

A Novel High Molecular Weight (HMW) Adiponectin ELISA: Validation by Size Exclusion Chromatography and Western Blot Analysis



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Abstract

HMW adiponectin of the 3 circulating multimeric isoforms have been shown to be a better index of insulin sensitivity than total adiponectin using size exclusion and/or immuno-blotting techniques. We have developed a size specific HMW adiponectin ELISA only requiring simple pretreatment of serum and plasma samples. HMW and total adiponectin levels are determined in 108 subjects and in 20 morbidly obese subjects before and after (1, 3, 6 and 12 months) gastric bypass surgery. The ELISA has a concentration range of 3.125 - 200 ng/mL with 0.8 ng/mL sensitivity. Intra- and inter-assay CV are < 4% and 10%. Linearity of dilution curves are parallel to calibration curves. FPLC profiles of the enzyme treated samples shows recognition of predominantly HMW Adiponectin. HMW Adiponectin by this method closely compares to Western Blot analysis ($y = 0.77x - 0.15$; $r = 0.96$, $n = 56$). BMI and sex related changes are more pronounced and significant for HMW and % HMW compared to total Adiponectin. Gastric bypass surgery causes increases in HMW and total Adiponectin, but changes in HMW Adiponectin are more pronounced and precede to the changes in total Adiponectin. This novel HMW adiponectin ELISA after enzyme treatment recognizes predominantly HMW adiponectin, closely compares with Western Blot data, and magnifies BMI and gender associated differences than those for total adiponectin. The new ELISA is analytically robust, simple and rapid and can easily be adopted for high throughput analysis.

Introduction

Adiponectin, a fat cell secretory protein, decreases with body fat and it correlates well with insulin sensitivity in health and disease. Adiponectin circulates in complex multimeric isoforms comprising of HMW (12-18 mers), hexamers and trimers. Size exclusion and/or Western blot analysis suggests that HMW Adiponectin or HMW/Total Adiponectin is a better index of insulin sensitivity than total Adiponectin in a wide variety of insulin resistant states including obesity, diabetes and cardio-vascular diseases.

Objective

To develop a simple, robust and accurate method to measure HMW Adiponectin in human serum and plasma samples.

Materials and Methods

HMW Adiponectin determination: Twenty mL serum or plasma samples are pretreated with the sample digestion solution (Cat # ESDS) for 2 hrs at 37°C at 1/10 dilution with sample digestion buffer (Cat # EDGB), further diluted (1/200) with assay buffer (Cat # AB-10XP) and then assayed by HMW Adiponectin ELISA kit (EZH/MWA-64K).

Total Adiponectin is measured by Human Adiponectin ELISA kit (Cat # EHAD-61K).

Efficacy of digestion process is determined in control and enzyme treated samples after FPLC (Superdex 200) separation with Total and HMW adiponectin ELISAs.

Analytical comparison of this method is made with Western Blot data from 56 subjects.

Biological significance of HMW Adiponectin over total Adiponectin is determined in 108 control and 20 morbidly obese subjects undergoing Gastric Bypass Surgery.

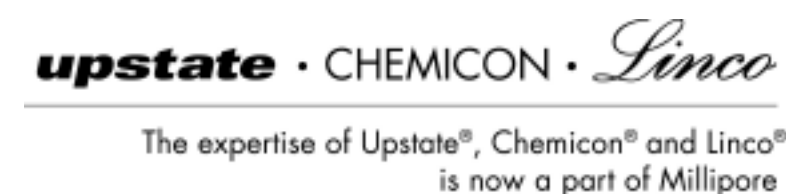


Table 1: Anthropometric Parameters of the Human Subjects

Groups	Gender	Age (Years)	Race	Systolic BP	Diastolic BP	BMI (Kg/m ²)
Lean (n = 25)	17 Male	39.0 +/- 12.3	5 C	128 +/- 12	77 +/- 8	21.56 +/- 1.85
	8 Female		16 AA			
Over Weight (n = 28)	12 Male	37.8 +/- 12.0	8 C	121 +/- 12	76 +/- 8	26.79 +/- 1.34
	16 Female		18 AA			
Obese (n = 55)	25 Male	36.9 +/- 10.7	16 C	126 +/- 14	79 +/- 7	35.76 +/- 5.13
	30 Female		30 AA			
			9 H			

All values are mean +/- SD

C: Caucasian AA: African American H: Hispanic

Figure 1a and 1b. Evaluation of HMW Adiponectin ELISA. (a) Calibration curve (closed square; n = 8) and impression profile (open circle; n = 8) and (b) Linearity of the dilution curves from 10 serum samples

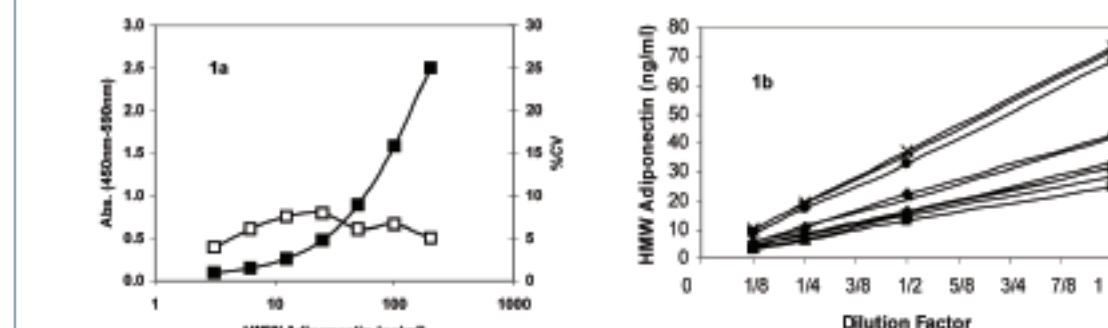


Table 2: Intra- and Inter-assay variation

Intra-assay variation			Inter-assay variation		
Sample #	Mean	% CV	Sample #	Mean	% CV
1	13.33	4.13	11	5.95	0.97
2	21.23	8.14	12	11.10	1.27
3	23.18	4.13	13	13.65	3.41
4	23.65	3.68	14	21.33	2.14
5	28.65	3.14	15	25.43	3.31
6	26.60	5.03	16	27.25	2.62
7	34.78	7.79	17	39.45	3.08
8	38.35	9.10	18	65.33	2.62
9	60.75	3.01			
10	61.50	3.80			

Figure 2. FPLC elution profiles of control (closed circles; Total ELISA) and enzyme treated (open circles; HMW ELISA) serum samples

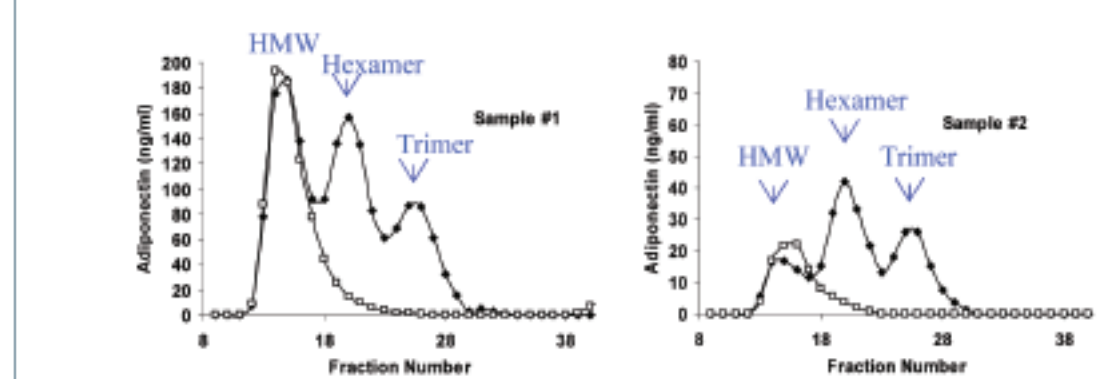


Figure 3. Comparison with Western Blot Analysis

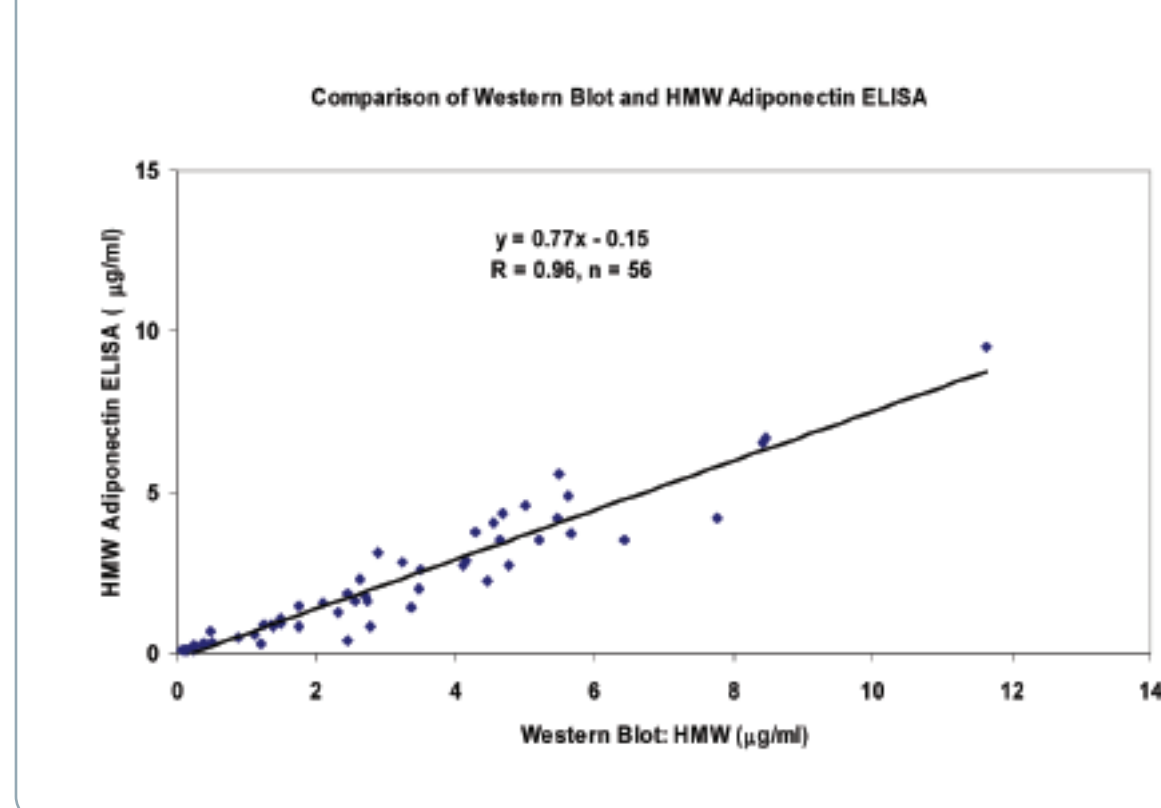
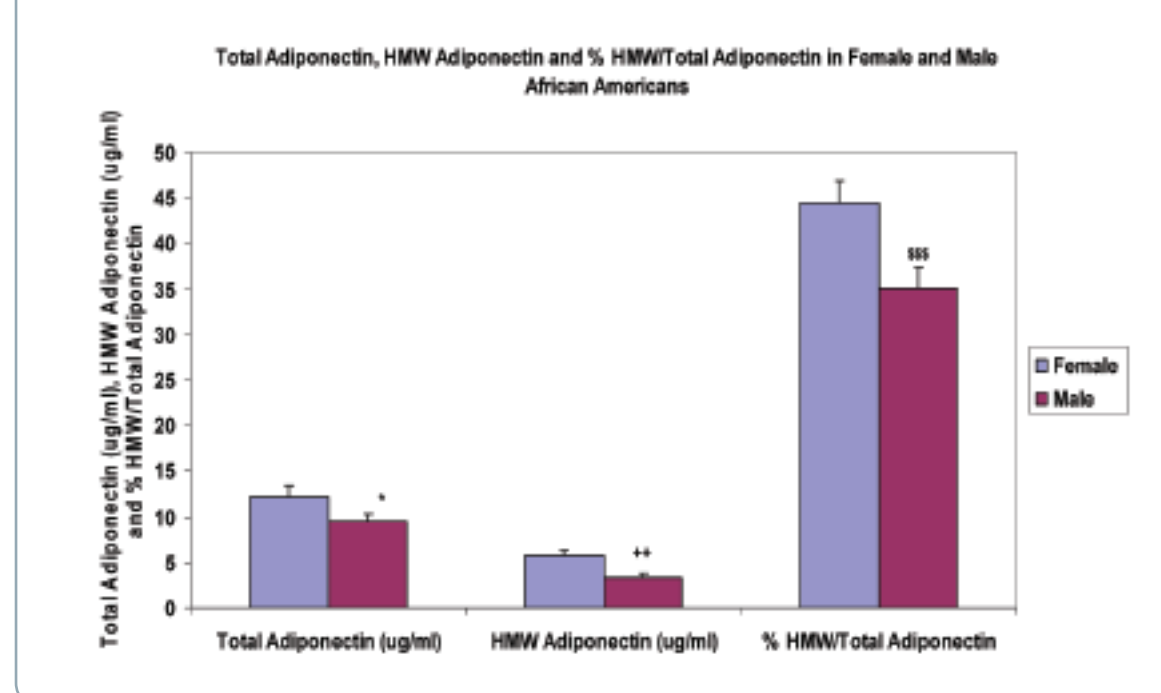


Figure 5. * (p < 0.05); HMW Adiponectin: ++ (p < 0.02) and % HMW Adiponectin/Total Adiponectin Ratio: \$\$\$ (p < 0.01).



Summary

We have developed a simple, robust, rapid and a specific ELISA method for the measurement of HMW Adiponectin which requires only simple sample pretreatment.

FPLC profiles of control and enzyme treated samples demonstrates the specificity for HMW adiponectin by this method.

HMW Adiponectin values determined by this method closely match with Western blot data.

More pronounced differences in HMW Adiponectin compared to total Adiponectin with regard to obesity, weight loss (gastric bypass surgery) and sexual dimorphism are observed and thus emphasizing the biological significance of this HMW Adiponectin ELISA.

Figure 4. The bars represent mean +/- SEM. Total Adiponectin: * (p < 0.05) and ** (p < 0.001); HMW Adiponectin: + (p < 0.005) and ++ (p < 0.0001) and % HMW Adiponectin/Total Adiponectin Ratio: \$ (p < 0.0002) and \$\$\$ (p < 0.000005).

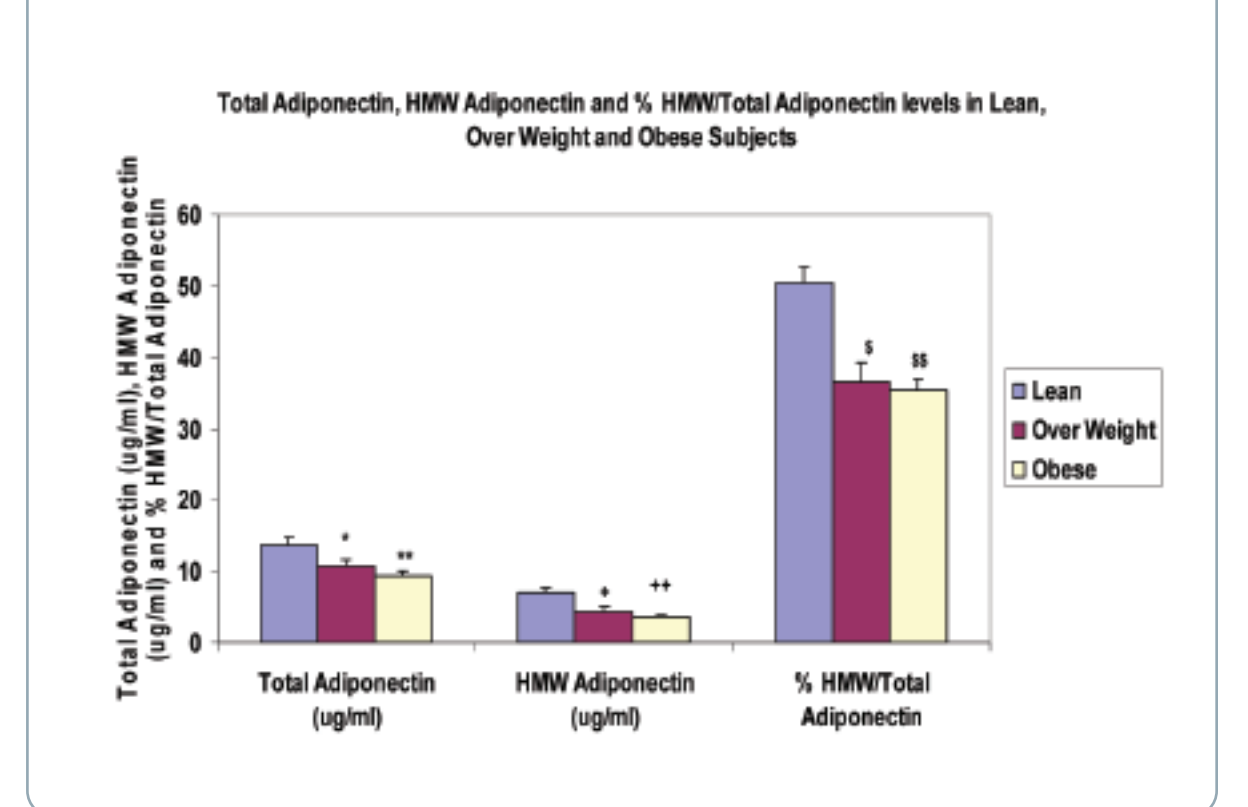
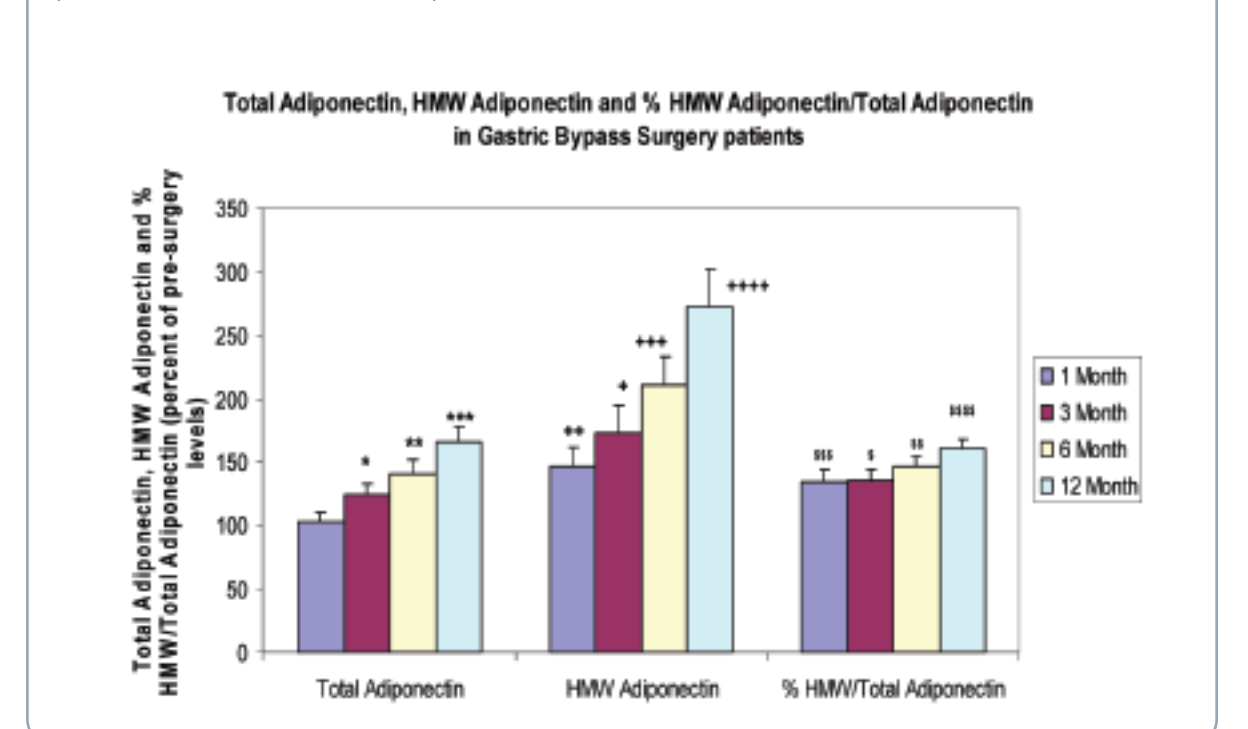


Figure 6. * (p < 0.02), ** (p < 0.001) and *** (p < 0.00002); HMW Adiponectin: + (p < 0.002), ++ (p < 0.0005), +++ (p < 0.0002) and ++++ (p < 0.00002) and % HMW Adiponectin/Total Adiponectin Ratio: \$ (p < 0.0005), \$\$ (p < 0.00001), \$\$\$ (p < 0.000005) and \$\$\$\$ (p < 0.0000005).



Conclusions

The availability of this assay (1) provides a useful tool for the high throughput analysis of HMW adiponectin in samples from various patho-physiological conditions associated with insulin resistance and other metabolic risk factors and (2) will facilitate further elucidation of the significance of HMW Adiponectin as a bio-marker and/or diagnostic method in the progression and treatment of diabetes and cardio-vascular diseases.

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