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Analytical Validation and Biological Evaluation of a High–Molecular-Weight Adiponectin ELISA

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Background: Of the 3 circulating multimeric forms of adiponectin, the high–molecular-weight (HMW) form, as measured by size-exclusion and/or immunoblotting techniques, is a better index of insulin sensitivity for monitoring health and disease than is total adiponectin. We aimed to develop a simple ELISA to measure HMW adiponectin.
Methods: We pretreated serum or plasma samples with digestion solution containing proteinase K (Millipore, ESDS). HMW (Millipore, EZHMWA-64K) and total adiponectin (Millipore, EZHADP-61K) concentrations were measured in treated and untreated samples, respectively, from 108 individuals and from 20 morbidly obese patients before and at 1, 3, 6, and 12 months after gastric-bypass surgery.
Results: The ELISA has a dynamic range of 3–200 μg/L and a detection limit of 0.8 μg/L. Intraassay and interassay CVs were <4% and <10%, respectively. Sample-dilution curves paralleled the calibration curves. Fast protein liquid chromatography profiles of the proteinase K-treated samples revealed predominantly HMW adiponectin. Values for HMW adiponectin produced with this method are comparable with those obtained with Western blot analysis ($y = 0.77x - 0.15$; $r = 0.96$; n = 56). Body mass index (BMI)- and sex-related changes were more pronounced for HMW adiponectin and percentage of HMW adiponectin than for total adiponectin. HMW and total adiponectin increased after bypass surgery, but changes in HMW adiponectin were more pronounced and preceded changes in total adiponectin.
Conclusion: This simple, rapid ELISA for HMW adiponectin recognizes the HMW isoform, produces results closely correlated with those obtained with Western blotting, and appears to better distinguish BMI-, sex-, and weight loss–associated differences than assays for total adiponectin.
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Adiponectin, the only secretory hormone of fat cells that is paradoxically decreased in obesity (1), also has insulin-sensitizing effects (2–4); all other known adipokines are increased in obesity and induce insulin resistance (5). Given that adipose tissue is the primary site of adiponectin secretion, the decrease in the production and secretion of adiponectin with increasing adiposity is an intriguing phenomenon. Substantial and convincing evidence demonstrates that hypoadiponectinemia is associated with insulin resistance and obesity, diabetes, and metabolic syndrome including cardiovascular abnormalities (6, 7). Dietary, lifestyle, and/or pharmacological interventions (thiazolidinediones) that improve insulin sensitivity also increase circulating adiponectin concentrations (8, 9). In addition, decreased adiponectin could be a risk factor for the progression of insulin resistance and type 2 diabetes in healthy individuals, with or without impaired glucose tolerance (10, 11).

Adiponectin is a 28-kDa protein with a collagen-like structure; it circulates predominantly in 3 multimeric forms, i.e., trimer, hexamer, and high–molecular-weight (HMW)[6] multimers (12- and 18-mers), which are evident

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[6] Nonstandard abbreviations: HMW, high–molecular-weight; BMI, body mass index; FPLC, fast protein liquid chromatography.

from size-fractionation (gel filtration, velocity gradient, and gel electrophoresis) and immunoblotting experiments (5). These 3 multimeric adiponectin isoforms have different biological activities, with HMW adiponectin probably being the active form (5, 12, 13). Tonelli et al. (14) and Pajvani et al. (15) initially showed that the distribution of these multimers, particularly as measured by the ratio of HMW adiponectin to total adiponectin (i.e., percentage of HMW adiponectin), correlates better with thiazolidinedione-mediated improvement in insulin sensitivity than does the total adiponectin concentration. Percentage of HMW adiponectin appears to be a better index of insulin sensitivity (14, 15) and of metabolic syndrome trait cluster(s) (16).

To measure HMW adiponectin and/or percentage of HMW adiponectin, most studies have combined size fractionation and immunoblotting (14–21), a cumbersome approach that cannot easily be adopted for high-throughput analyses of several samples. Therefore, we have developed an ELISA to measure HMW adiponectin in human serum and plasma samples.

Materials and Methods

STUDY PARTICIPANTS

We obtained human serum and/or plasma samples from 4 different sources. In the initial development and validation of the HMW adiponectin ELISA, plasma (containing EDTA dibasic sodium salt, 1.5 g/L) and serum samples from 30 individuals (4 men and 26 women) were obtained from a commercial source (Bioreclamation). After assay development, we obtained another 56 plasma samples from Bioreclamation to compare HMW adiponectin values obtained with the new ELISA and with Western blotting; we obtained no biochemical or anthropometric data on these individuals. In a retrospective study, we obtained another 108 plasma samples from Bioreclamation and measured HMW adiponectin and total adiponectin to evaluate the effect of body mass index (BMI) and sex. Table 1 summarizes the anthropometric data for these individuals. Plasma samples obtained from 20 morbidly obese patients before and at 1, 3, 6, and 12 months after Roux-en-Y gastric-bypass surgery were also measured to

evaluate the effect of weight loss on HMW adiponectin as measured with this new ELISA method. The changes in body composition, measures of insulin resistance, and the concentrations of adiponectin and its isoforms that occurred after gastric-bypass surgery have previously been described (22). The Institutional Review Board of the University of California, Davis, approved the experimental protocol, and all study individuals provided written informed consent to participate in this study.

FAST PROTEIN LIQUID CHROMATOGRAPHY ANALYSIS

Samples of human serum and plasma (100 μL of a 1/5 dilution in sample-digestion buffer, EDGB) were fractionated before and after proteinase K (sample-digestion solution, ESDS) treatment by size-exclusion fast protein liquid chromatography (FPLC) on a Superdex 200 10/300 Tricorn column (GE Healthcare). Sample treatment with the enzyme selectively degrades hexamer and trimer isoforms without affecting HMW adiponectin. The mobile phase was 25 mmol/L sodium phosphate, pH 7.5, containing 100 mg/L phenylmethylsulfonyl fluoride, with a flow rate of 0.5 mL/min and a 0.25-mL fraction size. FPLC fractions of the enzyme-treated and untreated plasma sample were then assayed for adiponectin immunoreactivity with the Human Adiponectin ELISA (EZHADP-61K; Millipore) and Human HMW Adiponectin ELISA (EZHMWA-64K, Millipore) reagent sets.

WESTERN BLOT ANALYSIS

We used Western blotting to measure the trimer, hexamer, and HMW adiponectin isoforms in 56 human plasma samples. We first fractionated plasma samples by polyacrylamide gel electrophoresis under nondenaturing and nonreducing conditions with a 1.5-mm 4%–20% polyacrylamide Tris-glycine gel cassette (Invitrogen, EC6028BOX), 10× Novex TrisGlycine SDS Running Buffer (Invitrogen, LC2675; diluted 1/10), and 2× TrisGlycine SDS Sample Buffer (Invitrogen, LC2675) in a XCell SureLock MiniCell (Invitrogen, EI0001) for 1.5 h at 150 V. We ran SeeBlue Plus2 Pre-Stained Standard (Invitrogen, LC5925) with each electrophoresis separation. Proteins were transferred from the gel to a nitrocellulose membrane at 100 V for 2 h in a Mini Trans-Blot

Table 1. Anthropometric variables for the study individuals.[a]

Group	Sex, n	Age, years	Race, n	Systolic BP[b], mmHg	Diastolic BP, mmHg	BMI, kg/m²
Lean (n = 25)	M, 17 F, 8	39.0 (12.3)	C, 5 AA, 16 H, 4	128 (12)	77 (8)	21.56 (1.85)
Overweight (n = 28)	M, 12 F, 16	37.8 (12.0)	C, 8 AA, 18 H, 2	121 (12)	76 (8)	26.79 (1.34)
Obese (n = 55)	M, 25 F, 30	36.9 (10.7)	C, 16 AA, 30 H, 9	126 (14)	79 (7)	35.76 (5.13)

[a] Data are presented as the mean (SD) where appropriate.

[b] BP, blood pressure; C, Caucasian; AA, African American; H, Hispanic; M, Male; F, Female.

Cell with 200 mL/L methanol, 2 g/L sodium dodecyl sulfate and 40 mL 25× Novex Tris-Glycine Transfer Buffer (Invitrogen, LC3675) in 760 mL distilled water. After blocking overnight at 4 °C with Blocker® Casein in Tris-buffered saline (1% by weight Casein Hammersten Grade in Tris-buffered saline, Kathon as preservative, pH 7.4, Pierce, 37532), we incubated the nitrocellulose membrane in the same buffer for 1.5 h with mouse antihuman adiponectin monoclonal antibody labeled with horseradish peroxidase (1/1000 dilution) (R&D Systems, MAB10651). After washing away the excess antibody, we added detection reagents 1 and 2 in equal proportions (Amersham/GE Healthcare, RPN2106V1 and RPN2106V2), incubated the membrane for 1 min, and exposed the membrane for 1–4 min to BioMax Light Film (Kodak, 869358). We digitally photographed the immunoblot and quantified the trimer, hexamer, and HMW adiponectin bands with AlphaImager and AlphaEase FC software V.4.1.0 (Alpha Innotech).

MEASUREMENT OF TOTAL HUMAN ADIPONECTIN AND HMW ADIPONECTIN

We measured total adiponectin in serum and plasma samples with Millipore's Human Adiponectin ELISA. We used the newly developed HMW Adiponectin ELISA to measure HMW adiponectin. We diluted 20-μL serum samples 1/10 with sample-digestion buffer (Millipore, EDGB) and pretreated the samples with sample-digestion solution (Millipore, ESDS) for 2 h at 37 °C. Enzyme-treated samples were diluted further to 1/20 with 1× sample dilution buffer (Millipore, ESDB) and then assayed for HMW adiponectin with the Human HMW Adiponectin ELISA. The pairs of antibodies for capture and detection were different for the total (Millipore, EZHADP-61K) and HMW (Millipore, EZHMWA-64K) adiponectin ELISA reagent sets. The calibrator for the total adiponectin ELISA was pooled human serum calibrated against recombinant human adiponectin produced in mammalian cells (Millipore, 1061-K). We used the same calibrator for the HMW Adiponectin ELISA after we corrected for the proportion of HMW adiponectin, as measured by FPLC analysis.

STATISTICAL ANALYSIS

We carried out a regression analysis to compare HMW adiponectin values obtained with the HMW ELISA reagent set and with Western blotting. Differences in the concentrations of total adiponectin and HMW adiponectin between lean, overweight, and obese individuals, between male and female individuals, and before and after gastric-bypass surgery were compared by means of 2-tailed Student t-tests under the assumption of unequal variances. Multivariate analysis was performed by stepwise regression (Minitab 14; Minitab). BMI was entered as a response variable, and sex, age, race, diastolic and systolic blood pressures, total adiponectin, HMW adiponectin, and percentage of HMW adiponectin were entered as predictor variables. HMW, percentage of

HMW, and total adiponectin values are expressed as the mean (SE), whereas all other data including those of anthropometric variables and assay characteristics are expressed as the mean (SD).

Results

CHARACTERISTICS OF THE HMW ADIPONECTIN ASSAY

The calibration curve for this HMW Adiponectin ELISA (n = 8, Fig. 1A) has a concentration range of 3–200 μg/L. Assay imprecision (CV) ranged between 4% and 8% for absorbance and was <2% for the back-calculated values, as evaluated by fixed weighted 5-parameter (asymmetrical) logistic curve fitting (StatLIA software; Brendan Technologies). This program yielded an assay sensitivity of 0.8 μg/L, which is the mean + 2 SDs of the minimum detection limit (n = 8). Interassay imprecision (4 assays, 10 samples) and intraassay imprecision (5 results within a single assay, 8 samples; Table 2) were measured after the samples had each been pretreated and diluted (1/200 final dilution). HMW adiponectin values reported in Table 2 are after sample pretreatment and dilution from HMW adiponectin assays. Intraassay CVs were between 1.0% and 3.4% for sample HMW adiponectin values between 6.0 μg/L and 65.3 μg/L. Interassay CVs were between 3.0% and 8.1% for HMW adiponectin values between 13.3 μg/L and 61.5 μg/L. To evaluate the robustness of the assay, we analyzed 18 different serum samples on 2 different occasions. We observed no significant differ-
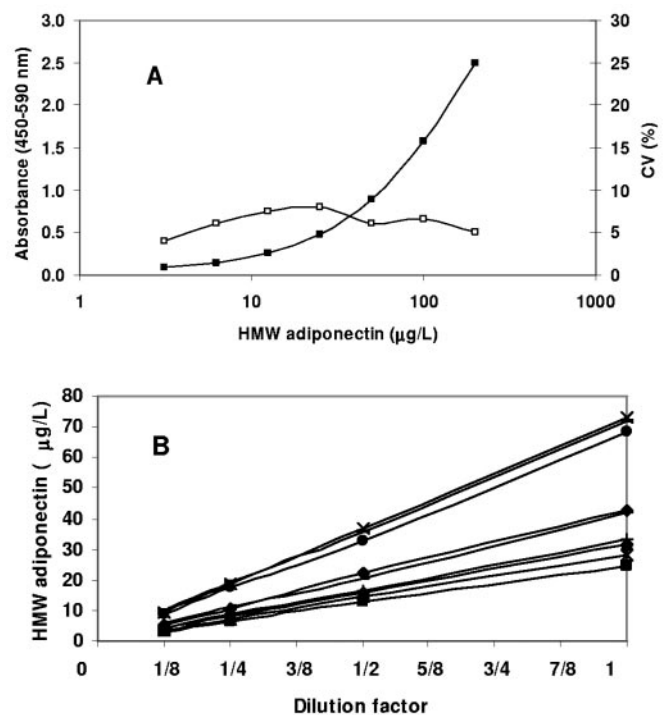

Fig. 1. Evaluation of the HMW Adiponectin ELISA.

(*A*), calibration curve with mean absorbance values (n = 8; ■) and an imprecision profile (□) of CVs based on the same 8 calibration curves. (*B*), linearity of the dilution curves for 10 serum samples after pretreating and diluting the sample; serial dilutions were with ELISA buffer.

Table 2. Interassay and intraassay CVs.

Interassay (n = 4)			Intraassay (n = 5)		
Sample no.	Mean concentration, μg/L	CV, %	Sample no.	Mean concentration, μg/L	CV, %
1	13.3	4.1	11	6.0	1.0
2	21.2	8.1	12	11.1	1.3
3	23.2	4.1	13	13.7	3.4
4	23.7	3.7	14	21.3	2.1
5	28.7	3.1	15	25.4	3.3
6	26.6	5.0	16	27.3	2.6
7	34.8	7.8	17	39.5	3.1
8	38.4	9.1	18	65.3	
9	60.8	3.0			
10	61.5	3.8			

ences (i.e., P >0.05) in HMW adiponectin values [mean (SD), 8.7 (4.5) mg/L vs 9.0 (4.8) mg/L] with a mean difference of −1.6% (7.1%) in the mean values obtained with the 2 replicate assays. Recovery was evaluated by adding different concentrations of HMW adiponectin (obtained from human serum after enzyme digestion and calibration with the HMW Adiponectin ELISA) to 8 pretreated and diluted samples. The data in the recovery experiments were expressed as a percentage of the expected value (basal plus added HMW adiponectin). The mean recovery was 101.2% (1.9%) for 3.125 μg/L, 106.6% (2.3%) for 25 μg/L, and 112.4% (3.1%) for 100 μg/L. An evaluation of the linearity of endogenous HMW adiponectin concentrations in serial dilutions (1/8, 1/4, and 1/2) of 10 initially diluted and pretreated serum samples yielded values between 95% and 110% of the expected values (value of a 1/1 dilution times the dilution factor). The dilution curves were linear for all 8 serum samples (Fig. 1B) and paralleled the calibration curve. Insulin, glucagons, leptin, glucagon-like peptide 1, amylin, resistin, acylation-stimulating protein, different interleukins, interferon γ, tumor necrosis factor α, transforming growth factor β, and plasminogen activator inhibitor 1 diluted in assay buffer (20–1000 μg/L) showed no cross-reactivity (<0.01%) with the HMW Adiponectin ELISA in direct tests. Values for paired serum (x) and plasma (y) samples (n = 21) showed excellent correlation in the same assay ($y = 0.95x - 0.23$; $r = 0.99$); however, plasma values [7.0 (5.1) mg/L] were slightly but significantly lower [mean difference, 8.0% (5.7%); P <0.01] than serum values [7.5 (5.3) mg/L].

FPLC PROFILES

Six serum samples with or without proteinase K pretreatment were fractionated by FPLC on a Superdex G-200 column. The total adiponectin ELISA for FPLC fractions of untreated samples detected 3 distinct peaks for the HMW, hexamer, and trimer isoforms. These results suggest that the total adiponectin ELISA recognizes all 3 adiponectin multimeric isoforms (Fig. 2A). The HMW Adiponectin ELISA recognizes all 3 isoforms, but hexamers and trimers are recognized to an appreciably lesser

extent before the simple enzyme pretreatment. After pretreatment, the HMW adiponectin peak remains unaltered, whereas both hexamer and trimer peaks virtually disappear because of their preferential degradation by the enzyme (Fig. 2A). The efficacy of the HMW Adiponectin ELISA for measuring HMW adiponectin can be ascribed to both this ELISA's lower sensitivity for recognizing hexamers and trimers and preferential enzymatic degradation of these 2 isoforms.
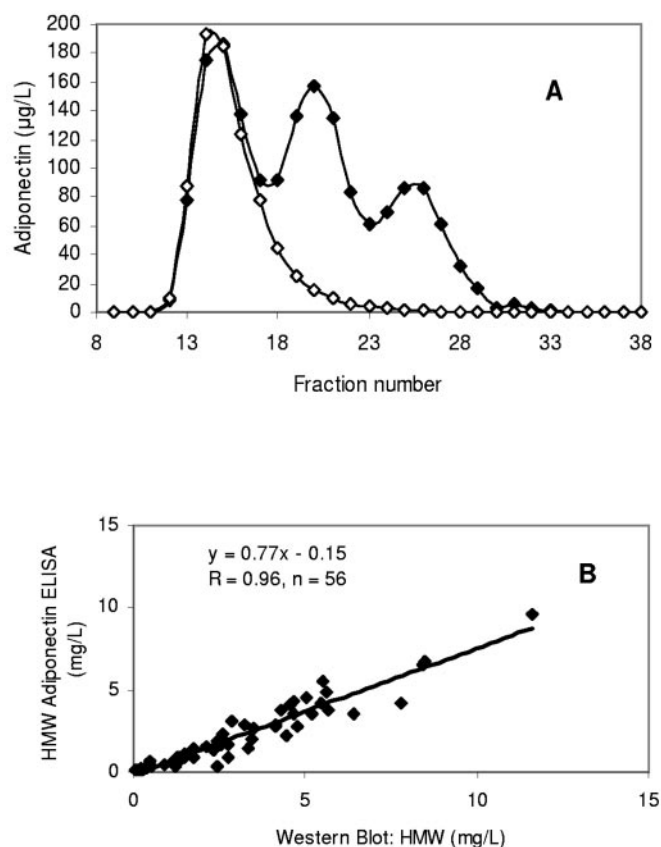
Fig. 2. Comparison of ELISA and Western blot analysis.

(*A*), a representative FPLC elution profile of human serum before (♦) and after (◇) sample pretreatment and subsequent ELISA of total and HMW adiponectin. (*B*), comparison with Western blotting results (ratio of HMW adiponectin to total adiponectin x total adiponectin).

WESTERN BLOT ANALYSIS

We measured the relative distributions of adiponectin isoforms in 56 human plasma samples by Western blotting with nondenaturing and nonreducing gels. HMW adiponectin concentrations in these samples were then extrapolated by multiplying the percentage HMW adiponectin values obtained by Western blotting by the total adiponectin concentrations obtained with the Millipore human adiponectin ELISA. The HMW adiponectin values indirectly derived via Western blotting were then compared with those obtained for the HMW Adiponectin ELISA after sample pretreatment. HMW adiponectin concentrations obtained with these 2 different methods show high correlation ($y = 0.77 x − 0.15$; $r = 0.96$) (Fig. 2B). Of note is that the apparent difference (approximately 23%) in absolute values could be due to these 2 very different methods and the possibilities of differential recognition and/or different affinities of the antibodies used.

EFFECT OF BODY WEIGHT AND SEX

Mean (SE) values for total adiponectin, HMW adiponectin, and percentage of HMW adiponectin (relative to total adiponectin) in lean [mean BMI, 21.56 (1.85) kg/m^2], overweight [mean BMI, 26.79 (1.34) kg/m^2], and obese [mean BMI, 35.67 (5.13) kg/m^2] individuals are shown in Fig. 3A. As expected, concentrations of total adiponectin were significantly decreased in overweight individuals

Fig. 3. Total adiponectin, HMW adiponectin, and percentage of HMW adiponectin according to body weight and sex.

(A), values for lean (n = 25), overweight (n = 28), and obese (n = 55) individuals. *, $P <0.05$; **, $P <0.001$; +, $P <0.005$; ++, $P <0.0001$; †, $P <0.0002$; ††, $P <0.000005$. (B), values for African American women (n = 26) and men (n = 35). *, $P <0.05$; ++, $P <0.02$; †††, $P <0.01$. Columns represent the mean (SE).

[10.75 (0.98) mg/L; $P <0.05$] and obese individuals [9.45 (0.51) mg/L; $P <0.001$], compared with lean individuals [13.80 (1.08) mg/L]; however, the differences for HMW adiponectin concentrations were even more pronounced in overweight individuals [4.44 (0.55) mg/L; $P <0.005$] and obese individuals [3.60 (0.32) mg/L; $P <0.0001$], compared with lean individuals [7.06 (0.68) mg/L]. Similarly, the differences in percentage of HMW adiponectin were also more pronounced than for the total adiponectin differences in comparisons of overweight individuals [38.71% (2.55%); $P <0.0002$] and obese individuals [35.44% (1.61%); $P <0.000005$] with lean individuals [50.48% (2.21%)]. The differences in HMW adiponectin ($P <0.0005$) and percentage of HMW adiponectin ($P <0.0005$) were also more pronounced in the obese group [mean BMI, 35.75 (5.13) kg/m^2] than in the combined lean and overweight groups [mean BMI, 24.32 (3.07) kg/m^2], compared with the differences for total adiponectin ($P <0.005$). The ratio of HMW adiponectin to total adiponectin has previously been demonstrated to be higher in female individuals than in male individuals (3, 15). Because of the disproportionately higher ratio of males to females in the lean group than in the overweight and obese groups, the weight-related decrease in HMW adiponectin concentration may have been compromised due to a suboptimal selection of the study population.

As shown in Fig. 3B with mean (SE) values, the subpopulation of African American women [mean BMI, 29.74 (5.81) kg/m^2; n = 26] and men [mean BMI, 28.97 (7.78) kg/m^2; n = 35], featured more pronounced alterations for HMW adiponectin [5.68 (0.64) mg/L and 3.63 (0.39) mg/L, respectively; $P <0.02$] and percentage of HMW adiponectin [44.42% (2.38%) and 35.04% (2.26%), respectively; $P <0.01$] than for total adiponectin [12.27 (1.08) mg/L and 9.54 (0.64) mg/L, respectively; $P <0.05$]. Analysis of the entire dataset of 54 female and 54 male individuals revealed significantly higher values in females for HMW adiponectin ($P <0.05$), but not for percentage of HMW adiponectin ($P >0.05$) and total adiponectin ($P >0.05$).

In a multivariate analysis with stepwise regression, the best-fitting model included HMW adiponectin ($\beta = −1.01$; $P <0.0001$), sex ($\beta = 3.5$; $P <0.01$), and diastolic pressure ($\beta = 0.16$; $P >0.05$). This model accounted for 20.5% of the variance in BMI (adjusted R^2). Total adiponectin was not a significant predictor and was thus left out of the model.

GASTRIC-BYPASS SURGERY

Fig. 4 summarizes the effects of weight loss on total, HMW, and percentage of HMW adiponectin at 1, 3, 6, and 12 months after gastric-bypass surgery in 20 morbidly obese adults (19 women and 1 man). Gastric-bypass surgery produced appreciable weight loss and improved insulin sensitivity in all of the patients (22). Before surgery, the mean (SE) concentrations of total adiponectin and HMW adiponectin in the morbidly obese patients
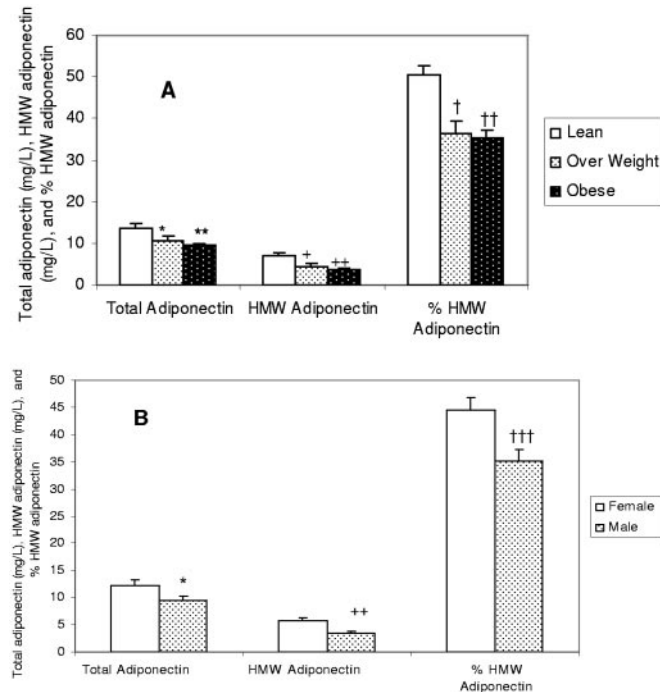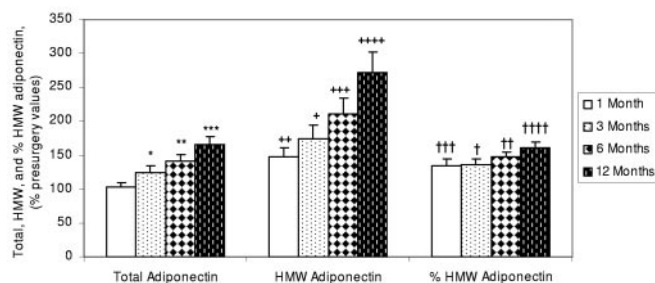
Fig. 4. Total, HMW, and percentage of HMW adiponectin in morbidly obese individuals after gastric-bypass surgery.

*, $P < 0.02$; **, $P < 0.001$; ***, $P < 0.00002$; +, $P < 0.002$; ++, $P < 0.0005$; +++, $P < 0.0002$; ++++, $P < 0.00002$; †, $P < 0.0005$; ††, $P < 0.00001$; †††, $P < 0.000005$; ††††, $P < 0.0000005$. Columns (percentage of baseline value) represent the mean (SE).

were 7.2 (0.5) mg/L and 2.2 (0.3) mg/L, respectively, and the mean (SE) percentage of HMW adiponectin value was 28.1% (2.3%). Total adiponectin concentrations were unaltered at 1 month after surgery [102.47% (7.66%) of presurgery concentrations, $P > 0.05$] but were significantly increased at 3 months [124.52% (9.02%), $P < 0.02$], 6 months [141.3% (10.50%), $P < 0.001$], and 12 months [166.12% (11.44%), $P < 0.00002$]; however, the changes in HMW adiponectin were significantly more pronounced than for total adiponectin at 1 month [147.00% (14.48%), $P < 0.0005$], 3 months [173.65% (20.58%), $P < 0.002$], 6 months [210.5% (23.44%), $P < 0.0002$], and 12 months [271.72% (30.67%), $P < 0.00002$] after gastric-bypass surgery. In addition, the changes in percentage of HMW adiponectin were also more significant than for total adiponectin at 1 month [134.61% (9.17%), $P < 0.00005$], 3 months [136.08% (8.26%), $P < 0.0005$], 6 months [146.81% (7.64%), $P < 0.00001$], and 12 months [160.74% (7.74%), $P < 0.0000005$] after surgery.

Discussion

That adiponectin has an insulin-sensitizing effect (2–4) and is an important biomarker for insulin sensitivity in various pathophysiological conditions associated with insulin resistance is well documented (1, 5–7). In addition, the circulating adiponectin concentration seems to be a good predictor of the progression of insulin resistance in healthy and/or glucose-intolerant individuals (10, 11). Therapeutic or dietary interventions intended to improve insulin sensitivity are usually associated with increased adiponectin concentrations (8, 9). Of the 3 distinct multimeric isoforms in the circulation, HMW adiponectin (12- to 18-mers) is believed to be the most biologically active (5, 12, 13). The HMW adiponectin concentration and/or percentage of HMW adiponectin are reported to be a better index of insulin sensitivity than total adiponectin (14, 21).

Testing the importance of HMW adiponectin in various metabolic states requires a simple, accurate, and rapid methodology. A majority of the studies have fractionated the different adiponectin multimers by size with gel electrophoresis, gel filtration, and velocity gradient centrifugation and then used immunoblotting with anti-adiponectin antibody to recognize the multimers. These time-consuming methods require experience in protein-separation techniques. In addition, these methods are not practical for analyzing several samples. Immunoassays, particularly ELISA, can be an alternative. Currently, 2 different ELISAs are commercially available (23, 24). Blu-her et al. (25) evaluated this HMW Adiponectin ELISA (ALPCO Diagnostics), an RIA for total adiponectin, and another ELISA (Mediagnost) to determine the best adiponectin assay for predicting improvement in insulin sensitivity after exercise in healthy individuals, those with impaired glucose tolerance, and diabetic individuals. These investigators found that the total adiponectin RIA, not the HMW Adiponectin ELISA, was the better predictor of insulin sensitivity. Another HMW Adiponectin ELISA developed by Nakano et al. (24) uses an antibody raised against HMW adiponectin but involves no enzyme pretreatment of the sample. Although this adiponectin ELISA is simple, it also measures the hexameric isoform to some degree (24).

Our HMW Adiponectin ELISA method is simple, rapid, and specific with respect to the multimeric (HMW) isoform. After a simple enzyme pretreatment of the sample, the new ELISA recognizes predominantly the HMW adiponectin isoform. This HMW Adiponectin ELISA meets most of the analytical-robustness criteria of a good immunoassay. In addition, the HMW adiponectin values obtained with this method and with a more conventional size-exclusion and Western blotting method are well correlated ($r = 0.96$) over a wide range of HMW adiponectin concentrations; however, there is a 23% difference in the absolute values, which is not surprising considering the 2 very different methodologies.

The relevance of this new HMW Adiponectin ELISA is demonstrated by comparing the values for HMW adiponectin and total adiponectin in the context of BMI, sex, and weight loss in morbidly obese patients. Obesity-related changes were more apparent for HMW adiponectin concentration and percentage of HMW adiponectin than for total concentration when these variables were evaluated for lean, overweight, and obese individuals (26–28). Similarly, we noted larger differences between men and women for HMW adiponectin concentration and percentage of HMW adiponectin than for the concentration of total adiponectin (3, 15, 29); however, our studies may have been compromised by confounding factors introduced by a suboptimal control population. Gastric-bypass surgery is an effective means of weight loss and leads to improved insulin sensitivity (30). Similar to the findings of Swarbrick et al. (22) with a different ELISA (23), the present study also demonstrated a significant increase in HMW adiponectin concentration and percentage of HMW adiponectin (but not for the concentration of total adiponectin) at 1 month after gastric-bypass surgery, when appreciable weight loss and an improvement in

insulin resistance are typically observed. The concentration of HMW adiponectin increased progressively at 3, 6, and 12 months after surgery, along with further decreases in body weight and insulin resistance (homeostasis model assessment), and the increases were proportionately greater than for total adiponectin concentrations.

In summary, we have developed a simple, robust, rapid, and size-specific ELISA for measuring HMW adiponectin. The results are comparable with those obtained with a Western blotting method. More pronounced differences were observed for HMW adiponectin than for total adiponectin with respect to obesity, weight loss, and sex. This assay may become a useful tool for high-throughput analyses of HMW adiponectin and may facilitate further elucidation of the importance of HMW adiponectin as a biomarker for monitoring the progression and treatment of diabetes and cardiovascular diseases.

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