ELISA For The Measurement of High Molecular Weight Adiponectin

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INTRODUCTION

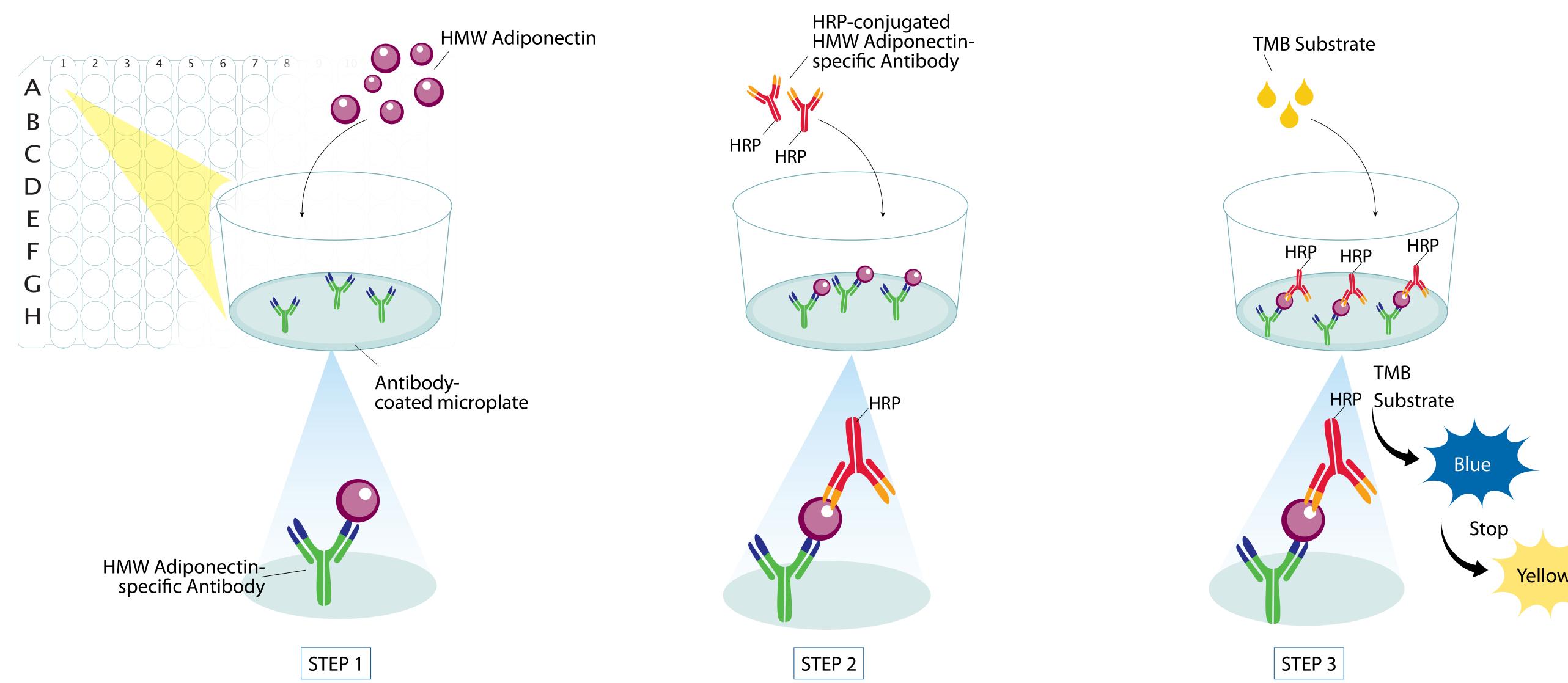
Adiponectin is an adipocyte-derived protein with diverse biological functions. It circulates in plasma as low molecular weight (LMW) trimers, medium molecular weight (MMW) hexamers, and high molecular weight (HMW) multimers. The different oligomeric forms have been suggested to exert distinct actions on specific target tissues. The HMW Adiponectin is the primary bioactive form with insulin-sensitizing activity and its circulating levels negatively correlate with obesity, insulin resistance and coronary artery disease (CAD). Current ELISA assays to detect HMW Adiponectin require the laborious process of selective enzyme digestion of the LMW and MMW forms. We have developed a novel ELISA that utilizes HMW Adiponectin-specific antibodies. This easy-to-use assay does not require any pretreatment of the sample and provides excellent reproducibility.

GENERAL METHODS

ANTIBODY GENERATION

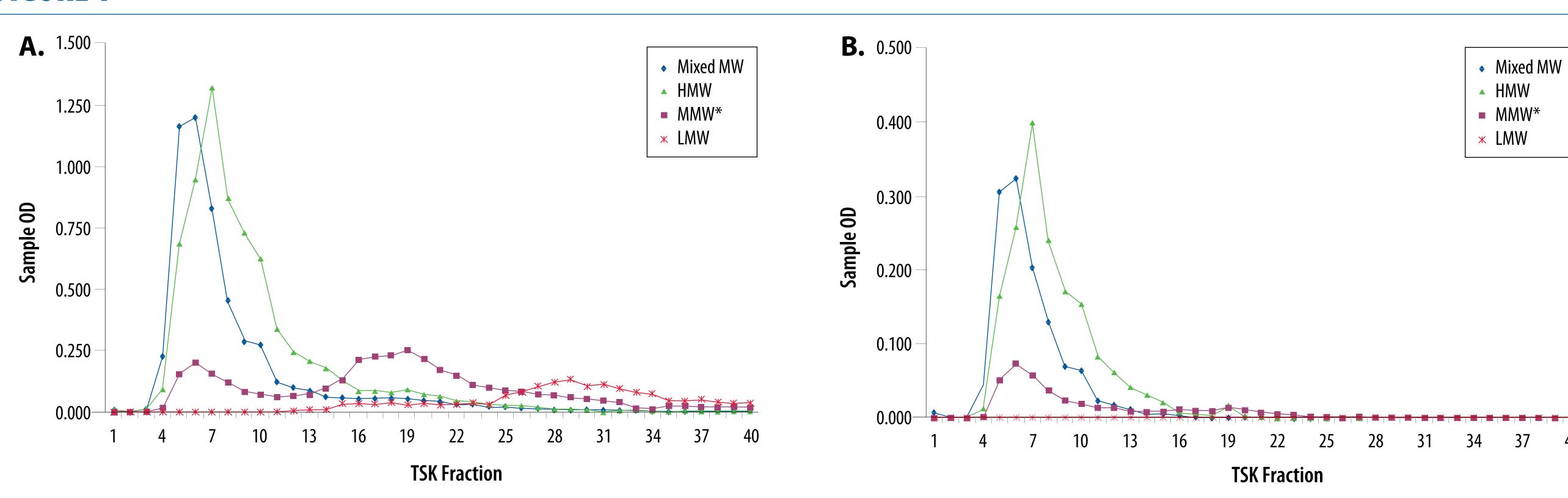
HMW Adiponectin was purified from a heterogeneous mixture of recombinant Adiponectins using gel filtration chromatography and used to generate anti-HMW Adiponectin monoclonal antibodies. The antibodies were rigorously tested to ensure specificity for the HMW isoform of Adiponectin. Two antibodies specific for HMW Adiponectin were paired as the capture antibody and detection antibody in a standard quantitative sandwich enzyme immunoassay.

ASSAY PROCEDURE



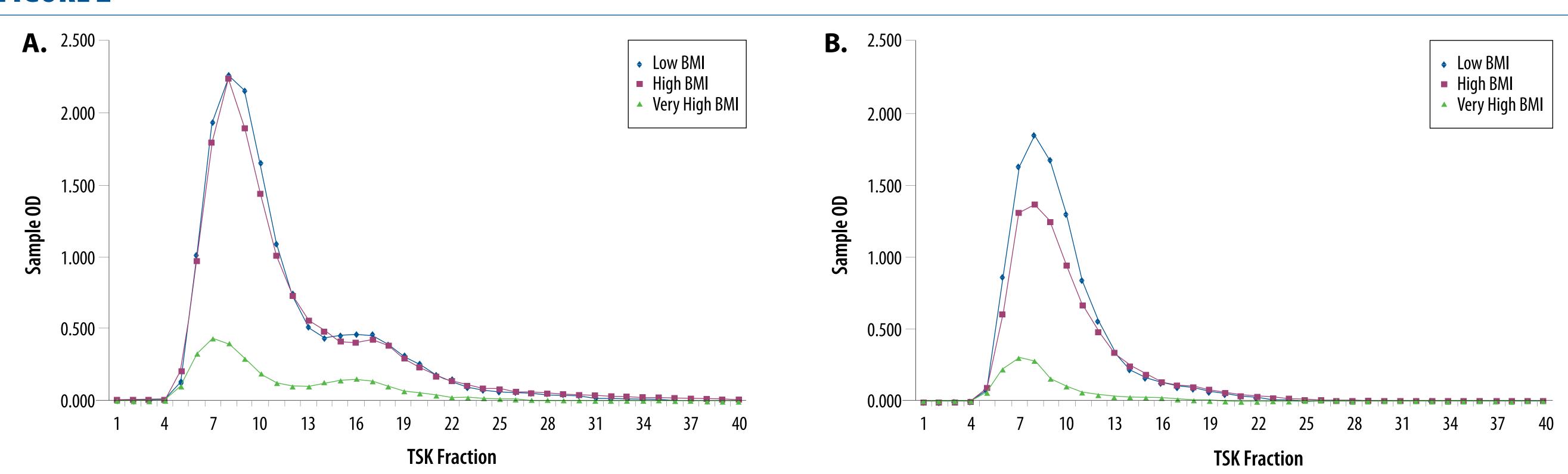
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FIGURE



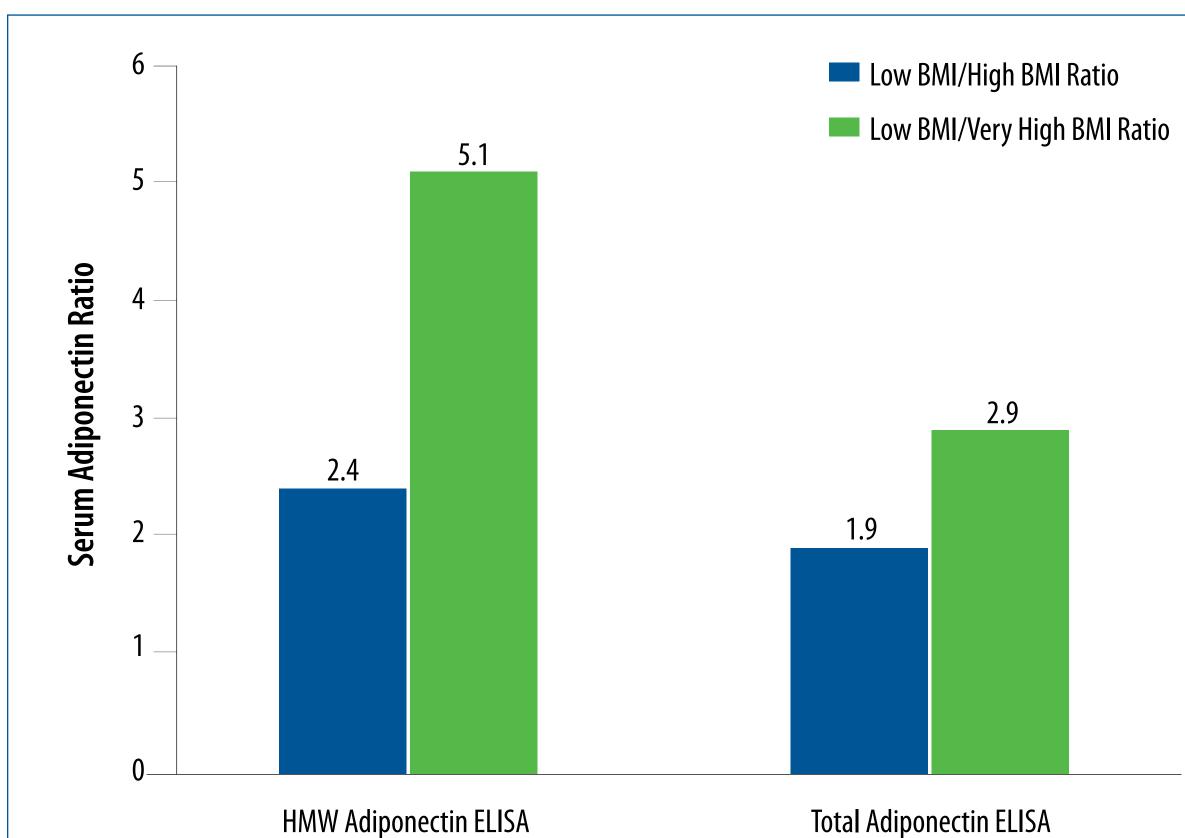
Detection of Adiponectin Isoforms using the Quantikine® Human Total Adiponectin/Acrp30 and Quantikine Human HMW Adiponectin/Acrp30 ELISA Kits. The HMW, MMW, and LMW isoforms of Adiponectin were purified from a heterogeneous mixture of recombinant Adiponectins using size exclusion chromatography. The HMW (green line), MMW* (pink line), and LMW (red line) fractions, as well as a pool of mixed molecular weight Adiponectins (blue line), were reapplied to a TSK gel chromatography column, and the individual fractions collected were analyzed using the Quantikine Human Total Adiponectin/Acrp30 ELISA Kit (Catalog # DRP300, A) and the Quantikine Human HMW Adiponectin/Acrp30 ELISA Kit (Catalog # DHWAD0, B). The Human Total Adiponectin/Acrp30 Immunoassay detected all three molecular weight Adiponectin isoforms, while the Human HMW Adiponectin/Acrp30 Immunoassay only detected HMW Adiponectin. *The MMW Adiponectin fraction contained a small amount of HMW Adiponectin.

FIGURE 2



Detection of Adiponectin Isoforms in Human Serum using the Quantikine Human Total Adiponectin/Acrp30 and Quantikine Human HMW Adiponectin/Acrp30 ELISA Kits. Serum from individuals with low BMI (blue line), high BMI (pink line), and very high BMI (green line) was applied to a TSK gel chromatography column. Individual fractions collected were analyzed using the Quantikine Human Total Adiponectin/Acrp30 ELISA Kit (A) and the Quantikine Human HMW Adiponectin/Acrp30 ELISA Kit (B). The Human Total Adiponectin/Acrp30 Immunoassay detected all three molecular weight Adiponectin isoforms, while the Human HMW Adiponectin/Acrp30 Immunoassay only detected HMW Adiponectin. In addition, a clearer discernment between the levels of HMW Adiponectin in the different BMI serum pools was observed using the Human HMW Adiponectin/Acrp30 Immunoassay (low BMI average=19.8, n=4; high BMI average=32.8, n=4; very high BMI average=55.4, n=3).

FIGURE 3



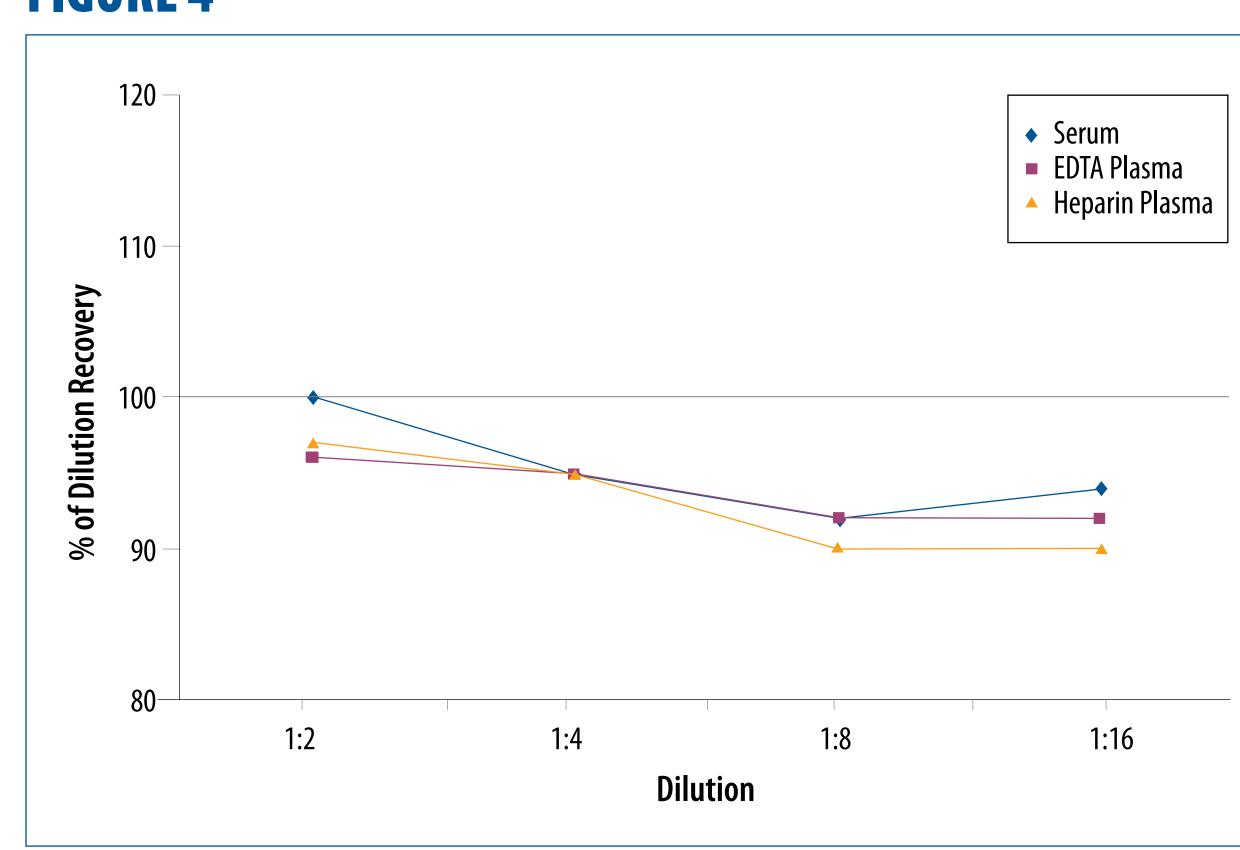
Serum Adiponectin Ratio of Low BMI to High and Very High BMI. The levels of Adiponectin in serum from individuals with low BMI, high BMI, and very high BMI were measured using the Quantikine Human Total Adiponectin/Acrp30 and Quantikine Human HMW Adiponectin/Acrp30 ELISA Kits. The fold difference in serum Adiponectin levels between individuals with low BMI and high BMI (blue bar), and individuals with low BMI and very high BMI (green bar) were calculated for each ELISA kit. A 2.4 fold and a 5.1 fold difference in Adiponectin levels between individuals with low and high BMI, and low and very high BMI, respectively, was detected with the Human HMW Adiponectin/Acrp30 Immunoassay. However, the Human Total Adiponectin/Acrp30 Immunoassay only detected a 1.9 fold and a 2.9 fold difference in Adiponectin levels between individuals with low and high BMI, and low and very high BMI, respectively (low BMI average=19.8, n=4; high BMI average=32.8, n=4; very high BMI average=55.4, n=3).

TARIF

	INTRA-ASSAY PRECISION			INTER-ASSAY PRECISION		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (ng/mL)	25.6	70.6	141	27.7	70.4	134
Standard Deviation	0.67	2.64	3.97	2.4	6.0	11.1
CV (%)	2.6	3.7	2.8	8.6	8.5	8.3

Assay Precision. Intra-assay precision was assessed by testing three samples of known concentration twenty times on one plate. Inter-assay precision was assessed by testing three samples of known concentration with twenty separate assays.

FIGURE 4



Assay Linearity. Serum (blue line) and plasma samples collected using EDTA (pink line) or heparin (gold line) as an anticoagulant were serially diluted and the levels of HMW Adiponectin were analyzed using the Quantikine Human HMW Adiponectin/Acrp30 ELISA Kit. The percent of dilution recovery was calculated for all samples (serum n=4; EDTA plasma n=4; heparin plasma n=4).

DISCUSSION

- The Quantikine Human HMW Adiponectin/Acrp30 ELISA Kit (Catalog # DHWAD0) is a complete, fully validated, ready-to-run sandwich ELISA that provides the highest levels of specificity, accuracy, precision, and sensitivity in quantifying HMW Adiponectin.
- The Human HMW Adiponectin/Acrp30 Kit is the first commercially available ELISA designed to measure HMW Adiponectin without the need for proteolytic pretreatment of the sample.
- Measuring only HMW Adiponectin reveals a greater fold difference in Adiponectin levels between individuals with low and very high BMI, suggesting that HMW Adiponectin levels may serve as a more useful indicator of conditions related to metabolic syndrome, such as obesity and insulin resistance, than total Adiponectin levels.
- The Human HMW Adiponectin/Acrp30 ELISA Kit provides a simple, straight-forward method for assessing HMW Adiponectin levels in serum and plasma for the study of obesity, Type II Diabetes, and CAD.

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