Total and High–Molecular Weight Adiponectin in Relation to Metabolic Variables at Baseline and in Response to an Exercise Treatment Program

Comparative evaluation of three assays

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OBJECTIVE — Adiponectin, an adipocyte-secreted hormone, circulates in the serum in several multimeric forms. Compared with total adiponectin, high–molecular weight (HMW) adiponectin has been suggested to be a better predictor of metabolic parameters and insulin sensitivity in humans. Our objective was to compare total adiponectin with HMW adiponectin as predictors of metabolic variables and insulin sensitivity at both baseline and after an exercise intervention.

RESEARCH DESIGN AND METHODS — We obtained blood samples from 60 men and women with normal glucose tolerance ($n = 20$), impaired glucose tolerance (IGT) ($n = 20$), or type 2 diabetes ($n = 20$) at baseline and after 4 weeks of training to measure metabolic variables. Using commercially available assays, we measured plasma total adiponectin using LINCO, Mediagnost, and ALPCO assays and HMW adiponectin using an ALPCO assay.

RESULTS — HMW adiponectin and total adiponectin (ALPCO) had similar ability to predict the presence of insulin resistance. Total adiponectin, as measured by radioimmunoassay (LINCO) and enzyme-linked immunosorbent assay (ELISA) (Mediagnost), correlated most strongly with measures of insulin sensitivity ($P < 0.01$) and lipid profile ($P < 0.01$) at baseline, showed greater improvements of adiponectin levels ($P < 0.001$), was more closely associated with improvements of lipid measures with exercise training ($P < 0.01$), and more accurately predicted insulin resistance and IGT in comparison with total adiponectin or HMW measured with the ALPCO ELISA.

CONCLUSIONS — These results do not support the superiority of HMW over total adiponectin (measured using currently available assays) in assessing metabolic variables at baseline or in response to physical training. Moreover, there are significant differences in the ability of commercially available assays for total adiponectin to predict metabolic variables.

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Abbreviations: AUC, area under the curve; ELISA, enzyme-linked immunosorbent assay; HMW, high molecular weight; IGT, impaired glucose tolerance; NGT, normal glucose tolerance; OGTT, oral glucose tolerance test.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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Adiponectin is an adipocyte-secreted hormone that has been proposed to play a central role in metabolism in humans (1–3). Cross-sectional studies have linked decreased adiponectin levels with several metabolic traits, including insulin resistance, dyslipidemia, and the metabolic syndrome (2,4,5). In addition, low adiponectin levels have been shown to predict future development of diabetes (6), cardiovascular disease (7), and obesity-associated malignancies (8,9) in observational studies.

Adiponectin has been shown to circulate in serum in several multimeric forms, and these different forms have been postulated to have differing biologic activity (10,11). High–molecular weight (HMW) adiponectin recently has been proposed to be the biologically active form of the hormone, and, thus, it has been hypothesized that HMW adiponectin may better predict metabolic parameters than total adiponectin (12). Several studies (12–15) have reported an association between HMW adiponectin and insulin sensitivity, but whether this relationship is stronger than the well-documented associations of total adiponectin with insulin sensitivity and metabolic variables remains to be determined.

We previously have shown that total adiponectin levels in serum and expression of adiponectin receptors in skeletal muscle are correlated with insulin resistance, lipid levels, and obesity in Caucasian men and women (5). Moreover, we have demonstrated that 4 weeks of exercise resulted in an increase in total adiponectin and an increase in the expression of adiponectin receptors in skeletal muscle from subjects with normal glucose tolerance (NGT), impaired glucose tolerance (IGT), and type 2 diabetes (5). However, the effect of exercise on HMW adiponectin has not been conclusively demonstrated. Bobbert et al. (14) have reported that total adiponectin increases with exercise in 17 obese men and

women and showed that exercise resulted in a relative increase in the HMW form (assessed by Western blotting), but these changes did not correlate with improved insulin sensitivity.

Progress in the study of HMW adiponectin has been impaired by lack of a commercially available assay to measure individual multimers of adiponectin with high sensitivity and accuracy in large numbers of samples. A novel enzyme-linked immunosorbent assay (ELISA) system for the selective measurement of human adiponectin multimers recently has been described (16), and, using this method, Hara et al. (12) have reported that HMW adiponectin has better predictive power to detect the presence of insulin resistance and the metabolic syndrome than total adiponectin measured using the same ELISA.

To study whether measuring HMW adiponectin provides a better predictive value than total adiponectin in assessing metabolic variables, and to identify the assay method that correlates most closely with insulin sensitivity and best predicts improvements in metabolic factors at baseline and after exercise training, we measured serum total adiponectin using three different commercially available assay methods: $ADIPO_L$ (LINCO), $ADIPO_M$ (Mediagnost), and $ADIPO_A$ (ALPCO), as well as HMW adiponectin before and after an exercise intervention program in 60 men and women.

RESEARCH DESIGN AND METHODS

— A total of 60 Caucasian men and women were studied in the context of a study on insulin resistance, with 20 subjects each having NGT, IGT, and type 2 diabetes on the basis of a 75-g oral glucose tolerance test (OGTT) according to American Diabetes Association criteria (17). Subjects were enrolled in 60 min of supervised physical training sessions 3 days per week. Each training session included 20 min of biking or running, 20 min of swimming, and 20 min of warming up/cooling down periods, as previously described (5). At baseline and after 4 weeks of training, blood samples were obtained in the fasting state and measurements of anthropometric parameters were performed. The study was approved by the ethics committee of the University of Leipzig. All subjects gave written informed consent.

Metabolic assessment

Metabolic and anthropometric assessment was performed as previously described (5). Insulin sensitivity was assessed in all subjects at baseline and after 4 weeks of training using the euglycemic-hyperinsulinemic clamp method. Briefly, after an overnight fast intravenous catheters were inserted into antecubital veins in both arms. One was used for the infusion of insulin and glucose; the other was used for the frequent sampling. After a priming dose of 1.2 nmol/m^2 insulin, the infusion with insulin was started with a constant infusion rate of 0.28 nmol/m^2 body surface per min and continued for 120 min. After 3 min, the variable 20% glucose infusion rate was added. The glucose infusion rate was adjusted during the clamp to maintain the blood glucose at 5.0 mmol/l. Bedside blood glucose measurements were performed every 5 min.

Adiponectin assays

Serum adiponectin levels were measured using radioimmunoassay (LINCO Research, St. Charles, MO) ($ADIPO_L$) with a sensitivity of 1 ng/ml and an intra-assay coefficient of variation (CV) of 6.6% and also using ELISA (Mediagnost, Reutlingen, Germany) ($ADIPO_M$), as previously described (18). In addition, serum levels of total adiponectin, as well as HMW adiponectin, were determined using a novel ELISA (ALPCO Diagnostics, Salem, NH) ($ADIPO_A$). The sensitivity of this assay was 0.04 ng/ml. The recovery rate was 99–103% for total adiponectin and 97–105% for HMW adiponectin. The effect of serial dilutions has been tested on human serum samples, and linearity and specificity of the assay has been documented (16). Total and HMW adiponectin values per subject time point were obtained together in the same assay, and the ratio of HMW to total adiponectin per subject time point was calculated by dividing the respective values. All respective samples before and after exercise were measured together in the same assay.

In the current study, 10 different serum samples were used as internal controls to estimate precision for both HMW and total adiponectin. Concentrations of adiponectin, ranging from 2.02 to 11.54 μg/ml, were consecutively measured four times. For total adiponectin, the intra-assay CV was 5.3% (range 2.8–8.4) and the average interassay CV was 7.4% (3.2–9.5). The same human serum samples also were analyzed after treatment with proteinase K (used to digest adiponectin multimers), and precision was estimated at different concentrations of HMW adiponectin (0.4–6.6 μg/ml). For HMW adiponectin, the intra-assay CV was 6.8% (range 5.4–8.4) and the interassay CV was 8.7% (7.2–11.5). Based on our serum samples, the intra-assay CV was 4.2% and the interassay CV was 5.6% for total adiponectin, while for HMW adiponectin the intra-assay CV was 6.4% and the interassay CV was 7.9%. The CV of the assay was also evaluated in heparin-treated plasma samples and was 9.1% for total adiponectin and 7.9% for HMW adiponectin based on 16 pairs of internal controls. To our knowledge, this is the first study to evaluate the precision of this assay for both serum and plasma based on a large number of samples (16).

Statistical analysis

Comparisons of descriptive characteristics, expressed as means ± SD, were conducted using one-way ANOVA with Bonferroni-corrected post hoc tests and were repeated using nonparametric Kruskal-Wallis. Nonparametric Spearman correlation coefficients were calculated among baseline measures of study variables, HMW, and total adiponectin, as well as between changes in total and HMW adiponectin with changes in measures of insulin sensitivity. Comparisons of baseline and after training measures were made using both paired *t* tests and Wilcoxon's rank-sum tests among all subjects and then stratified by sex and glucose tolerance group (NGT, IGT, or type 2 diabetes). A level of $\alpha = 0.05$ was used to determine statistical significance. Statistical analyses were performed using SPSS version 8 (SPSS, Chicago, IL).

RESULTS

— Participants with IGT and type 2 diabetes were significantly older and had higher BMI, waist-to-hip ratio, percentage body fat, fasting plasma glucose, 2-h OGTT glucose, fasting plasma insulin, fasting leptin, and total and LDL cholesterol compared with subjects with NGT (Table 1). $ADIPO_L$ and $ADIPO_M$ total adiponectin measures were strongly correlated to each other ($r = 0.96$) and inversely associated with baseline athropometric variables (BMI, waist-to-hip ratio, and percentage fat mass), as well as baseline parameters of insulin resistance (fasting plasma glucose, 2-h OGTT glucose, and fasting plasma insulin and positively associated with whole-blood glucose uptake) (Table 2). Moderately strong associations were also appar-

Table 1—*Baseline characteristics by glucose tolerance group*

	NGT	IGT	Type 2 diabetes	*P* value
n	20	20	20	
Men/women	9/11	9/11	11/9	0.77
Age (years)	32.8 ± 2.5	56.0 ± 2.6*	53.1 ± 1.5*	<0.001
Body weight (kg)	69.6 ± 3.2	87.6 ± 3.7†	94.7 ± 4.4*	<0.001
BMI (kg/m^2)	24.3 ± 0.3	29.8 ± 0.9*	31.4 ± 0.7*	<0.001
Waist-to-hip ratio	0.84 ± 0.02	1.21 ± 0.04*	1.28 ± 0.03*	<0.001
Fat mass (%)	24.5 ± 0.7	34.9 ± 1.9*	38.2 ± 1.8*	<0.001
Tobacco use (*n*)	1 (5)	4 (20)	8 (40)‡	0.03
Fasting plasma glucose (mmol/l)	5.2 ± 0.1	5.7 ± 0.1‡	6.2 ± 0.1†	<0.001
2-h OGTT glucose (mmol/l)	6.0 ± 0.2	9.4 ± 0.2*	13.1 ± 0.3*	<0.001
Fasting plasma insulin (pmol/l)	66 ± 8	695 ± 110*	319 ± 47‡	<0.001
Whole-blood glucose uptake (μmol · kg^{-1} · min^{-1})	75.9 ± 3.8	18.7 ± 9.0*	21.5 ± 9.2*	<0.001
Free fatty acids (mmol/l)	0.41 ± 0.04	0.54 ± 0.06	0.56 ± 0.06	0.11
Fasting leptin (pmol/l)				
Male	2.8 ± 0.7	20.7 ± 3.0†	31.9 ± 3.5*	<0.001
Female	6.1 ± 0.8	42.2 ± 7.2*	53.2 ± 5.3*	<0.001
Total cholesterol (mmol/l)	4.62 ± 0.11	5.34 ± 0.12†	5.60 ± 0.16*	<0.001
Total HDL (mmol/l)	1.62 ± 0.07	1.21 ± 0.04*	1.11 ± 0.04*	<0.001
Total LDL (mmol/l)	2.34 ± 0.10	3.22 ± 0.12*	3.30 ± 0.19*	<0.001
Triglycerides (mmol/l)	2.03 ± 0.06	2.01 ± 0.10	2.11 ± 0.07	0.63
$ADIPO_L$ (μg/ml)	8.95 ± 0.55	3.38 ± 0.26*	3.48 ± 0.42*	<0.001
$ADIPO_M$ (μg/ml)	8.81 ± 3.43	3.51 ± 1.47*	3.82 ± 2.16*	<0.001
$ADIPO_A$ (μg/ml)	6.72 ± 0.65	6.25 ± 0.68	5.15 ± 0.90	0.32
HMW adiponectin (μg/ml)	2.88 ± 0.32	2.80 ± 0.44	2.42 ± 0.63	0.78

Data are means ± SD. *$P < 0.001$ for Bonferroni-corrected comparison with the group with NGT. †$P < 0.01$ for Bonferroni-corrected comparison with the group with NGT. ‡$P < 0.05$ for Bonferroni-corrected comparison with the group with NGT.

ent with baseline lipid profile, with negative correlations of total adiponectin with total and LDL cholesterol and positive associations with HDL. The novel ELISA, $ADIPO_A$, produced values of total and HMW adiponectin that were highly correlated (r = 0.96) but did not show strong associations with baseline body composition, measures of insulin sensitivity, or lipid profile. HMW and total adiponectin as quantified by $ADIPO_A$ showed weak associations with $ADIPO_L$ and $ADIPO_M$ (Table 2). Strength of associations between $ADIPO_L$ and $ADIPO_M$ total adiponectin with anthropometric measures, parameters of insulin sensitivity, and lipid profile ranged from ±0.32 to 0.61, whereas the correlations of these variables were much weaker with total and HMW adiponectin as measured by $ADIPO_A$ (±0.03 to 0.27).

With respect to identifying insulin resistance, $ADIPO_L$ and $ADIPO_M$ better predicted these conditions than $ADIPO_A$ total or HMW (Fig. 1). The area under the curve (AUC) for insulin resistance, as defined by whole-body glucose uptake <40 μmol · kg^{-1} · min^{-1} was 0.96 (95% CI 0.91–1.00; $P < 0.0001$) and 0.92 (0.83–1.00; $P < 0.0001$) for $ADIPO_L$ and $ADIPO_M$, respectively, and each had sig-

Table 2—*Spearman correlation matrix of study variables with adiponectin measures*

	Total $ADIPO_L$	Total $ADIPO_M$	Total $ADIPO_A$	HMW $ADIPO_A$
$ADIPO_M$	0.96*			
$ADIPO_A$	0.25	0.25		
HMW adiponectin	0.20	0.20	0.96*	
Age	−0.39*	−0.32*	−0.14	−0.12
BMI	−0.53*	−0.42*	−0.16	−0.12
Waist-to-hip ratio	−0.51*	−0.41*	−0.25	−0.21
Fat mass (%)	−0.53*	−0.44*	−0.24	−0.21
Fasting plasma glucose	−0.52*	−0.44*	−0.21	−0.16
2-h OGTT glucose	−0.60*	−0.53*	−0.27*	−0.23
Fasting plasma insulin	−0.49*	−0.42*	−0.09	−0.04
Whole-blood glucose uptake	0.61*	0.54*	0.14	0.13
Total cholesterol	−0.58*	−0.50*	−0.22	−0.20
HDL cholesterol	0.47*	0.42*	0.11	0.09
LDL cholesterol	−0.50*	−0.40*	−0.07	−0.03

*$P < 0.01$.

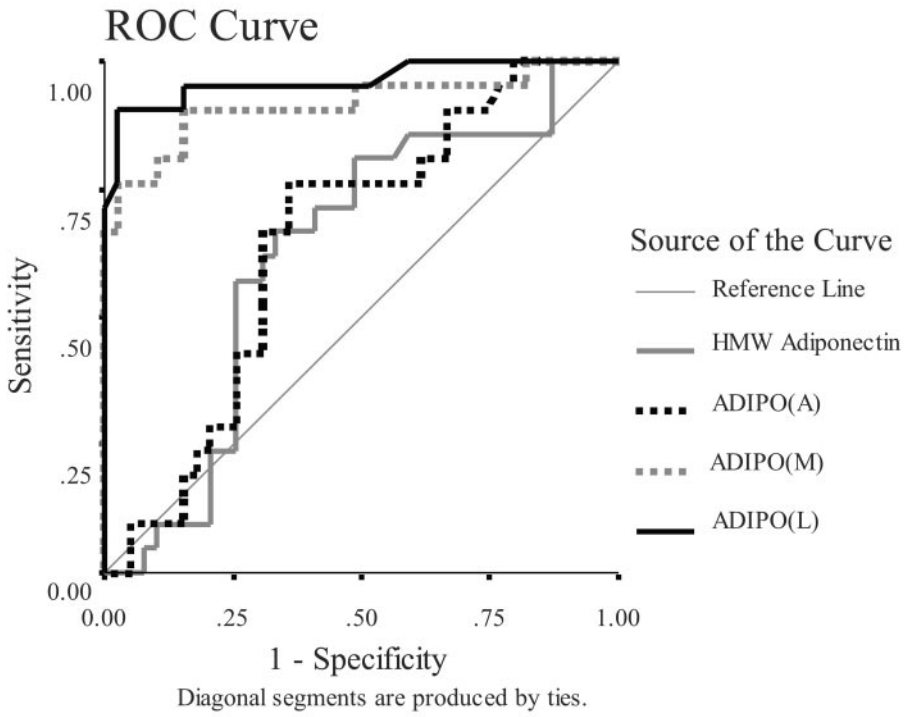

Figure 1—*Receiver-operating characteristic (ROC) curves for prediction of insulin resistance (defined by whole-body glucose uptake <40 μmol · kg^{-1} · min^{-1}) by total adiponectin by assay type: ADIPO$_L$ (LINCO), ADIPO$_M$ (Mediagnost), ADIPO$_A$ (ALPCO), and HMW adiponectin (novel ELISA HMW adiponectin [ALPCO]).*

nificantly better predictive performance over ADIPO$_A$ (P = 0.0001 for comparison with ADIPO$_L$ and P = 0.002 with ADIPO$_M$), with an AUC of 0.65 (0.51–0.79; P = 0.10), and HMW adiponectin (P = 0.0001 for comparison with ADIPO$_L$ and P = 0.001 with ADIPO$_M$), with an AUC of 0.63 (P = 0.20). Similar results were observed when defining insulin resistance by homeostasis model assessment >2.5 (ADIPO$_L$ AUC = 0.96 [95% CI 0.91–1.02], ADIPO$_M$ 0.92 [0.83–1.00], ADIPO$_A$ 0.65 [0.511–0.79], and HMW 0.628 [0.48–0.77]) with predictive values consistent with those reported recently (12). Analysis using the ratio of HMW to total adiponectin did not materially change the results (data not shown). Similarly, AUC indicated greater ability to predict IGT of ADIPO$_L$ (AUC = 0.95; P < 0.001) and ADIPO$_M$ (AUC = 0.90; P < 0.001) than ADIPO$_A$ for measuring total (AUC = 0.65; P = 0.05) and HMW adiponectin (AUC = 0.63; P = 0.10) .

The ADIPO$_L$ and ADIPO$_M$ techniques showed a more highly significant effect of exercise training on total adiponectin in all subjects than that seen on total or HMW adiponectin using ADIPO$_A$ (Table 3). No significant change in total adiponectin was observed among subjects with NGT using ADIPO$_L$ or ADIPO$_M$, but substantial increases in adiponectin were detected in participants with IGT or type 2 diabetes. In contrast, ADIPO$_A$ showed a similar magnitude of effect of training in subjects with NGT, IGT, and type 2 diabetes, and the change in total adiponectin

Table 3—*Measures of adiponectin by sex and glucose tolerance group*

	Baseline	Posttraining	Absolute change	% change	*P* value*
All subjects (n = 60)					
ADIPO$_L$	5.2 ± 3.1	6.9 ± 2.8	1.7 ± 2.3	32.7	<0.001
ADIPO$_M$	5.4 ± 3.5	7.1 ± 3.3	1.8 ± 2.9	33.3	<0.001
ADIPO$_A$	6.0 ± 3.4	7.7 ± 3.4	1.7 ± 4.5	28.3	0.004
HMW adiponectin	2.7 ± 2.1	3.8 ± 2.1	1.1 ± 3.0	40.7	0.008
Glucose tolerance group					
Subjects with NGT (n = 20)					
ADIPO$_L$	8.7 ± 2.5	8.6 ± 2.7	−0.2 ± 1.7	−2.3	0.97
ADIPO$_M$	8.8 ± 3.4	8.8 ± 3.7	0.0 ± 3.2	0.0	0.97
ADIPO$_A$	6.7 ± 2.9	8.7 ± 3.9	1.9 ± 3.9	28.4	0.04
HMW adiponectin	2.9 ± 1.4	4.1 ± 2.2	1.2 ± 2.1	41.4	0.02
Subjects with IGT (n = 20)					
ADIPO$_L$	3.4 ± 1.2	5.8 ± 2.2	2.4 ± 1.8	70.6	<0.001
ADIPO$_M$	3.5 ± 1.5	6.0 ± 2.4	2.5 ± 2.1	71.4	<0.001
ADIPO$_A$	6.3 ± 3.0	7.7 ± 3.5	1.5 ± 4.6	23.8393	0.17
HMW adiponectin	2.8 ± 1.9	3.9 ± 2.4	1.1 ± 3.3	39.3	0.14
Type 2 diabetic subjects (n = 20)					
ADIPO$_L$	3.5 ± 1.9	6.2 ± 2.5	2.7 ± 2.2	77.1	<0.001
ADIPO$_M$	3.8 ± 2.2	6.6 ± 2.9	2.8 ± 2.6	73.7	<0.001
ADIPO$_A$	5.1 ± 4.0	6.8 ± 2.6	1.7 ± 5.0	33.3	0.15
HMW adiponectin	2.4 ± 2.8	3.3 ± 1.7	0.8 ± 3.5	33.3	0.30

Data are means ± SD. **P* value from paired *t* test. Nonparametric Wilcoxon signed-rank test produced comparable results.

was less pronounced and did not achieve statistical significance in the IGT and type 2 diabetic groups. Stratifying by sex showed a similar pattern of more highly significant results using $ADIPO_L$ and $ADIPO_M$ methods than $ADIPO_A$, with a slightly stronger effect of training on adiponectin in men than women (data not shown). Additionally, changes with exercise correlated more strongly with changes in total adiponectin as measured by $ADIPO_L$ and $ADIPO_M$ than by total or HMW for free fatty acids ($r = 0.38$, $P < 0.01$ and $r = 0.30$, $P = 0.02$ vs. $r = 0.03$, $P = 0.85$ and $r = 0.03$, $P = 0.81$, for $ADIPO_L$, $ADIPO_M$, $ADIPO_A$, and HMW, respectively), total cholesterol ($r = -0.34$, $P < 0.01$ and $r = -0.28$, $P = 0.03$ vs. $r = 0.17$, $P = 0.20$ and $r = 0.18$, $P = 0.16$, respectively), triglycerides ($r = -0.34$, $P < 0.01$ and $r = -0.20$, $P = 0.13$ vs. $r = 0.11$, $P = 0.41$ and $r = 0.09$, $P = 0.48$, respectively), and insulin ($r = -0.20$, $P = 0.12$ and $r = -0.18$, $P = 0.18$ vs. $r = 0.06$, $P = 0.67$ and $r = 0.03$, $P = 0.82$, respectively). Similarly, the magnitudes of correlations of change in 2-h OGTT glucose and whole-blood glucose uptake were greater with change in $ADIPO_L$ and $ADIPO_M$ than $ADIPO_A$ or HMW adiponectin; however, all associations failed to reach statistical significance (not shown). Change in HDL with training was not significantly associated with changes in total adiponectin or HMW as measured by any assays. Finally, given our SD of 0.3, we had 80% power to detect a difference in AUC of 0.15 between different adiponectin assays.

CONCLUSIONS — We confirm herein that total adiponectin as measured by $ADIPO_L$ and $ADIPO_M$ is significantly correlated with insulin sensitivity and metabolic variables. Additionally, total adiponectin as measured by $ADIPO_L$ and $ADIPO_M$ is clearly superior to either HMW or total adiponectin as measured by $ADIPO_A$ at identifying the presence of insulin resistance and predicting levels of metabolic variables. Adiponectin levels increase with exercise and significantly correlate with improvements in lipid profile when total adiponectin is measured by $ADIPO_L$ and $ADIPO_M$ but not with total adiponectin or HMW adiponectin measured by $ADIPO_A$. Finally, we did not detect any clinically significant difference between HMW adiponectin and total adiponectin, when both are measured by $ADIPO_A$, at predicting insulin sensitivity and metabolic variables. The associations between metabolic variables and HMW adiponectin, when measured by techniques other than the assays used herein, remains to be seen.

Previous studies (10,13,14,19) investigating the differential associations of multimeric forms of adiponectin have used either Western blot technique or, subsequently, a recently described ELISA technique to quantify the proportion of total adiponectin circulating in the HMW form (12). A recent article (12) reporting that the HMW adiponectin–to–total adiponectin ratio is slightly superior to total adiponectin (AUC 0.713 vs. 0.615) at predicting the presence of insulin resistance and the metabolic syndrome used the same ELISA technique used herein. We replicated herein the predictive value of these measurements, as expressed by almost identical receiving-operating characteristic curves in this and the previous study (12). While we report that HMW and total adiponectin, as measured by the recently reported ALPCO ELISA (16), were not different in predicting the presence of insulin resistance or IGT, we also report that the other two total adiponectin assays evaluated herein are significantly better in this respect. This study was adequately powered to detect a 0.15 difference in AUC, which is clinically important.

We also report herein that both total and HMW adiponectin increase with exercise but that correlations with metabolic parameters are indistinguishable when total adiponectin and HMW adiponectin are considered (as measured using a commercially available ELISA from the same source). Our findings are in agreement with those reported by Bobbert et al. (14), who found that both HMW and total adiponectin increased to a similar degree with exercise and weight loss in 17 obese men and women, but there was no correlation between adiponectin (HMW or total) and insulin sensitivity either before or after weight loss in this study. HDL was the only metabolic variable correlated with total or HMW adiponectin in the previous study (14). The small study group, which included subjects with NGT, IGT, and type 2 diabetes and/or the interassay variability as well as the relative insensitivity of the Western methodology compared with radioimmunoassay or ELISA may have accounted for these prior findings (14). We have used a more sensitive and precise methodology, i.e., ELISA, to quantitate HMW adiponectin, and our sample size was 3.5 times larger. Importantly, both total adiponectin and HMW adiponectin showed similar correlation with insulin sensitivity and metabolic variables, although HMW adiponectin (measured using the current commercially available ELISA) was not significantly associated with any study variable in the current study. The magnitude of the correlation coefficients was similar to those previously reported using a larger sample size (16), however, supporting the validity of our measurements.

Similar to changes in total adiponectin, the process regulating the production of adiponectin oligomers is incompletely understood in humans. Posttranslational modification of lysine residues within the collagenous domain of the molecule appears to be involved and adiponectin glycosylation is reduced in individuals with diabetes, explaining the low HMW adiponectin observed in this population (11). Several studies where HMW adiponectin is measured by Western blot have reported a selective reduction in HMW adiponectin in subjects with type 2 diabetes (10,11,20) and a preferential elevation following treatment with the insulin sensitizer rosiglitazone (19). Two human mutations that impair the formation of HMW adiponectin have been described in individuals with type 2 diabetes, suggesting, albeit not proving, a possible role for HMW adiponectin in the pathogenesis of diabetes (10).

In the initial description of the ELISA system for selective measurement of adiponectin multimers, Ebinuma et al. (16) compared Western blotting to their selective ELISA. They found a strong correlation between multimer ELISA and densitometry results for HMW adiponectin using samples from 16 healthy volunteers, but the assay has not been tested in individuals with IGT and diabetes, in whom low HMW adiponectin levels have been described. It is also important to note that the precision of the total and selective ELISAs in the previous study are based on measurement of two human samples only (16). We expand herein by providing more extensive evaluation, and although we duplicate associations with-total adiponectin, we report that the specific assay used may not be as sensitive as other commercially available total adiponectin assays, possibly due to differences in the antibody used.

Strengths of our study include the larger study size compared with the previous study (14) and the detailed measurement of body composition, lipids,

and parameters of insulin sensitivity before and after a 4-week exercise intervention, allowing for assessment of associations both at baseline and of changes in study variables with exercise. While it is possible that laboratory measurement error may have played a role in our finding of lack of superiority of HMW adiponectin relative to total adiponectin in predicting insulin resistance, the consistency of the magnitudes of correlations from our study with the previous studies using $ADIPO_L$ (13), $ADIPO_M$ (21), and $ADIPO_A$ (12) indicate that it is more likely that our results are due to the relatively poor performance of the $ADIPO_A$ and HMW adiponectin assay. Further studies, using additional methods to measure HMW adiponectin, are needed to draw firm conclusions regarding the biological activity of HMW adiponectin.

In summary, using a recently developed, commercially available ELISA technique, we failed to demonstrate a stronger association of insulin sensitivity and metabolic variables with HMW adiponectin versus total adiponectin measured by existing assays in individuals with NGT or IGT or type 2 diabetes. This data does not support the superiority of HMW adiponectin, as measured by the novel ELISA, over total adiponectin in assessing insulin sensitivity and changes with physical training. Moreover, we document significant differences in the ability of total adiponectin to predict the presence of insulin resistance when measured using different commercially available assays.

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