

Adiponectin Multimeric Complexes and the Metabolic Syndrome Trait Cluster

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Adiponectin circulates in human plasma mainly as a 180-kDa low molecular weight (LMW) hexamer and a high molecular weight (HMW) multimer of ~360 kDa. We comprehensively examined the relationships between circulating levels of total adiponectin, adiponectin multimers, and the relative distribution (i.e., ratio) of multimeric forms with key features of the metabolic syndrome. Total adiponectin ($r = 0.45$), HMW ($r = 0.47$), LMW ($r = 0.31$), and HMW-to-total adiponectin ratio ($r = 0.29$) were significantly correlated with insulin-stimulated glucose disposal rate. Similarly, total ($r = -0.30$), HMW ($r = -0.38$), and HMW-to-total adiponectin ratio ($r = -0.34$) were correlated with central fat distribution but not with total fat mass or BMI. Regarding energy metabolism, although there were no effects on resting metabolic rate, total ($r = 0.41$) and HMW ($r = 0.44$) were associated with increasing rates of fat oxidation. HMW-to-total adiponectin ratio increased as a function of total adiponectin, and it was HMW quantity (not total or HMW-to-total adiponectin ratio or LMW) that was primarily responsible for all of these relationships. Impact on nuclear magnetic resonance lipoprotein subclasses was assessed. HMW and total adiponectin were correlated with decreases in large VLDL ($r = -0.44$ and -0.41); decreases in small LDL ($r = -0.41$ and -0.36) and increases in large LDL ($r = 0.36$ and 0.30) particle concentrations accompanied by increased LDL particle size ($r = 0.47$ and 0.39); and increases in large HDL ($r = 0.45$ and 0.37) and HDL particle size ($r = 0.53$ and 0.47). Most of these correlations persisted after adjustment for metabolic covariables. In conclusion, first, serum adiponectin is associated with increased insulin sensitivity, reduced abdominal fat, and high basal lipid oxidation; however, it is HMW quantity, not total or HMW-to-total adiponectin ratio, that is primarily responsible for these relationships. Second, reduced quantities of HMW independently recapitulate the lipoprotein subclass profile associated with insulin resistance after correcting for glucose disposal rate and BMI. Finally, HMW adiponectin is an important factor in explaining the metabolic syndrome. *Diabetes* 55:249–259, 2006

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AMPK, AMP-activated protein kinase; DEXA, dual-energy X-ray absorptiometry; HMW, high molecular weight; LMW, low molecular weight; NMR, nuclear magnetic resonance; RIA, radioimmunoassay; TTBS, Tris-buffered saline with 0.05% Tween 20.

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Low circulating levels of adiponectin have been linked to several components of the metabolic syndrome, such as insulin resistance, upper body fat distribution, and dyslipidemia, including low HDL levels (1). The molecular mechanisms establishing a causal role for adiponectin in the epidemiological associations are less clear. Two receptors have been identified, AdipoR1 and AdipoR2 (adiponectin receptor 1 and 2) (2), which are expressed in muscle and liver (2) and in human fat cells (3). In muscle cells, ligand binding may initiate signal transduction through phosphorylation and activation of AMP-activated protein kinase (AMPK) (4), which is known to play a pivotal role in regulating cholesterol synthesis, lipogenesis and lipid oxidation, and glucose transport and oxidation. In fact, adiponectin has been shown to enhance insulin sensitivity in muscle and liver, stimulate fatty acid oxidation in muscle, and exert various anti-inflammatory and antiatherogenic effects (5–7). Thus, accumulating evidence supports the hypothesis that adiponectin is a key metabolic regulator, and it could confer increased risk for metabolic and cardiovascular diseases when circulating levels are diminished.

Adiponectin is a 30-kDa protein that contains three principal domains: an NH₂-terminal sequence, a variable region, and a collagenous domain (8). Adiponectin does not circulate as a monomer, but rather associates into multimeric higher-order structures via disulphide bonds linking the collagenous domains. In human plasma (9) and in cultured adipocyte media (3), adiponectin exists largely as low molecular weight (LMW) hexamers of ~190 kDa and high molecular weight (HMW) multimers >300 kDa. Most studies of adiponectin bioeffects have used either the full-length protein, prepared using bacterial expression systems, or the "globular head," produced by proteolytic cleavage. Therefore, it is uncertain whether these effects are physiologically relevant because neither the bacterially produced whole-length protein nor the globular head aggregate into higher-molecular weight forms (10).

There are very little data assessing the metabolic significance of adiponectin multimeric forms. There was one recent article showing that the ratio of HMW to total adiponectin, not the total adiponectin level per se, was responsible for favorable metabolic effects (11). The authors found that the ratio of HMW to total adiponectin, and not total adiponectin, was correlated with insulin sensitivity in humans and rodents, and it was increased by thiazolidinedione drugs in proportion to increments in hepatic insulin sensitivity manifested by a decrease in fasting hepatic glucose output (11,12). In addition, administration of HMW but not LMW adiponectin lowered

TABLE 1
Demographic, anthropometric, and metabolic characteristics of study groups

Variable	Insulin-sensitive normoglycemic			Insulin-resistant normoglycemic			Type 2 diabetic		
	All	Women	Men	All	Women	Men	All	Women	Men
<i>n</i>	25	11	14	22	11	11	21	11	10
Age (years)	31 ± 9	37 ± 11*	27 ± 5	32 ± 8	33 ± 9	33 ± 7	45 ± 10†	45 ± 7	49 ± 6
BMI (kg/m ²)	26 ± 5	29 ± 5*	24 ± 3	33 ± 7‡	34 ± 6	32 ± 8	30 ± 6	32 ± 8	28 ± 4
Fat mass (kg)	23 ± 14	31 ± 14*	13 ± 6	39 ± 17‡	41 ± 13*	29 ± 15	30 ± 17	31 ± 14	24 ± 9
Waist (cm)	85 ± 9§	88 ± 11	83 ± 6	104 ± 16	100 ± 11	107 ± 20	102 ± 15‡	99 ± 13	105 ± 16
WHR	0.82 ± 0.04	0.79 ± 0.05*	0.84 ± 0.02	0.88 ± 0.06‡	0.86 ± 0.04*	0.94 ± 0.09	0.93 ± 0.08†	0.88 ± 0.06*	0.98 ± 0.07
Glucose (mmol/l)	4.8 ± 0.29	4.7 ± 0.30	4.8 ± 0.29	5.5 ± 1.0	5.5 ± 1.2	5.5 ± 0.7	14.0 ± 3.9	14.3 ± 3.6	13.8 ± 4.2
Insulin (μU/ml)	5.4 ± 3.7	6.5 ± 4.8	4.6 ± 2.5	17.1 ± 11.8	21.6 ± 12.6	10.3 ± 6.9	10.0 ± 11.1	14.9 ± 17.2	7.1 ± 4.1
GDR (mg · kg ⁻¹ · min ⁻¹)	16.6 ± 2.6	17.8 ± 3*	15.6 ± 1.6	9.2 ± 2.4‡	9.4 ± 2.7	8.0 ± 2.9	7.3 ± 3.2‡	8.3 ± 3.7	6.6 ± 2.6
Adiponectin RIA (μg/ml)	8.3 ± 4.3	10.1 ± 4.6	7.0 ± 3.7	6.8 ± 2.6	7.2 ± 2.3	5.4 ± 3.1	5.5 ± 2.9‡	6.3 ± 3.2	4.8 ± 2.3
HMW adiponectin (μg/ml)	5.4 ± 2.9	6.4 ± 3.0	4.5 ± 2.7	4.1 ± 1.6	4.7 ± 1.4	3.2 ± 1.7	3.2 ± 2.0‡	3.8 ± 2.4	2.7 ± 1.6
LMW adiponectin (μg/ml)	2.9 ± 1.6	3.6 ± 1.7	2.4 ± 1.3	2.7 ± 1.2	2.9 ± 0.9	2.5 ± 1.6	2.2 ± 1.2	2.1 ± 1.4	2.3 ± 1.1
HMW-to-total adiponectin ratio	0.62 ± 0.08	0.63 ± 0.05	0.62 ± 0.1	0.58 ± 0.07	0.61 ± 0.07	0.55 ± 0.08	0.58 ± 0.10	0.63 ± 0.07*	0.54 ± 0.1

Data are the means ± SE. *P* < 0.05 was considered statistically significant. *Significantly different from the subgroup; †significantly different from insulin sensitive and resistant; ‡significantly different from insulin sensitive; §significantly different from insulin resistant. GDR, maximal insulin-stimulated glucose disposal rate; WHR, waist-to-hip ratio.

glucose in mice. In contrast, other studies have shown that LMW (hexamer) and HMW adiponectin are equally effective in activating nuclear factor-κB (13) and that both the adiponectin monomer and trimer are capable of stimulating AMPK, whereas LMW and HMW adiponectin produce no effect (4). Thus, the manner and extent to which adiponectin multimeric forms may exert differential biological activity remains unclear. Nevertheless, these data have important and far-ranging implications regarding previous data because epidemiological studies have assessed total immunoreactive adiponectin, not multimeric forms.

The aim of this study was to comprehensively examine relationships between circulating levels of total adiponectin, adiponectin multimers, and the relative distribution (i.e., ratio) of multimeric forms with key features of the metabolic syndrome, including insulin resistance, body fat distribution, and lipoprotein subclasses in subjects with and without type 2 diabetes across a wide range of insulin sensitivity, as defined by the hyperinsulinemic-euglycemic clamp. Because adiponectin has been shown to modulate fat oxidation in animal models, we also determined the association of adiponectin and its multimeric forms with rates of whole-body substrate oxidation by indirect calorimetry. The data strongly support the contention that adiponectin could play a pathophysiological role in the development of the metabolic syndrome trait cluster.

RESEARCH DESIGN AND METHODS

We studied 68 (33 women and 35 men) subjects with and without type 2 diabetes (14), and the clinical characteristics of the study group are listed in Table 1. Before the study, all patients with type 2 diabetes (*n* = 21) were being treated with diet or sulfonylurea and/or metformin oral hypoglycemic agents, but they were withdrawn from therapy for at least 3 weeks and followed on an outpatient basis. All subjects were allowed to equilibrate on a weight maintenance diet (28–32 kcal · kg⁻¹ · day⁻¹) consisting of 50% carbohydrate, 30% fat, and 20% protein. Nondiabetic and untreated subjects with type 2 diabetes were then admitted to the General Clinical Research Center for three nights. During this period, they remained active, and the isocaloric diet was maintained throughout. Weight had to be stable (±3%) for at least 3 months before the study, and none of the study subjects engaged in regular exercise. None of the volunteers had cardiovascular, renal, or hepatic disease, and all were chemically euthyroid. No subjects were ingesting any pharmacological agents known to affect carbohydrate homeostasis, lipids, or lipoprotein metabolism. Protocols were approved by the institutional review board, and written informed consent was obtained from every subject.

Standard 75-g oral glucose tolerance tests were performed after a 12-h overnight fast (14). According to this test, 35 subjects were classified as normal, 1 was found to have impaired fasting glucose, 6 were found to have impaired glucose tolerance, and 16 had type 2 diabetes. The nondiabetic subjects were categorized as insulin sensitive or insulin resistant based on the maximally insulin-stimulated glucose uptake rates > or ≤12.8 mg · kg⁻¹ · min⁻¹, respectively, during hyperinsulinemic-euglycemic clamp studies. This cutoff placed 46% of the randomly recruited nondiabetic patients in the insulin-resistant category (*n* = 22), and it was selected because logistic analyses indicated that values <12.8 mg · kg⁻¹ · min⁻¹ were highly associated with the trait cluster of the insulin resistance syndrome (15). Percentage of body fat, regional percentage of body fat, and lean body mass were measured by dual-energy X-ray absorptiometry (DEXA; Lunar Radiation, Madison, WI), as previously described (16).

Insulin sensitivity. In vivo insulin sensitivity was assessed, using the euglycemic-hyperinsulinemic glucose clamp technique as previously described (17,18). Glucose uptake was normalized per kilogram of lean body mass (excluding bone mass) determined by DEXA to yield the glucose disposal rate per kilogram of lean body mass.

Separation of adiponectin and Western blot analysis. Human serum was diluted 10-fold with deionized water. We separated 2 μl of this diluted human serum sample plus 2 μl of protein loading buffer under nonreducing conditions by NuPAGE 3–8% Tris-acetate gel (Invitrogen) electrophoresis. Proteins were electrophoretically transferred into an Invitrogen polyvinylidene fluoride membrane and incubated overnight at 4°C with blocking solution (5%

nonfat milk in Tris-buffered saline with 0.05% Tween 20 [TTBS]). The blocked membrane was incubated with a human adiponectin monoclonal antibody from BD Bioscience (1:10,000 dilution with 1% nonfat milk in TTBS) for 1 h at room temperature and then washed five times with TTBS buffer for 10 min each time at room temperature with constant shaking. Then, the membrane was incubated with a horseradish peroxidase-conjugated second antibody (1:3,000 dilution; Santa Cruz Biotechnology) for 1 h at room temperature and washed five times with TTBS buffer for 10 min each time at room temperature with constantly shaking. Immunodetection analysis was accomplished using a Western blot detection system (LumiGLO reagent and peroxide; Cell Signaling Technology). Images of the developed film were scanned using an Epson Silverfast scanner. Densitometries of the high and low molecular bands were measured, using Image software (Scion, Frederick, MD), and were confirmed to be linearly related to the amount of target protein through the use of standards run on each gel. In human sera, we observed that adiponectin circulated in two principal multimeric forms, corresponding to a LMW band at ~180 kDa, which has been shown to represent adiponectin hexamer, and an HMW band at ~360 kDa (9). We calculated the proportions of HMW and LMW protein as a function of total adiponectin protein by dividing the band densitometry of either multimer by total density in each lane. In each subject, the absolute adiponectin concentration was quantified in the same serum sample by radioimmunoassay (RIA; Linco Research). The relative proportion of HMW and LMW adiponectin determined from the immunoblots was multiplied by the total adiponectin concentration assessed by RIA to determine the amount of adiponectin that resides in HMW and LMW multimers. This analysis assumes that adiponectin monomers display an equal degree of immunoreactivity either as constituents of HMW or LMW multimers on the immunoblots.

Nuclear magnetic resonance lipoprotein subclass profile. Fasting blood for the nuclear magnetic resonance (NMR) lipoprotein subclass profile was obtained from the same sample as the conventional lipid panel. Venipuncture did not involve intravenous fluids or heparin administration. Serum was isolated by centrifugation (3,000 rpm, 20 min, 4°C) promptly after blood clotting and stored at -80°C until assay. The NMR lipoprotein subclass profile was determined using a 400-MHz proton NMR analyzer at LipoScience (Raleigh, NC). The technique for NMR lipoprotein determination has previously been described in detail (18–20).

Indirect calorimetry. After an overnight fast, and while at the General Clinical Research Center, resting energy expenditure was measured by indirect calorimetry using a Deltratrac metabolic monitor (Deltratrac II; SensorMedics, Yorba Linda, CA). Measurements began after 30 min of rest, while supine in bed. The instrument was calibrated by ethanol combustion tests on a monthly basis and against standard gases before each test. Expired air was collected using the adult-size ventilated canopy system for 20 min after a 10-min equilibration. Whole-body oxygen consumption (V_{O_2}) and CO_2 production (V_{CO_2}) were calculated by measuring gradients across the face and the flow rates of air using the Haldane transformation. In our lab, the coefficient of variation between resting energy expenditure measured in a walk-in room calorimeter and the Deltratrac metabolic monitor is 7.3%. Energy expenditure and rates of lipid and carbohydrate oxidation were determined from the respiratory quotient value and the tables reported by Lusk (21). Metabolic rates and rates of lipid and carbohydrate oxidation were normalized per kilogram of metabolically active body mass according to the method of Ravussin et al. (22).

Other assays. Plasma glucose was measured by the glucose oxidase method using a glucose analyzer (YSI 2300; Yellow Springs Instruments, Yellow Springs, OH). Serum insulin levels were measured using an electrochemiluminescence immunoassay (Roche Diagnostics, Mannheim, Germany).

Statistical analyses. All data are the means \pm SD, unless otherwise indicated. Differences in outcomes of interest between the sexes were compared using Student's *t* test. Comparisons of more than two variables were calculated with ANOVA followed by post hoc Tukey test, if appropriate. The correlations between total adiponectin, HMW, LMW, and the ratio of HMW to total adiponectin and different body fat, glucose disposal rate, and lipid measurements (as continuous variables) were examined using Spearman correlation coefficients. In some instances, we performed partial correlation analyses considering the effects of age, sex, BMI, waist, waist-to-hip ratio, total fat mass, percentage fat mass, or trunk/leg fat by DEXA. Student's *t* tests for differences between correlation coefficients comparing a metabolic parameter with both LMW and HMW were also calculated where indicated. Subjects were also classified into three distinct groups, comprised of insulin-sensitive and insulin-resistant normoglycemic individuals and patients with type 2 diabetes; separate simple and partial correlation analyses were performed in these subgroups. The SAS program (version 9.0; SAS Institute, Cary, NC) was used for analyses. Differences were accepted as significant at $P < 0.05$.

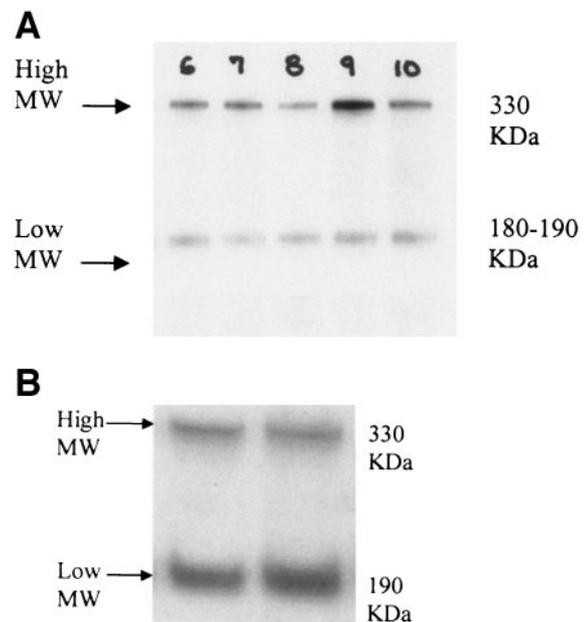


FIG. 1. Representative immunoblot illustrating the two main adiponectin complexes, HMW and LMW bands, from human serum in five different patients (A). These same bands were also resolved on immunoblots of culture media from human adipose cells (B).

RESULTS

Study subgroups. Table 1 shows anthropometric and metabolic characteristics of the insulin-sensitive, insulin-resistant, and type 2 diabetic subgroups stratified by sex. Mean values in the insulin-resistant and type 2 diabetic subgroups were similar for these characteristics, including the degree of insulin resistance as measured by the insulin-stimulated glucose disposal rate; however, the diabetic subjects were older and were hyperglycemic relative to insulin resistant subjects. Both type 2 diabetic and insulin-resistant subgroups exhibited higher waist-to-hip ratio, BMI, fat mass, and fasting insulin level, together with decreased glucose disposal rates, compared with insulin sensitive subjects.

Adiponectin and adiponectin multimers. Fig. 1 depicts an immunoblot of adiponectin multimers in sera from five subjects (Fig. 1A). The dominant forms detected in human sera were represented by an LMW 180-kDa protein corresponding to an adiponectin hexamer and an HMW form migrating at 330–360 kDa. Figure 1B demonstrates that these same dominant forms are released into media from cultured differentiated human adipocytes. Figure 2 demonstrates that total serum adiponectin levels by RIA were highest in insulin-sensitive subjects, tended to decrease in insulin-resistant subjects (24% decline, $P = NS$), and were further diminished in type 2 diabetic subjects to the point where the concentration was significantly less than that observed in insulin-sensitive subjects (41% decline, $P < 0.05$). When adiponectin multimeric forms were examined, levels of HMW adiponectin followed this same pattern; however, in contrast, LMW adiponectin levels did not differ among these subgroups. Thus, the progressive decline in adiponectin in insulin-resistant and type 2 diabetic compared with insulin-sensitive subjects was entirely attributable to decrements in HMW adiponectin, only with no change in LMW adiponectin. The ratio of HMW to total adiponectin also did not differ significantly among insulin-sensitive (0.62), insulin-resistant (0.58), and type 2 diabetic

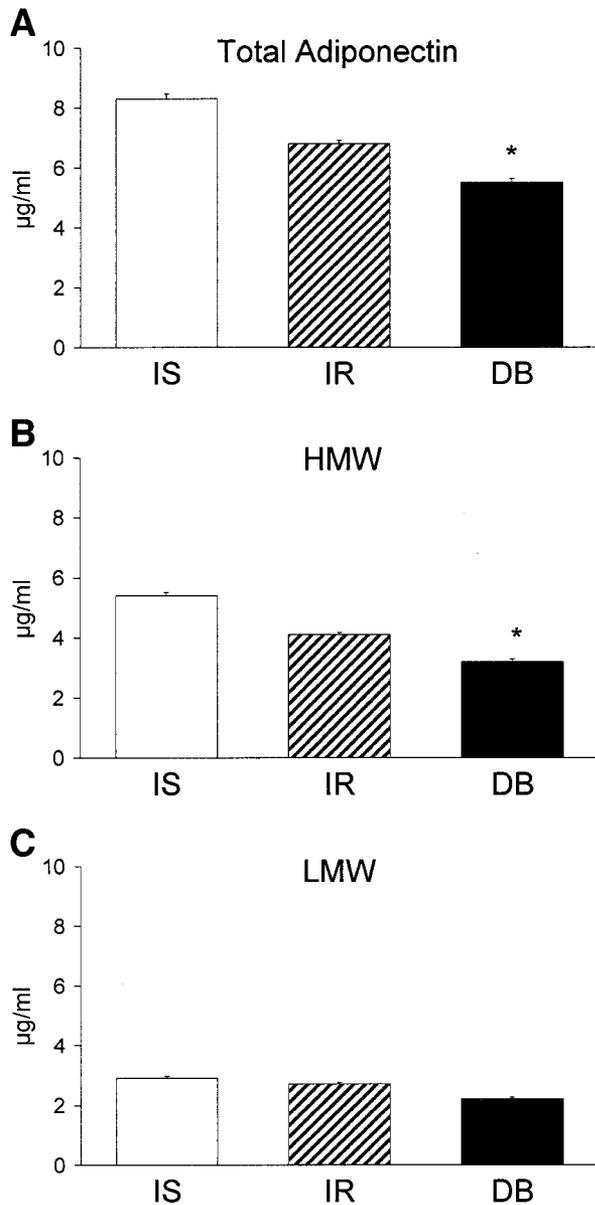


FIG. 2. Serum levels of total adiponectin (A), HMW adiponectin (B), and LMW adiponectin (C) according to insulin sensitivity category. Individuals with normal fasting glucose levels were categorized as insulin sensitive (IS) or insulin resistant (IR) based on maximally insulin-stimulated glucose disposal rate, and untreated patients with type 2 diabetes (DB). Data are the means \pm SE of adiponectin levels ($\mu\text{g/ml}$). *Significantly different from insulin sensitive, $P < 0.05$.

(0.58) subgroups ($P = \text{NS}$), as delineated in Table 1. Even so, as total adiponectin levels increase, so too does the proportion of adiponectin residing in the HMW form; that is, total adiponectin was correlated with the ratio of HMW to total adiponectin ($r = 0.30$, $P < 0.05$). Similar adiponectin results were obtained when the subjects were categorized as normal glucose tolerant with the highest adiponectin level ($5.1 \pm 2.6 \mu\text{g/ml}$), impaired glucose tolerant (3.3 ± 1.4), and diabetic with the lowest adiponectin level (3.4 ± 2.0), based on the 2-h glucose measurement during the oral glucose tolerance test (data not shown).

When the combined data in all study subjects were stratified by sex, women were found to have significantly higher total adiponectin (7.9 ± 3.7 vs. 5.9 ± 3.2 , $P = 0.02$), HMW adiponectin (5.0 ± 2.4 vs. 3.6 ± 2.2 , $P = 0.02$), and

ratio of HMW to total adiponectin (0.62 ± 0.06 vs. 0.57 ± 0.10 , $P = 0.04$) than men. The amount of LMW adiponectin was similar in men and women such that the higher level of total adiponectin in women was mainly caused by the increased amounts of HMW adiponectin. When the insulin-sensitive, insulin-resistant, and type 2 diabetic subgroups were separately stratified by sex in Table 1, women in every subgroup tended to have higher total and HMW adiponectin than men, but these differences did not reach statistical significance.

Adiponectin complexes, body fat, and body fat distribution. We next analyzed the relationships between total adiponectin and its HMW and LMW forms on one hand and body fat and distribution on the other hand, as assessed by BMI, waist and waist-to-hip ratio, and direct measurements of total and regional body fat (trunk/leg fat) by DEXA. Simple regression analyses showed no correlation between measures of generalized adiposity (i.e., BMI, waist circumference, total fat mass, and percent fat mass) with adiponectin, HMW or LMW complexes, or the ratio of HMW to total adiponectin (data not shown). In contrast, waist-to-hip ratio, the index of central body fat redistribution, was highly correlated with adiponectin ($r = -0.37$, $P < 0.01$), HMW adiponectin ($r = -0.43$, $P < 0.01$), and ratio of HMW to total adiponectin ($r = -0.38$, $P < 0.01$), but not with LMW adiponectin, as shown in Fig. 3. Table 2 shows the results of partial correlation analyses taking into account effects of age and sex. Neither BMI, total fat, nor percent body fat were correlated with total adiponectin or with adiponectin HMW and LMW complexes. Waist-to-hip ratio was again correlated with total adiponectin ($r = -0.30$, $P < 0.05$), HMW adiponectin ($r = -0.38$, $P < 0.01$), and ratio of HMW to total adiponectin ($r = -0.34$, $P < 0.01$), but not with LMW adiponectin. The ratio of trunk to leg fat on DEXA was correlated with total adiponectin ($r = -0.30$, $P < 0.05$) and HMW adiponectin ($r = 0.31$, $P < 0.05$). Waist circumference was associated only with the ratio of HMW to total adiponectin ($r = -0.31$, $P < 0.05$), but not with HMW and LMW adiponectin forms. Because waist circumference is highly correlated with total fat mass, we examined the relationship of waist circumference with adiponectin multimers after controlling for total fat mass; these analyses showed that higher waist circumference was correlated with lower levels of total adiponectin ($r = -0.30$, $P < 0.05$), HMW adiponectin ($r = -0.34$, $P < 0.05$), and the ratio of HMW to total adiponectin ($r = -0.29$, $P < 0.05$), independent of total fat levels. Thus, adiponectin and the HMW multimer were primarily correlated with redistribution of body fat to the central compartment and not with measures of generalized obesity.

Adiponectin, insulin resistance, and substrate oxidation. To study the impact of adiponectin complexes on insulin resistance, we measured maximally stimulated glucose disposal rates during hyperinsulinemic-euglycemic clamps, normalized for the amount of lean body mass. The glucose disposal rate was positively correlated with total adiponectin ($r = 0.45$), HMW adiponectin ($r = 0.47$), LMW adiponectin ($r = 0.31$), and the ratio of HMW to total adiponectin ($r = 0.29$), as shown in Fig. 4 (all $P < 0.05$). Because glucose disposal rate was highly correlated with BMI ($r = -0.36$, $P < 0.01$), waist circumference ($r = -0.55$, $P < 0.01$), and waist-to-hip ratio ($r = -0.59$, $P < 0.01$), we explored whether the associations between adiponectin measures and glucose disposal rate occur independently of total body fat and body fat distribution

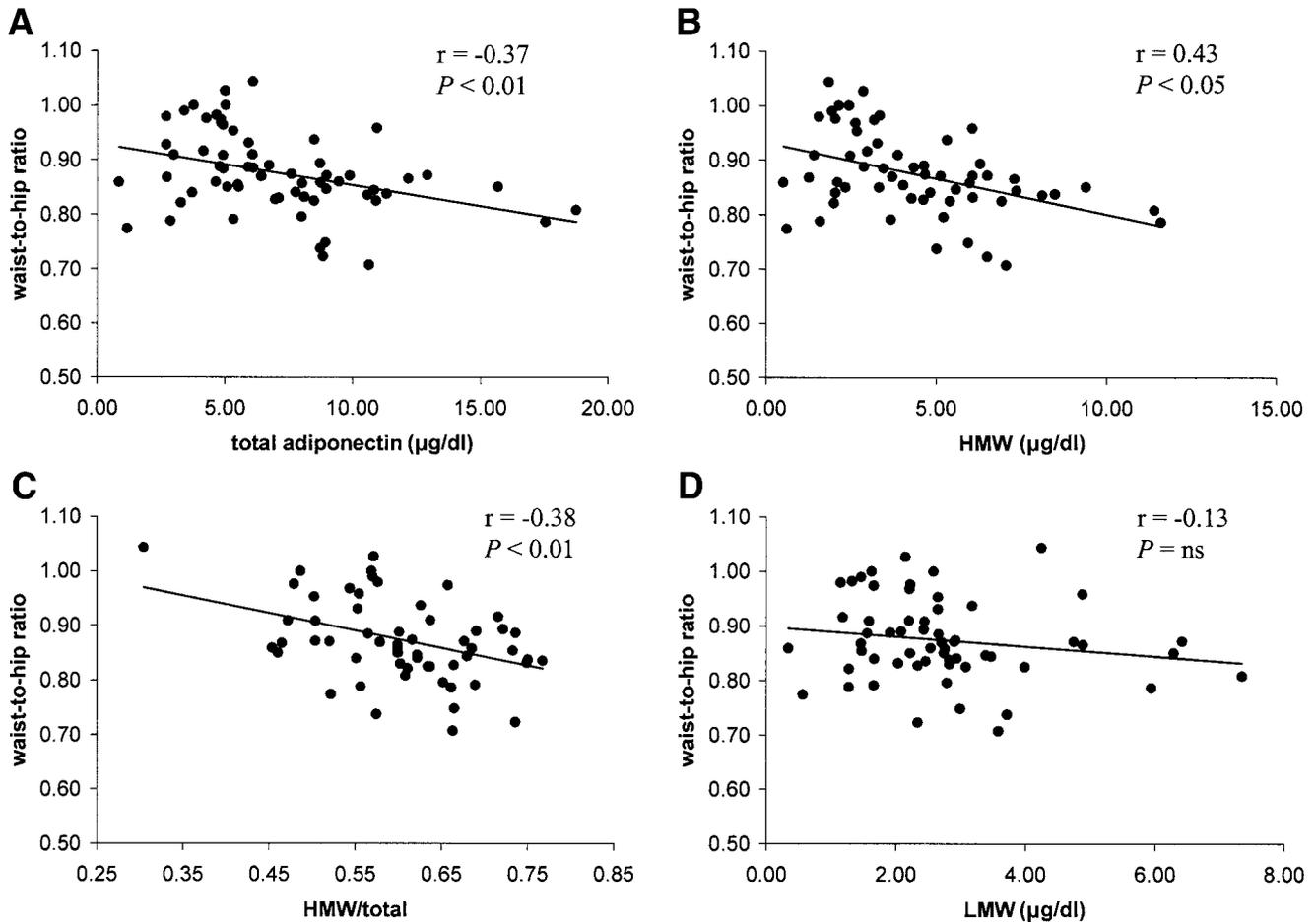


FIG. 3. Correlations between waist-to-hip ratio and total adiponectin (A), HMW adiponectin (B), HMW-to-total adiponectin ratio (C), and LMW adiponectin (D).

(Table 3). After controlling for sex and BMI, increasing glucose disposal rate still remained correlated with higher levels of total ($r = 0.46$), HMW ($r = 0.48$), and LMW adiponectin ($r = 0.35$) (all $P < 0.05$). Similar results were obtained after controlling for waist circumference. After controlling for waist-to-hip ratio, the associations between glucose disposal rate and total ($r = 0.32$), HMW ($r = 0.33$), and LMW adiponectin ($r = 0.27$) became weaker, but they were still significant (all $P < 0.05$). On the other hand, the correlations between glucose disposal rate and ratio of HMW to total adiponectin were not statistically significant after adjustment for BMI, waist circumference, or waist-to-hip ratio. In all these situations, even though the correlation between glucose disposal rate and HMW adiponectin was stronger than with LMW adiponectin, the difference between these two correlation coefficients did not quite reach statistical significance ($P = 0.06$).

When separate analyses were performed in type 2

diabetic and nondiabetic subjects (insulin sensitive + insulin resistant), glucose disposal rate was strongly correlated with total adiponectin ($r = 0.59$, $P < 0.01$, and $r = 0.31$, $P < 0.05$, respectively) and HMW adiponectin ($r = 0.68$, $P < 0.01$, and $r = 0.32$, $P < 0.05$, respectively) in both subgroups. Neither LMW nor the ratio of HMW to total adiponectin was correlated with glucose disposal rate in any of the subgroups.

Because adiponectin has been reported to increase both insulin sensitivity and fatty acid oxidation in skeletal muscle from mice (4), we examined whether adiponectin or its multimeric forms were associated with increased lipid oxidation and energy expenditure at the whole-body level in humans. This analysis was confined to nondiabetic subjects (insulin sensitive + insulin resistant) because the diabetic state per se influences fuel utilization. There was no relationship between adiponectin and its complexes with resting energy expenditure (data not shown). Adi-

TABLE 2

Correlations between total, HMW, and LMW adiponectin and the ratio of HMW to total adiponectin versus body fat and body fat distribution

	BMI	Waist	Waist-to-hip ratio	Total fat mass	Trunk/leg fat
Total adiponectin ($\mu\text{g/ml}$)	-0.01	-0.11	-0.30†	-0.02	-0.30*
HMW ($\mu\text{g/ml}$)	-0.06	-0.17	-0.38†	-0.07	-0.31*
LMW ($\mu\text{g/ml}$)	0.08	0.02	-0.13	0.06	-0.22
HMW-to-total adiponectin ratio	-0.21	-0.31*	-0.34†	-0.18	-0.12

Adjusted for age and sex ($n = 68$). * $P < 0.05$; † $P < 0.01$.

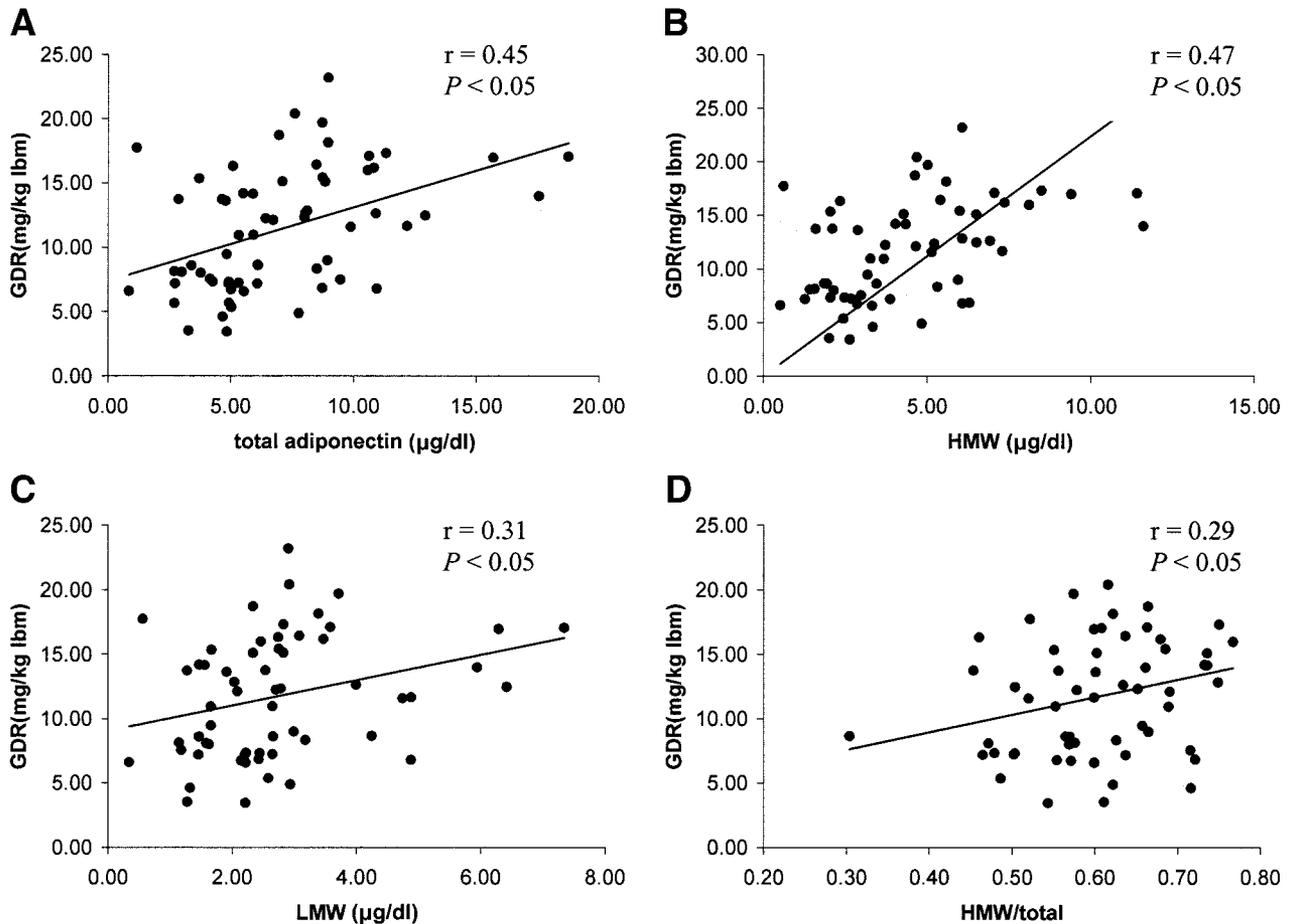


FIG. 4. Correlations between glucose disposal rate (GDR), as measured by hyperinsulinemic-euglycemic clamp, and total adiponectin (A), HMW (B), LMW (C), and HMW-to-total adiponectin ratio (D) in a group of 68 diabetic and nondiabetic subjects.

adiponectin was, however, linked with differences in substrate oxidation. Among nondiabetic subjects ($n = 47$), total adiponectin was negatively correlated with rates of carbohydrate oxidation ($r = -0.33$, $P < 0.05$) and positively correlated with fat oxidation ($r = 0.41$, $P < 0.01$), and these associations were even stronger with HMW adiponectin ($r = -0.38$, $P < 0.01$, and $r = 0.44$, $P < 0.01$, respectively). The association between LMW adiponectin and fat oxidation was weaker but statistically significant ($r = 0.31$, $P < 0.05$); however, even though HMW adiponectin showed higher correlations with fat oxidation than LMW adiponectin, the difference between these two correlation coefficients did not reach statistical significance ($P = 0.10$).

Because sex differences in fuel oxidation, particularly fat oxidation, have been previously reported (23), we performed stratified analyses by sex in the nondiabetic

subjects. Similar to the analysis of the entire sample, total adiponectin and HMW multimer concentrations were inversely related to carbohydrate oxidation ($r = -0.51$, $P < 0.05$, and $r = -0.56$, $P < 0.01$, respectively) and positively correlated with lipid oxidation ($r = 0.45$, $P < 0.05$, and $r = 0.48$, $P < 0.05$) in men. These correlations were not statistically significant in women.

Adiponectin complexes and lipid panel, blood pressure, and lipoprotein subclasses. In the conventional lipid panel, total and HMW adiponectin were correlated with HDL cholesterol ($r = 0.25$ and 0.28 , $P < 0.05$) and triglycerides ($r = -0.31$, $P < 0.05$, and $r = -0.37$, $P < 0.01$, respectively). The ratio of HMW to total adiponectin was only correlated with triglycerides ($r = -0.37$, $P < 0.01$), whereas no correlation was found between LMW adiponectin and components of the conventional lipid panel. After controlling for age, sex, BMI, glucose disposal rate,

TABLE 3

Correlations between maximal insulin-stimulated glucose disposal rate versus HMW and LMW adiponectin and the ratio of HMW-to-total adiponectin unadjusted and adjusted for body fat variables in the study sample

	Unadjusted	Adjusted*		
		BMI	Waist	Waist-to-hip ratio
Total adiponectin ($\mu\text{g/ml}$)	0.45 [†]	0.46 [‡]	0.43 [‡]	0.32 [‡]
HMW ($\mu\text{g/ml}$)	0.47 [†]	0.48 [‡]	0.44 [‡]	0.33 [‡]
LMW ($\mu\text{g/ml}$)	0.31 [†]	0.35 [‡]	0.35 [‡]	0.27 [‡]
HMW-to-total adiponectin ratio	0.29 [†]	0.22	0.13	0.08

$n = 68$. *Adjusted for sex and column variable; [†] $P < 0.05$; [‡] $P < 0.01$.

TABLE 4

Correlation coefficients of total, HMW, and LMW adiponectin and HMW-to-total adiponectin ratio versus NMR lipoprotein subclass particle concentration and lipoprotein size

	Unadjusted				Adjusted for age, sex, BMI, GDR, and fasting glucose			
	Total	HMW	LMW	Ratio	Total	HMW	LMW	Ratio
Particle concentration								
VLDL								
Total	-0.25*	-0.26*	-0.18	-0.20	-0.19	-0.20	-0.13	-0.19
Small	-0.13	-0.14	-0.08	-0.14	-0.02	-0.04	0.00	-0.14
Intermediate	-0.32*	-0.31*	-0.25	-0.15	-0.33*	-0.32*	-0.27*	-0.13
Large	-0.41†	-0.44†	-0.30*	-0.27*	-0.35*	-0.36†	-0.27	-0.17
LDL								
Total	-0.29*	-0.32*	-0.20	-0.24	-0.11	-0.13	-0.05	-0.16
Small	-0.36†	-0.41†	-0.25	-0.26*	-0.19	-0.22	-0.11	-0.13
Intermediate	0.02	0.01	0.07	-0.13	0.04	0.02	0.08	-0.18
Large	0.30*	0.36†	0.15	0.36†	0.14	0.20	0.01	0.27*
HDL								
Total	0.02	-0.01	0.02	-0.02	-0.12	-0.11	-0.10	-0.01
Small	-0.20	-0.25	-0.12	-0.22	-0.31*	-0.33*	-0.21	-0.22
Intermediate	-0.10	0.06	0.10	0.05	0.11	0.12	0.09	0.12
Large	0.37†	0.45†	0.20	0.41†	0.30*	0.36†	0.14	0.33*
Particle size								
VLDL	-0.21	-0.23	-0.15	-0.16	-0.21	-0.23	-0.14	-0.13
LDL	0.39†	0.47†	0.24	0.39†	0.23	0.30*	0.08	0.32*
HDL	0.47†	0.53†	0.28*	0.39†	0.41†	0.47†	0.24	0.32*

$n = 68$. * $P < 0.05$; † $P < 0.01$. GDR, maximal insulin-stimulated glucose disposal rate; ratio, HMW-to-total adiponectin ratio.

and fasting glucose, again only triglyceride levels correlated negatively with the ratio of HMW to total adiponectin ($r = -0.29$, $P < 0.05$). In the entire sample of diabetic and nondiabetic subjects, there was no correlation between adiponectin (and its complexes) and systolic or diastolic blood pressure, whereas total, HMW, and LMW adiponectin were correlated with fasting glucose (all $r = -0.29$, $P < 0.05$).

Alterations in lipoprotein subclasses are also a key feature of the insulin resistance syndrome (18). Therefore, total adiponectin and adiponectin multimers were analyzed together with parameters from the fasting NMR lipoprotein subclass profile. Table 4 shows the simple and partial correlations between total, HMW, LMW, and the ratio of HMW to total adiponectin and the lipoprotein subclass particle concentrations and sizes. Figure 5 shows associations of total, HMW, LMW, and the ratio of HMW to total adiponectin with selected lipoprotein subclasses. In the entire sample, as shown in Table 4, simple correlation analyses showed that total adiponectin was positively correlated with large LDL particle concentration and LDL particle size together with large HDL concentration and HDL particle size, and it was inversely correlated with small LDL particle concentration, intermediate and large VLDL particle concentrations, and VLDL size. Similar correlations were observed for HMW adiponectin and these lipoprotein parameters, although in every case the strength of the associations was stronger compared with the correlations with total adiponectin. Thus, adiponectin was associated with the full complement of lipoprotein subclass alterations that occur as a function of insulin resistance (18). Both HMW and LMW adiponectin showed correlations with HDL particle size and intermediate and large VLDL, as shown in Table 4. The strength of these associations was significantly higher for HMW adiponectin than for LMW adiponectin with HDL particle size ($P = 0.025$), but not with intermediate and large VLDL ($P = 0.10$

and 0.06, respectively). In addition, partial correlation analyses were performed considering the effects of age, sex, BMI, insulin sensitivity (glucose disposal rate), and fasting glucose (Table 4). Positive associations between both total adiponectin and the HMW multimer and large HDL particle concentration and HDL size remained significant, as did inverse correlations with intermediate and large VLDL concentrations. These observations are consistent with the hypothesis that total adiponectin and the HMW multimer have effects on HDL and VLDL that are independent of differences in insulin sensitivity, obesity, and glycemia. On the other hand, effects of adiponectin on LDL appear to be secondary and attributable to colinearity with these other variables.

DISCUSSION

Our data provide the first comprehensive examination regarding the relationships of adiponectin and circulating multimeric forms with clinical and metabolic traits that comprise the metabolic syndrome. We studied subjects over a broad range of insulin sensitivity, including those with and without type 2 diabetes. Our overall results indicate that the amount of HMW adiponectin is highly correlated with multiple traits within the metabolic syndrome complex. Reduced levels of HMW adiponectin were associated with upper body fat distribution, insulin resistance, impaired lipid oxidation, and dyslipidemia affecting multiple lipoprotein subclasses. In fact, associations with total adiponectin could largely be explained as a consequence of the effects of HMW adiponectin, whereas LMW adiponectin exhibited diminished or no relationship with most of the metabolic and clinical traits. In this study, we observed that there were two predominant adiponectin multimers circulating in human sera that migrate on nondenaturing gels at 180 kDa, corresponding putatively to an adiponectin hexamer, and at ~330–360 kDa (a

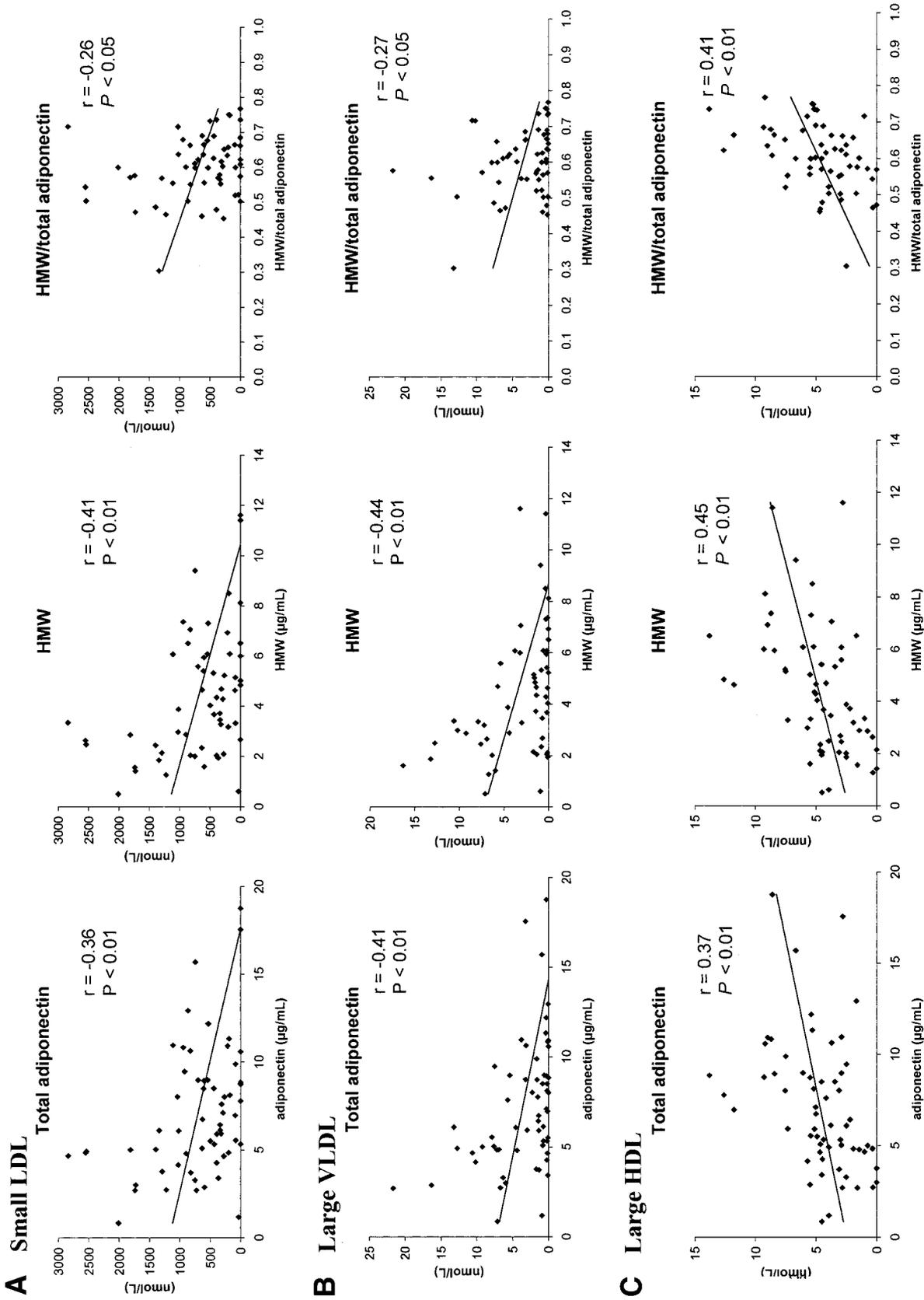


FIG. 5. Correlations between total adiponectin, HMW, LMW, and ratio of HMW to total adiponectin versus lipoprotein subclass concentrations and sizes. Total adiponectin was measured by RIA, its isoforms were determined by immunoblot, and lipoprotein subclass profiles were assessed by NMR. Data are from 68 individuals over a broad range of insulin sensitivity. The correlation coefficient (r) and P values are shown for each graph. The graphs show the relationship between total adiponectin, HMW adiponectin, and HMW-to-total adiponectin ratio versus small LDL (A), large VLDL (B), and large HDL (C).

higher molecular weight form). We measured total immunoreactive adiponectin by RIA and then assessed the relative amounts and proportions of LMW and HMW multimers by immunoblot and densitometric analyses. The circulating concentrations of HMW and LMW adiponectin were quantified by multiplying the LMW-to-total adiponectin and HMW-to-total adiponectin ratios measured in each lane on the immunoblots by adiponectin RIA results. This quantification of LMW and HMW adiponectin assumes that each adiponectin monomer is equally immunoreactive on the immunoblots independent of which multimeric form it comprises. However, our conclusions regarding correlations with quantitative traits do not depend on this assumption because only relative measures of adiponectin multimers among the study subjects are germane to these analyses. The statistical significance of all correlations is retained whether LMW and HMW adiponectin were measured as relative immunoblot band densities (not shown) or as the amount of total immunoreactive adiponectin that is detected in each multimeric form (used in the current study).

Increased central fat accumulation is considered a key pathophysiological feature of the metabolic syndrome, and our results support those of previous studies showing negative associations between total adiponectin and measures of central fat accumulation (24). We extended these observations to show that higher central fat distribution, as estimated by either the waist-to-hip ratio or trunk-to-leg-fat by DEXA, was correlated with lower HMW adiponectin and the ratio of HMW to total adiponectin, but not LMW adiponectin. The associations of HMW adiponectin and ratio of HMW to total adiponectin with measures of central fat distribution were stronger than those for total adiponectin. Interestingly, we did not find a correlation between total adiponectin (and its complexes) and measures of total adiposity, which underscores the importance of body fat distribution rather than total body fat on adiponectin levels. To further support these findings, we observed that higher waist circumference was associated with lower total adiponectin ($r = -0.30$), HMW adiponectin ($r = -0.34$), and the ratio of HMW to total adiponectin ($r = -0.29$) after controlling for the effect of total fat mass, indicating that these associations are driven mainly by central fat accumulation, as indicated by the waist circumference level rather than by total body adiposity. Overall, these results are confirmatory of previous reports indicating that lower adiponectin levels are correlated with higher central body fat distribution independent of total adiposity (25), and they support the contention that regional fat depot differences in adiponectin production exist. This is also consistent with reports indicating that adiponectin mRNA levels were lower in visceral versus subcutaneous adipose tissue from lean, obese, and diabetic subjects (26,27). Thus, our study suggests that increases in central adiposity may result in downregulation of adiponectin, and it raises the possibility that this may occur by a preferential decrease in the HMW form of adiponectin.

Adiponectin plays important roles in glucose homeostasis, particularly through acting as an insulin sensitizer. The primary mechanism by which adiponectin enhances insulin sensitivity appears to be through increased fatty acid oxidation and inhibition of hepatic glucose production. In this study, we measured insulin-stimulated glucose disposal and found that levels of total adiponectin are higher in insulin-sensitive subjects. We also showed that the

lower levels of total adiponectin observed in insulin-resistant and diabetic subjects are predominantly explained by lower HMW and not by LMW adiponectin, which in turn does not significantly change according to insulin sensitivity group (Fig. 1). When glucose disposal rate was analyzed as a continuous variable, HMW and LMW adiponectin and the ratio of HMW to total adiponectin were positively correlated with glucose disposal rate. These associations persist even after controlling for effects of body adiposity and fat distribution, indicating that the relationships between total, HMW, and LMW adiponectin are independent of total body fat or fat distribution. These findings are in agreement with functional studies indicating that adiponectin multimers activate the AMPK pathway, which in turn induces fatty acid oxidation, glucose uptake, and lactate production in myocytes (4,28).

Increased intramyocellular fat accumulation has been shown to be highly correlated with insulin resistance (29). Although the mechanisms underlying this relationship have not been elucidated, they may be partially related to impaired intramyocellular fat oxidation. To examine a role for adiponectin in this relationship, we assessed the association between adiponectin and its multimers with whole-body fat oxidation. For the first time, we demonstrated that total and HMW adiponectin were positively correlated with whole-body fat oxidation and negatively correlated with carbohydrate oxidation; these data in humans support previous rodent studies indicating that adiponectin administration augments lipid and glucose metabolism in muscle and liver through AMPK and peroxisome proliferator-activated receptor- α ligand activity (2,4). However, these rodent studies used bacterially expressed adiponectin, which does not aggregate into multimers. The current data indicate that both HMW and LMW adiponectin were associated with whole-body fat oxidation.

To the best of our knowledge, this is the first study reporting the associations between adiponectin multimers and circulating lipoprotein subclasses. In general, adiponectin was associated with the full complement of alterations in lipoprotein subclass particle concentration and particle size that occur as a function of insulin resistance (18). Higher levels of total adiponectin and HMW adiponectin were correlated with higher levels of the more cardioprotective large HDL particles as well as with higher HDL particle size, together with decreased concentrations of both large VLDL particles and small LDL particles. Typically, the associations between HMW adiponectin and the various lipoprotein subclass parameters were higher than those observed for total adiponectin; these data reflect the colinearity between total and HMW adiponectin and suggest that the lipoprotein effects of total adiponectin are mainly driven by the more biologically active HMW multimer. Consistent with this idea, the ratio of HMW to total adiponectin was positively correlated with large LDL and HDL particle concentrations as well as with LDL and HDL particle size. These correlations with HMW and the ratio of HMW to total adiponectin persisted even after controlling for possible confounding variables, including age, sex, BMI, glucose disposal rate, and fasting glucose, indicating an independent effect of HMW adiponectin in lipoprotein metabolism. Our novel results support those from a previous study reporting the inverse correlation between total adiponectin and small LDL particles (1). Interestingly, we observed a shift from small, dense LDL particles to larger, more buoyant LDL particles as a function of total adiponectin and HMW levels, similar

to that reported by the use of certain thiazolidinediones (30). However, in the current study, the associations between total adiponectin and small and large LDL particle concentrations disappeared after controlling for possible confounding variables, suggesting that such correlations were mainly driven by associated metabolic traits such as insulin resistance and central body fat distribution. There is considerable evidence suggesting that adiponectin may exert antiatherogenic properties, including direct effects on the vasculature that would prevent the development of atherosclerosis (31–33). These effects may also be mediated preferentially by the HMW multimer because HMW adiponectin has been shown to promote the survival of endothelial cells by preventing apoptosis (34). Therefore, it appears that HMW adiponectin could act to prevent cardiovascular disease both through effects on the vascular wall and by promoting a more favorable lipoprotein subclass profile.

In conclusion, we observed that serum adiponectin is associated with increased insulin sensitivity, reduced abdominal fat, and high basal lipid oxidation; however, it is HMW quantity, not total adiponectin or the ratio of multimeric forms, which is primarily responsible for these relationships. Still, the role of LMW adiponectin deserves further exploration because it was also correlated with insulin sensitivity and basal lipid oxidation. Additionally, reduced quantities of HMW adiponectin independently recapitulate the lipoprotein subclass profile associated with insulin resistance after correcting for glucose disposal rate and BMI. HMW adiponectin is an important biomarker for the metabolic syndrome and could play a pathogenic role in the development of the insulin resistance trait cluster.

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