

# A Novel Protease-based ELISA for Measuring Mouse Total & High Molecular Weight Adiponectin

Hiroyuki Ebinuma<sup>1</sup>, Kelly B. Scribner Doering<sup>2</sup>, Jo Ann Davis<sup>3</sup>, Collin M. Shaw<sup>2</sup>, Sean G. Conley<sup>2</sup>  
<sup>1</sup>Sekisui Medical Co Ltd, Japan; <sup>2</sup>ALPCO Diagnostics, Salem, NH; <sup>3</sup>Tanabe Research Laboratories, San Diego, CA

## Abstract

High molecular weight (HMW) adiponectin (ADP) and/or the HMW/total ADP ratio (HMWR) may be more informative than the measurement of total ADP alone, especially as it relates to insulin resistance. A traditional approach for quantification of ADP multimers is gel filtration followed by semi-quantitative Western blot (gel/WB), and although widely accepted, this method poses a challenge for many laboratories because of the drain on time, labor and sample volume, which is of the utmost concern when working with a mouse model. In the current study, we describe the development and application of the first and only ELISA that enables simultaneous measurement of mouse total and HMW ADP on the same microtiter plate.

The assay utilizes a 20 minute protease pretreatment of serum or plasma to separate HMW from other ADP multimers; capture and detection are carried out using a mono/polyclonal antibody sandwich and O-phenylenediamine as substrate.

To demonstrate physiological utility of this assay, we assessed the total, HMW and HMWR profiles for male db/db mice treated with 10 mg/kg rosiglitazone (TZD) (n=7), a therapeutic known to increase HMW ADP, specifically, or vehicle (n=7) for 29 days. As expected, mice treated with TZD had significantly higher ADP levels than vehicle-treated mice: 3-fold greater total ADP, 6-fold greater HMW ADP and 2-fold greater HMWR (p=0.001, p=0.004, p=0.001, respectively). Inter-group differences in HMWR mirrored data generated by conventional gel/WB (r<sup>2</sup>=0.80).

Attributes of this novel assay include the small sample volume requirement (10 µl), manageable assay duration (<3 hr) and the straightforward ELISA format, all of which provide substantial advantage over traditional methodology for most laboratories. In all, the Mouse HMW and Total ADP ELISA allows for a simplified means to assess the HMWR in mouse samples and to effectively discriminate between treatment groups in a relatively short time.

## Study Objectives

### Study 1:

**Objective** – To assess the utility of ALPCO/Sekisui Mouse HMW and Total Adiponectin ELISA using ob/ob mice treated with vehicle, rosiglitazone (TZD), or Compounds A, B or C, with lean counterparts as reference. Results are shown in Figure 4.

### Study 2:

**Objective** – To assess the relationship of total adiponectin values from the ALPCO/Sekisui Mouse HMW and Total Adiponectin ELISA with an alternative commercially available kit, the Millipore Mouse Adiponectin ELISA, using lean, db/db + rosiglitazone (TZD) or vehicle or BALB/c mice. The Millipore ELISA only measures total adiponectin. Results are shown in Figure 5.

### Study 3:

**Objective** – To assess the relationship of the ALPCO/Sekisui Mouse HMW and Total Adiponectin ELISA with gel filtration chromatography/semi-quantitative Western blot (gel/WB). Contracted services from the Mouse Metabolic Phenotyping Core at the University of Texas Southwestern (Dallas, TX) were employed for gel/WB analyses and were performed according to Schraw et al.<sup>2</sup> Results are shown in Figure 6.

## Assay Background

### Assay Procedure

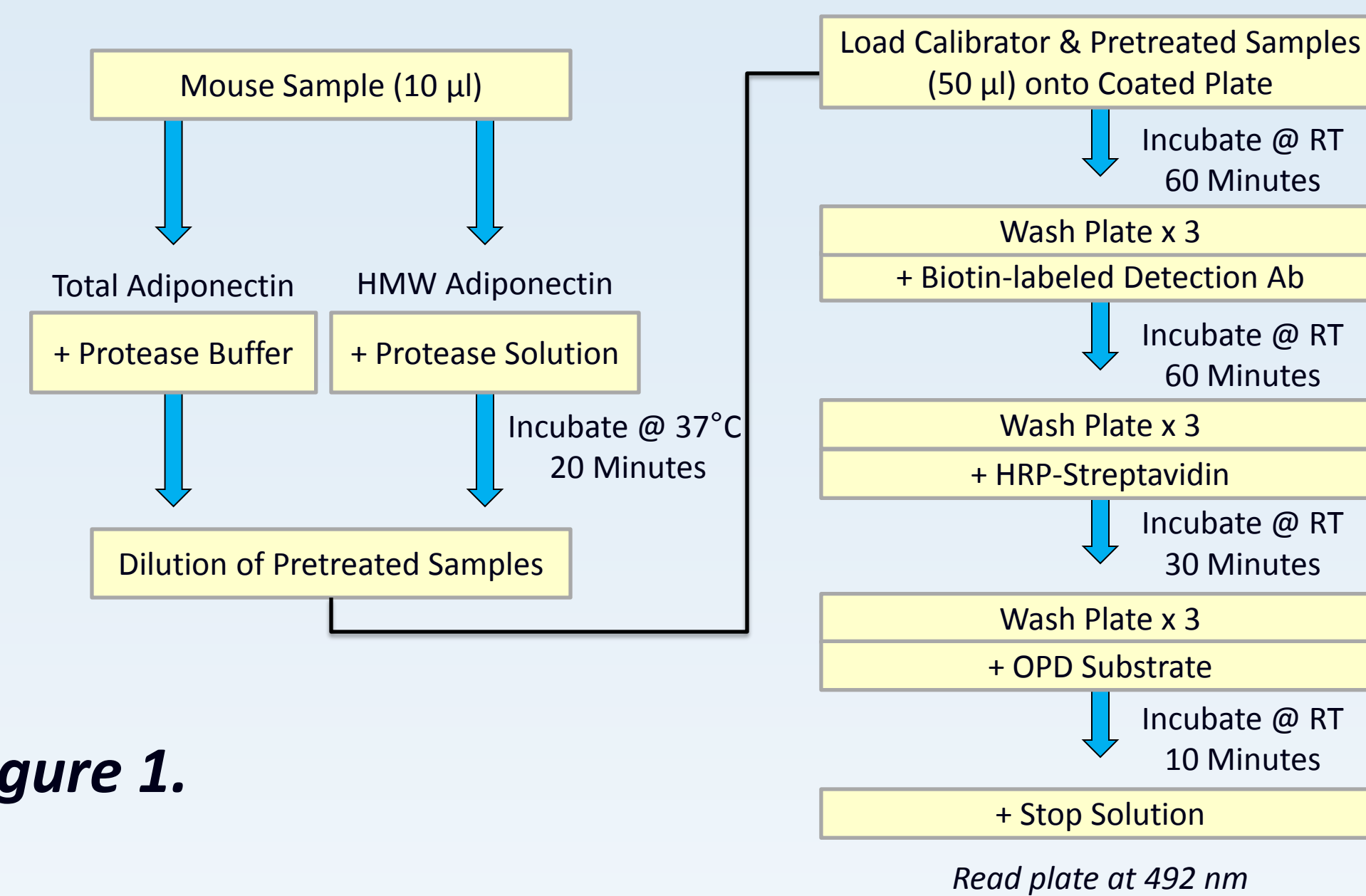


Figure 1.

### Protease Specificity

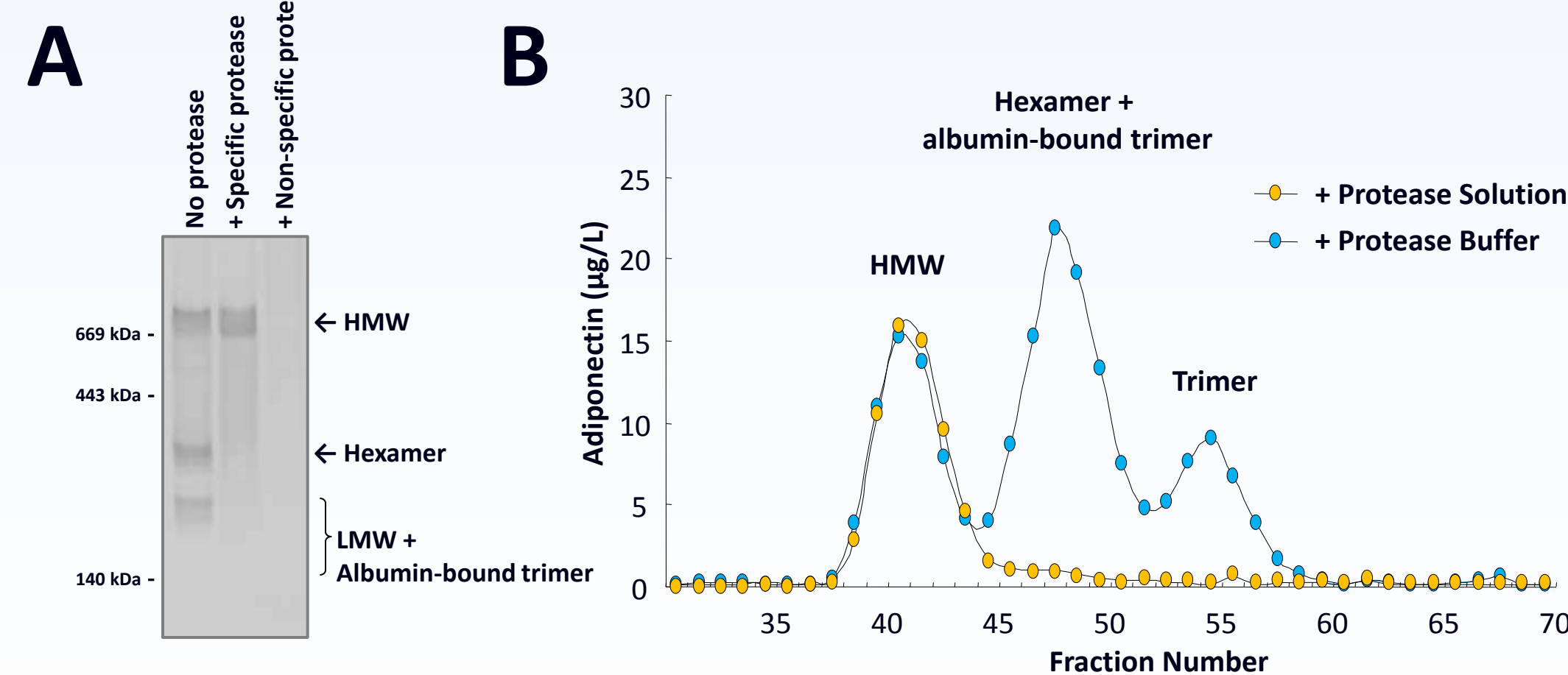


Figure 2.

- A) Mouse serum (+/- protease pretreatment) was separated by native PAGE and analyzed by Western blot using the polyclonal detection antibody from the ALPCO/Sekisui Mouse HMW and Total Adiponectin ELISA.
- B) Mouse serum (+/- protease pretreatment) was fractionated by gel filtration chromatography; fractions were assayed in the ALPCO/Sekisui Mouse HMW and Total Adiponectin ELISA. Figures adapted from ref 1.

### Sample Volume Adjustment

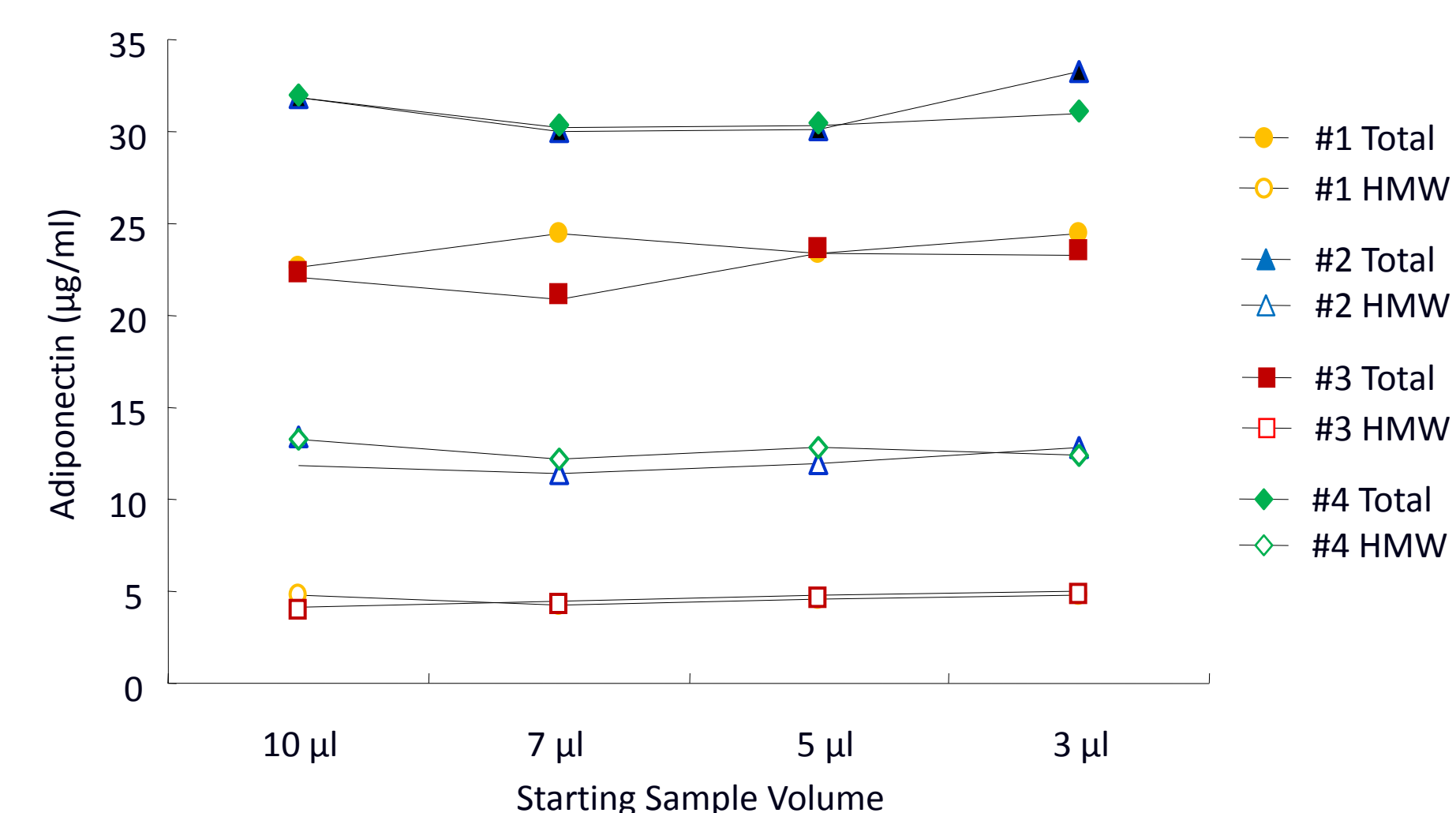


Figure 3.

Four serum samples were assessed in the ALPCO/Sekisui Mouse HMW and Total Adiponectin ELISA using starting volumes of 3, 5, 7 and 10 µl; protease solution and related buffers were adjusted proportionally. Each yielded similar intra-sample values for HMW and total Adiponectin.

### Study 1

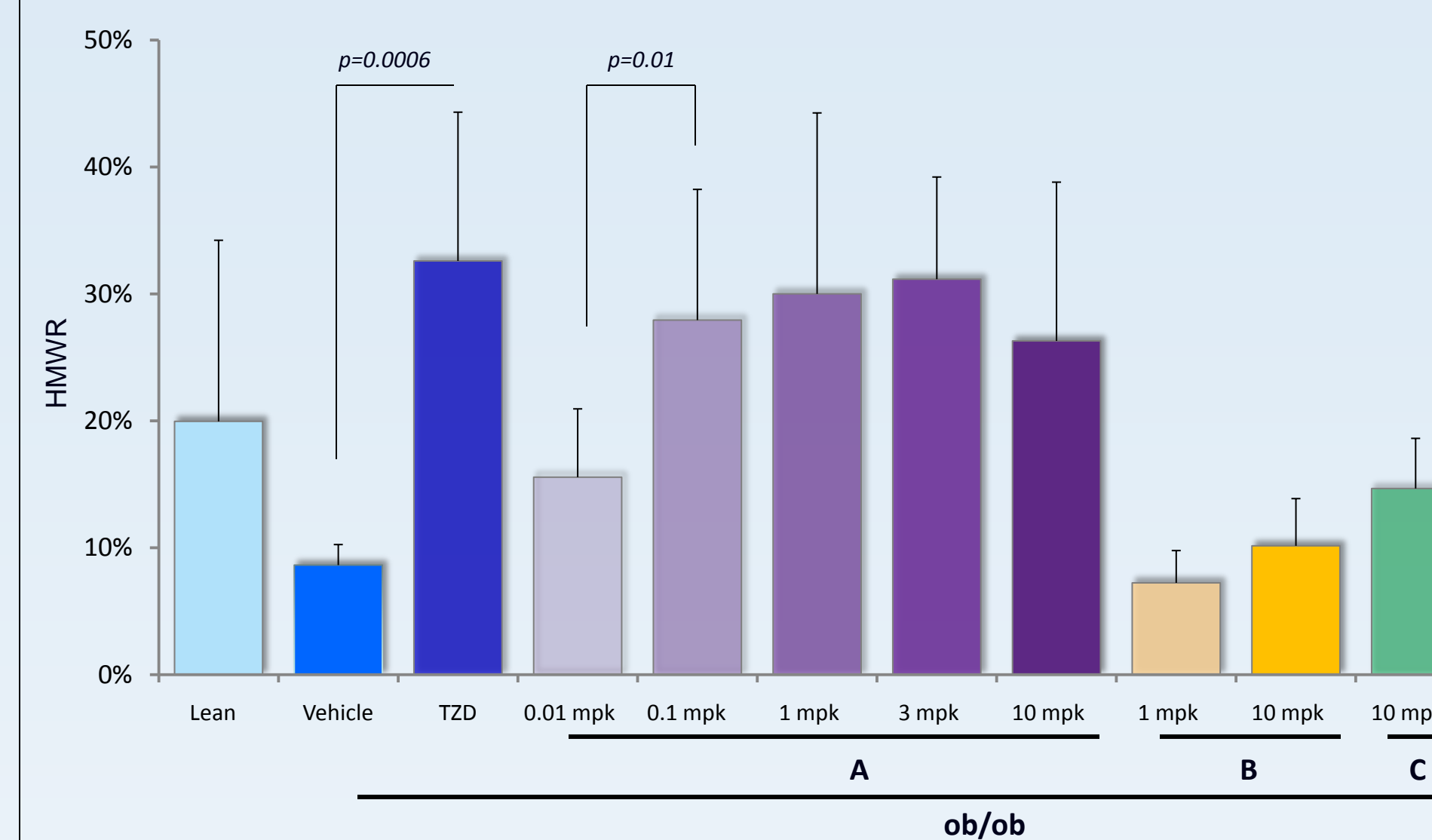


Figure 4.

Seven-week old male ob/ob mice were fed a normal chow diet and treated with TZD, vehicle or Compound A, B or C for 4 weeks; age-matched, lean controls were included for reference (n=8 for all groups). Serum samples were assayed in the ALPCO/Sekisui Mouse HMW and Total Adiponectin ELISA, and results for %HMWR are shown. mpk = mg/kg body weight

### Study 2

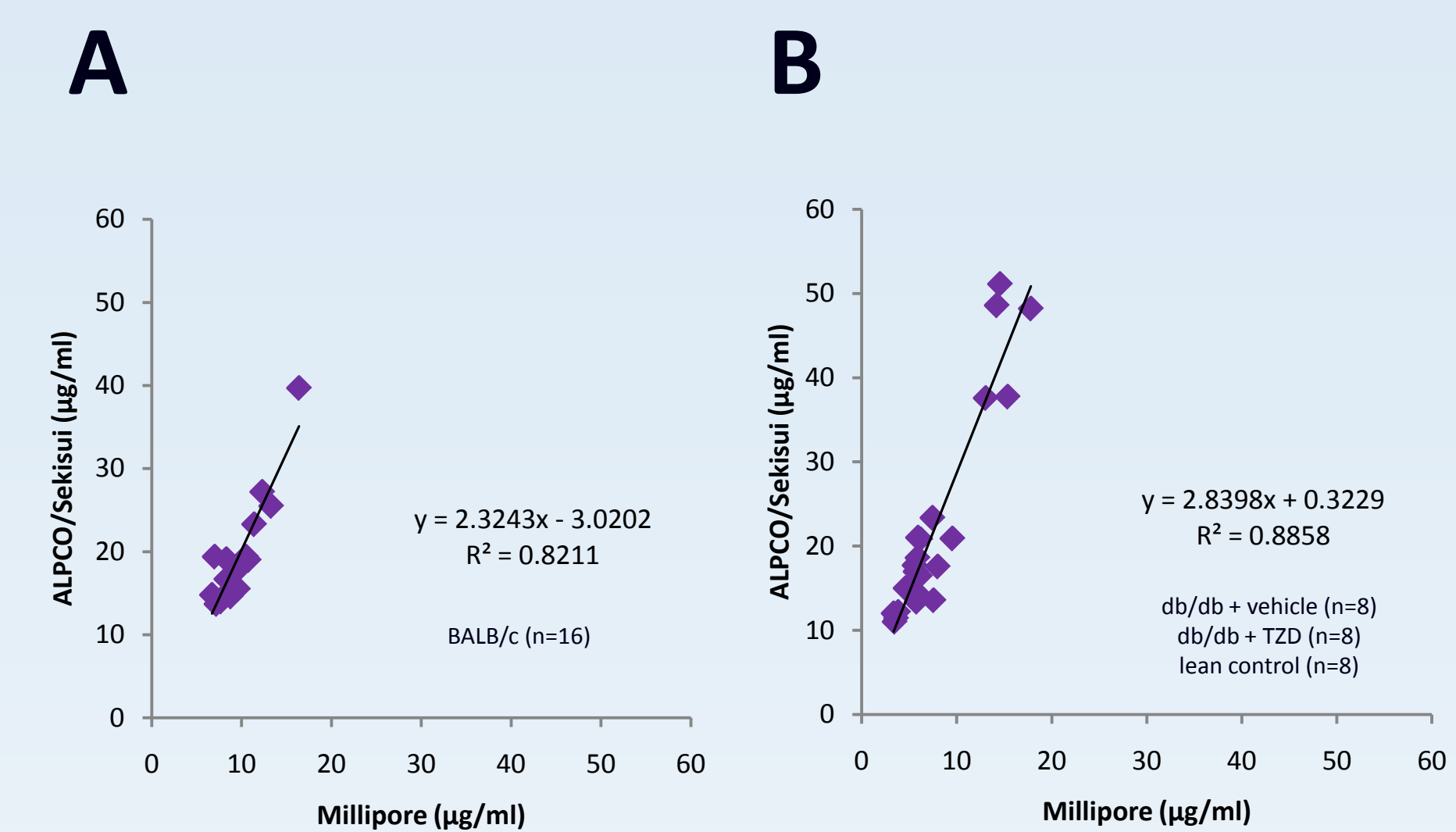


Figure 5.

Sera from adult male BALB/c (A) or 6-week old male db/db (+ 10 mg/kg TZD or vehicle for 29 days) and lean controls (B) were assayed in the ALPCO/Sekisui Mouse HMW and Total Adiponectin ELISA and results for total adiponectin were compared to values generated using the Millipore Mouse Adiponectin ELISA.

### Study 3

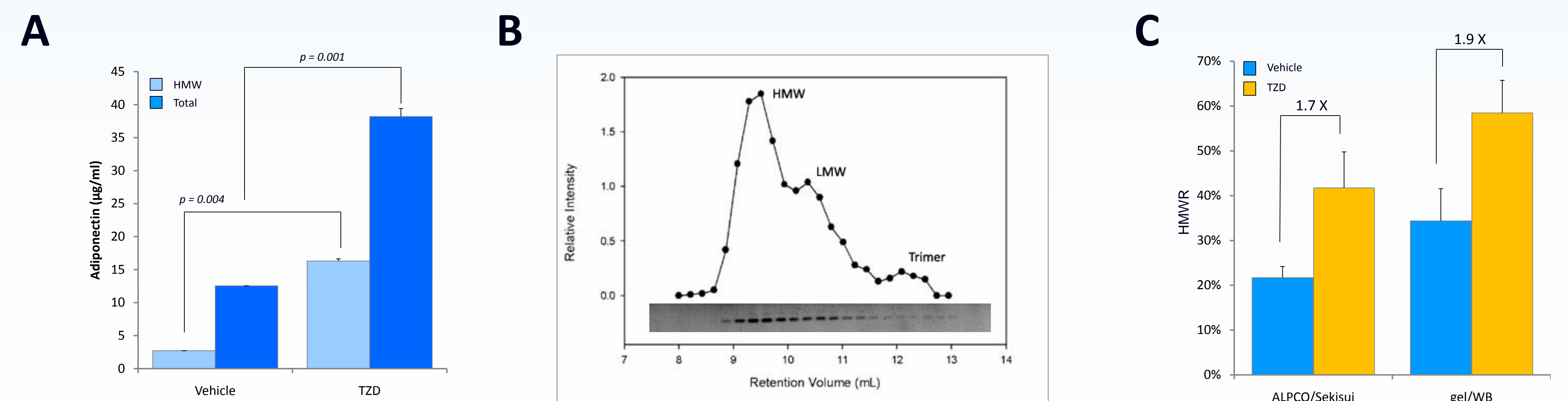


Figure 6.

Sera from db/db +TZD or vehicle (described in Figure 5B) were assayed in the ALPCO/Sekisui Mouse HMW and Total Adiponectin ELISA (A) and by gel/WB. Panel B shows a representative WB and densitometric graph for a single serum sample. Relative HMWR from the ALPCO/Sekisui ELISA and gel/WB are compared (C). For gel/WB, the HMWR is calculated by dividing the area under the curve (AUC) associated with the HMW peak in the densitometric graph by the total AUC.

## Conclusions

- ❖ We describe a novel ELISA that allows for simultaneous measurement of mouse total and HMW adiponectin
- ❖ Studies 1 and 3 demonstrate that the assay captures expected changes in HMWR due to TZD treatment of ob/ob and db/db mice
- ❖ Total adiponectin values from the ALPCO/Sekisui ELISA correlate well with the Millipore ELISA, despite differences in calibration
- ❖ Comparison with gel/WB illustrates utility of the ALPCO/Sekisui ELISA in that it equally discriminates between treatment groups
- ❖ Advantages of the ALPCO/Sekisui ELISA over gel/WB include:
  - ❖ small sample volume (≤10µl)
  - ❖ ease-of-use
  - ❖ short assay duration

## Acknowledgements

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## References

1. Ebinuma et al (2008) Clinica Chimica Acta 401: 181-3.
2. Schraw et al (2008) Endocrinology 149: 2270-82.