and a 3-month follow-up investigated the effects of weight reduction on adiponectin oligomers. This study primarily aimed to investigate the effects of HMW adiponectin on endothelial cell apoptosis. Due to the small number of participants, the precise relationship of adiponectin oligomers to metabolic changes after weight loss was not further evaluated (32). However, in agreement with the results of this study, we found substantial changes of adiponectin oligomer composition by moderate weight loss, and a shift from LMW adiponectin to MMW and HMW adiponectin was observed. The mechanism that regulates adiponectin oligomer composition has not been identified yet. However, data from intervention studies with thiazolidinediones suggest that the process is peroxisome proliferator–activated receptor (PPAR)-γ dependent (38). As PPAR-γ is activated by negative energy balance and weight reduction (39), the effect on adiponectin oligomers seen in this study might also depend on PPAR-γ–related pathways, although this remains speculative.

Previous studies suggested a correlation between insulin sensitivity and HMW adiponectin. We were unable to confirm such a relationship in our study. Several reasons might explain this difference. First, the correlation between insulin sensitivity and HMW adiponectin was observed in patients with type 2 diabetes, while only a trend was found in healthy subjects. We primarily investigated individuals with normal glucose tolerance. Thus, the effect of HMW adiponectin on insulin sensitivity might be more prominent in individuals with existing type 2 diabetes. We found only slight nonsignificant improvement of insulin sensitivity after moderate weight loss, which is in agreement with various previous studies (40,41). While insulin sensitivity was determined by euglycemic-hyperinsulinemic clamp, we did not analyze endogenous glucose production as a measure of hepatic insulin sensitivity. Possibly, HMW adiponectin is primarily associated with hepatic rather than total insulin sensitivity. Clearly, future studies addressing this important aspect are desirable. In addition, our patients were not treated with thiazolidinediones, which might have considerable impact on the results, as the effects of weight reduction and PPAR-γ agonists certainly differ not only in terms of insulin sensitivity.

Many studies have demonstrated a correlation between total adiponectin and HDL cholesterol, and most of these studies suggested that hypoadiponectinemia is more closely related to adiposity and dyslipidemia rather than insulin sensitivity, which is in agreement with our results at oligomer levels (42). Indeed, we found a strong correlation between HMW adiponectin and HDL cholesterol, which suggests that the relationship between total adiponectin and HDL cholesterol is primarily driven by HMW adiponectin rather than total adiponectin. HDL cholesterol is basically generated from lipid-free apolipoprotein A-I or lipid-poor pre–β1-HDL as precusors. These precursors are partially produced by the liver, and it is well known that adiponectin oligomers specifically affect liver metabolism (22). However, to our knowledge, no studies investigated the effects of adiponectin oligomers on pathways involved in the generation of HDL cholesterol, such as adenosine triphosphate–binding cassette transporter A1, lecithin: cholesterol acyltransferase, or cholesteryl ester transfer protein, which appears to be mandatory given the amount of data showing a relation between HDL cholesterol and adiponectin. Especially, the consideration of adiponectin oligomers may be important, which is supported by our multivariate analyses that revealed that HMW adiponectin explained ~30% of the variability of HDL cholesterol. Actually, these results confirm those of Baratta et al. (43), who demonstrated that adiponectin is correlated with serum lipid improvement independently of insulin sensitivity changes after weight loss. We also found this correlation but extend the picture by showing that HMW adiponectin is the key for this relationship. Definitely, different adiponectin oligomers and the varying appearance of the two adiponectin receptors AdipoR1 and AdipoR2 may be responsible for the different actions of adiponectin oligomers on fat or glucose metabolism. However, the precise molecular mechanism for the oligomer-specific effect is yet unclear.

In summary, this study demonstrated changes of adiponectin oligomer composition and fat metabolism by moderate weight reduction. While HMW and MMW adiponectin increased during weight loss, a corresponding reduction of LMW adiponectin was found. Specifically, HMW adiponectin was closely related to HDL cholesterol. Multivariate linear regression demonstrated that HMW and FFAs predicted ~60% of HDL cholesterol changes during weight loss. Our data suggest that adiponectin is associated with dyslipidemia rather than insulin sensitivity. Beneficial effects of moderate weight loss may be medi-

ated by changes of the adiponectin oligomer composition. In more general terms, we show that adiponectin oligomers might have specific biological functions in vivo in humans, especially with respect to lipid metabolism.

ACKNOWLEDGMENTS

J.S. was supported by the Bundesministerium für Bildung und Forschung (0313036B), the Eli Lilly International Foundation (ELIF21001), and the German Diabetes Association (DDG021006).

We thank Petra Exner and Katrin Sprengel for excellent technical assistance.

REFERENCES

1. Linton MF, Fazio S: A practical approach to risk assessment to prevent coronary artery disease and its complications. *Am J Cardiol* 92:19i–26i, 2003
2. Scott CL: Diagnosis, prevention, and intervention for the metabolic syndrome. *Am J Cardiol* 92:35i–42i, 2003
3. Matsuzawa Y, Funahashi T, Nakamura T: Molecular mechanism of metabolic syndrome X: contribution of adipocytokines adipocyte-derived bioactive substances. *Ann N Y Acad Sci* 892:146–154, 1999
4. Funahashi T, Nakamura T, Shimomura I, Maeda K, Kuriyama H, Takahashi M, Arita Y, Kihara S, Matsuzawa Y: Role of adipocytokines on the pathogenesis of atherosclerosis in visceral obesity. *Intern Med* 38:202–206, 1999
5. Hotamisligil GS, Shargill NS, Spiegelman BM: Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science* 259:87–91, 1993
6. Shimomura I, Hammer RE, Ikemoto S, Brown MS, Goldstein JL: Leptin reverses insulin resistance and diabetes mellitus in mice with congenital lipodystrophy. *Nature* 401:73–76, 1999
7. Maeda K, Okubo K, Shimomura I, Funahashi T, Matsuzawa Y, Matsubara K: cDNA cloning and expression of a novel adipose specific collagen-like factor, apM1 (AdiPose Most abundant Gene transcript 1). *Biochem Biophys Res Commun* 221:286–289, 1996
8. Scherer PE, Williams S, Fogliano M, Baldini G, Lodish HF: A novel serum protein similar to C1q, produced exclusively in adipocytes. *J Biol Chem* 270:26746–26749, 1995
9. Hu E, Liang P, Spiegelman BM: AdipoQ is a novel adipose-specific gene dysregulated in obesity. *J Biol Chem* 271:10697–10703, 1996
10. Nakano Y, Tobe T, Choi-Miura NH, Mazda T, Tomita M: Isolation and characterization of GBP28, a novel gelatin-binding protein purified from human plasma. *J Biochem (Tokyo)* 120:803–812, 1996
11. Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, Hotta K, Shimomura I, Nakamura T, Miyaoka K, Kuriyama H, Nishida M, Yamashita S, Okubo K, Matsubara K, Muraguchi M, Ohmoto Y, Funahashi T, Matsuzawa Y: Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochem Biophys Res Commun* 257:79–83, 1999
12. Matsubara M, Maruoka S, Katayose S: Decreased plasma adiponectin concentrations in women with dyslipidemia. *J Clin Endocrinol Metab* 87:2764–2769, 2002
13. Yamauchi T, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K, Mori Y, Ide T, Murakami K, Tsuboyama-Kasaoka N, Ezaki O, Akanuma Y, Gavrilova O, Vinson C, Reitman ML, Kagechika H, Shudo K, Yoda M, Nakano Y, Tobe K, Nagai R, Kimura S, Tomita M, Froguel P, Kadowaki T: The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nat Med* 7:941–946, 2001
14. Hotta K, Funahashi T, Arita Y, Takahashi M, Matsuda M, Okamoto Y, Iwahashi H, Kuriyama H, Ouchi N, Maeda K, Nishida M, Kihara S, Sakai N, Nakajima T, Hasegawa K, Muraguchi M, Ohmoto Y, Nakamura T, Yamashita S, Hanafusa T, Matsuzawa Y: Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. *Arterioscler Thromb Vasc Biol* 20:1595–1599, 2000
15. Yang WS, Lee WJ, Funahashi T, Tanaka S, Matsuzawa Y, Chao CL, Chen CL, Tai TY, Chuang LM: Weight reduction increases plasma levels of an adipose-derived anti-inflammatory protein, adiponectin. *J Clin Endocrinol Metab* 86:3815–3819, 2001
16. Reinehr T, Roth C, Menke T, Andler W: Adiponectin before and after weight loss in obese children. *J Clin Endocrinol Metab* 89:3790–3794, 2004
17. Diez JJ, Iglesias P: The role of the novel adipocyte-derived hormone adiponectin in human disease. *Eur J Endocrinol* 148:293–300, 2003
18. Shapiro L, Scherer PE: The crystal structure of a complement-1q family protein suggests an evolutionary link to tumor necrosis factor. *Curr Biol* 8:335–338, 1998
19. Yokota T, Oritani K, Takahashi I, Ishikawa J, Matsuyama A, Ouchi N, Kihara S, Funahashi T, Tenner AJ, Tomiyama Y, Matsuzawa Y: Adiponectin, a new member of the family of soluble defense collagens, negatively regulates the growth of myelomonocytic progenitors and the functions of macrophages. *Blood* 96:1723–1732, 2000
20. Crouch E, Persson A, Chang D, Heuser J: Molecular structure of pulmonary surfactant protein D (SP-D). *J Biol Chem* 269:17311–17319, 1994
21. McCormack FX, Pattanajitvilai S, Stewart J, Possmayer F, Inchley K, Voelker DR: The Cys6 intermolecular disulfide bond and the collagen-like region of rat SP-A play critical roles in interactions with alveolar type II cells and surfactant lipids. *J Biol Chem* 272:27971–27979, 1997
22. Tsao TS, Murrey HE, Hug C, Lee DH, Lodish HF: Oligomerization state-dependent activation of NF-kappa B signaling pathway by adipocyte complement-related protein of 30 kDa (Acrp30). *J Biol Chem* 277:29359–29362, 2002
23. Waki H, Yamauchi T, Kamon J, Ito Y, Uchida S, Kita S, Hara K, Hada Y, Vasseur F, Froguel P, Kimura S, Nagai R, Kadowaki T: Impaired multimerization of human adiponectin mutants associated with diabetes: molecular structure and multimer formation of adiponectin. *J Biol Chem* 278:40352–40363, 2003
24. Berg AH, Combs TP, Du X, Brownlee M, Scherer PE: The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. *Nat Med* 7:947–953, 2001
25. Fruebis J, Tsao TS, Javorschi S, Ebbets-Reed D, Erickson MR, Yen FT, Bihain BE, Lodish HF: Proteolytic cleavage product of 30-kDa adipocyte complement-related protein increases fatty acid oxidation in muscle and causes weight loss in mice. *Proc Natl Acad Sci U S A* 98:2005–2010, 2001
26. Maeda N, Shimomura I, Kishida K, Nishizawa H, Matsuda M, Nagaretani H, Furuyama N, Kondo H, Takahashi M, Arita Y, Komuro R, Ouchi N, Kihara S, Tochino Y, Okutomi K, Horie M, Takeda S, Aoyama T, Funahashi T, Matsuzawa Y: Diet-induced insulin resistance in mice lacking adiponectin/ACRP30. *Nat Med* 8:731–737, 2002
27. Kubota N, Terauchi Y, Yamauchi T, Kubota T, Moroi M, Matsui J, Eto K, Yamashita T, Kamon J, Satoh H, Yano W, Froguel P, Nagai R, Kimura S, Kadowaki T, Noda T: Disruption of adiponectin causes insulin resistance and neointimal formation. *J Biol Chem* 277:25863–25866, 2002
28. Tomas E, Tsao TS, Saha AK, Murrey HE, Zhang CC, Itani SI, Lodish HF, Ruderman NB: Enhanced muscle fat oxidation and glucose transport by ACRP30 globular domain: acetyl-CoA carboxylase inhibition and AMP-activated protein kinase activation. *Proc Natl Acad Sci U S A* 99:16309–16313, 2002
29. Yamauchi T, Kamon J, Minokoshi Y, Ito Y, Waki H, Uchida S, Yamashita S, Noda M, Kita S, Ueki K, Eto K, Akanuma Y, Froguel P, Foufelle F, Ferre P, Carling D, Kimura S, Nagai R, Kahn BB, Kadowaki T: Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nat Med* 8:1288–1295, 2002
30. Tsao TS, Tomas E, Murrey HE, Hug C, Lee DH, Ruderman NB, Heuser JE, Lodish HF: Role of disulfide bonds in Acrp30/adiponectin structure and signaling specificity: different oligomers activate different signal transduction pathways. *J Biol Chem* 278:50810–50817, 2003
31. Yamauchi T, Kamon J, Ito Y, Tsuchida A, Yokomizo T, Kita S, Sugiyama T, Miyagishi M, Hara K, Tsunoda M, Murakami K, Ohteki T, Uchida S, Takekawa S, Waki H, Tsuno NH, Shibata Y, Terauchi Y, Froguel P, Tobe K, Koyasu S, Taira K, Kitamura T, Shimizu T, Nagai R, Kadowaki T: Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. *Nature* 423:762–769, 2003
32. Kobayashi H, Ouchi N, Kihara S, Walsh K, Kumada M, Abe Y, Funahashi T, Matsuzawa Y: Selective suppression of endothelial cell apoptosis by the high molecular weight form of adiponectin. *Circ Res* 94:e27–e31, 2004
33. Spranger J, Kroke A, Mohlig M, Bergmann MM, Ristow M, Boeing H, Pfeiffer AF: Adiponectin and protection against type 2 diabetes mellitus. *Lancet* 361:226–228, 2003
34. Spranger J, Kroke A, Mohlig M, Hoffmann K, Bergmann MM, Ristow M, Boeing H, Pfeiffer AF: Inflammatory cytokines and the risk to develop type 2 diabetes: results of the prospective population-based European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam Study. *Diabetes* 52:812–817, 2003
35. DeFronzo RA, Tobin JD, Andres R: Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol* 237:E214–E223, 1979
36. Laemmli UK: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227:680–685, 1970
37. Abbasi F, Lamendola C, McLaughlin T, Hayden J, Reaven GM, Reaven PD:

Plasma adiponectin concentrations do not increase in association with moderate weight loss in insulin-resistant, obese women. *Metabolism* 53:280–283, 2004
38. Pajvani UB, Hawkins M, Combs TP, Rajala MW, Doebber T, Berger JP, Wagner JA, Wu M, Knopps A, Xiang AH, Utzschneider KM, Kahn SE, Olefsky JM, Buchanan TA, Scherer PE: Complex distribution, not absolute amount of adiponectin, correlates with thiazolidinedione-mediated improvement in insulin sensitivity. *J Biol Chem* 279:12152–12162, 2004
39. Verreth W, De KD, Pelat M, Verhamme P, Ganame J, Bielicki JK, Mertens A, Quarck R, Benhabiles N, Marguerie G, Mackness B, Mackness M, Ninio E, Herregods MC, Balligand JL, Holvoet P: Weight loss-associated induction of peroxisome proliferator-activated receptor-alpha and peroxisome proliferator-activated receptor-gamma correlate with reduced atherosclerosis and improved cardiovascular function in obese insulin-resistant mice. *Circulation* 110:3259–3269, 2004
40. Valsamakis G, McTernan PG, Chetty R, Al DN, Field A, Hanif W, Barnett AH, Kumar S: Modest weight loss and reduction in waist circumference after medical treatment are associated with favorable changes in serum adipocytokines. *Metabolism* 53:430–434, 2004
41. Reinehr T, Kiess W, Kapellen T, Andler W: Insulin sensitivity among obese children and adolescents, according to degree of weight loss. *Pediatrics* 114:1569–1573, 2004
42. Kazumi T, Kawaguchi A, Hirano T, Yoshino G: Serum adiponectin is associated with high-density lipoprotein cholesterol, triglycerides, and low-density lipoprotein particle size in young healthy men. *Metabolism* 53:589–593, 2004
43. Baratta R, Amato S, Degano C, Farina MG, Patane G, Vigneri R, Frittitta L: Adiponectin relationship with lipid metabolism is independent of body fat mass: evidence from both cross-sectional and intervention studies. *J Clin Endocrinol Metab* 89:2665–2671, 2004