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A method comparison of total and HMW adiponectin: HMW/total adiponectin ratio varies versus total adiponectin, independent of clinical condition

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ABSTRACT

Background: Total and high-molecular-weight (HMW) adiponectin have been associated with endocrine and cardiovascular pathology. As no gold standard is available, the discussion about biological relevance of isoforms is complicated. In our study we perform a method comparison between two commercially available assays measuring HMW and total adiponectin, in various patient groups, thus contributing further to this discussion.
Methods: We determined levels of HMW and total adiponectin using assays by Lumipulse® and Millipore® respectively, in 126 patients with different clinical characteristics (n = 29 healthy volunteers, n = 22 dialysis patients, n = 25 elderly with body mass index (BMI) <21 kg/m^2, n = 25 elderly with BMI 30–35 kg/m^2, n = 26 children).
Results: The Passing & Bablock regression analysis resulted in HMW adiponectin$_{LUMIPULSE}$ * 0.5 − 0.9 = total adiponectin$_{MILLIPORE}$, albeit with significant deviation from linearity ($p < 0.001$). Pearson's correlation was R = 0.987 ($p = 0.000$). No significant differences between patient groups were observed ($p = 0.190$).
Conclusions: The HMW/total adiponectin ratio varies with total adiponectin concentration independent of clinical conditions studied. Our results imply that total and HMW adiponectin have similar utility when assessing adiponectin levels in blood, as the ratio is independent of clinical condition.

1. Introduction

The adipocyte-derived hormone adiponectin circulates in blood in multiple isoforms: low-molecular- weight (LMW, trimer), medium-molecular-weight (MMW, hexamer) and high-molecular-weight (HMW, 12–32mer) [1]. Total and HMW adiponectin have previously been reported to be the most biologically active isoforms, and low concentrations have been associated with metabolic syndrome, obesity, insulin sensitivity, diabetes mellitus type 2, unfavourable cholesterol profiles and the development of coronary artery disease [2–6]. On the contrary, reports also show that high concentrations of total and HMW adiponectin are associated with heart failure and cardiovascular death, both in the general population and diabetics [7–10]. Patients with chronic kidney failure have high concentrations of total and HMW adiponectin [11], the concentration of adiponectin might even be used to predict decline in kidney function [12]. As with the high adiponectin levels found in some cardiovascular patients, the etiology of this is not completely understood.

Several mechanisms influence adiponectin levels and its isoform distribution. Genetic polymorphisms of the adiponectin gene are associated with altered HMW adiponectin levels and the development of cardiometabolic risk factors [13,14]. Weight loss is associated with an increase in total and HMW adiponectin concentrations [15,16]. In the absence of overt weight loss, different exercise regimens or type of diet may also influence adiponectin concentrations [17–19]. These changes are in part explained by more favourable insulin sensitivity

Abbreviations: LMW, low-molecular-weight; MMW, medium-molecular-weight; HMW, high-molecular-weight; RIA, radioimmunoassay; CLEIA, chemiluminescent enzyme immunoassay; BMI, body mass index; HMW/total adiponectin ratio, HMW adiponectin$_{LUMIPULSE}$/total adiponectin$_{MILLIPORE}$ ratio.

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profiles in lean, "healthy" individuals. Hajri et al. reported that insulin infusion significantly increased plasma adiponectin levels in lean subjects, but not in obese subjects, suggesting that the level of adiponectin in obese individuals is a consequence rather than a cause of insulin resistance [20]. On the contrary, adiponectin levels also affect insulin sensitivity, Rose et al. for example describe that ascorbic acid and thiazolidonedione have an insulin sensitising effect by increasing the secretion of HMW and total adiponectin [21]. Apart from insulin, various other inflammatory markers have been reported to influence total and HMW adiponectin [20,22–24].

Since the discovery of adiponectin and its isoforms, many different assays to define the serum level of HMW adiponectin have been developed [25–28]. As no gold standard is available, it is difficult to determine whether these assays are indeed specific for HMW adiponectin. This analytical issue can lead to contradictory results in association studies as mentioned above. Little knowledge is available about the relationship between different adiponectin assays in various clinical conditions known to influence adiponectin levels.

We performed a method comparison between a commercially available total adiponectin radioimmunoassay (RIA) and a commercially available HMW adiponectin chemiluminescent enzyme immunoassay (CLEIA) in several patient groups with different clinical conditions associated with a high range of adiponectin concentrations [29,30].

2. Materials and methods

2.1. Patients and samples

Serum samples were obtained from 29 apparently healthy individuals, 22 dialysis patients, 25 elderly individuals, with body mass index (BMI) 30–35 kg/m^2 and 25 elderly individuals with BMI <21 kg/m^2 with their informed consent. The apparently healthy individuals were employees at the local laboratory and between 20 and 65 years old. None of the employees had any health issues as far as the authors were aware. The dialysis patients were above 30 years of age. Elderly was defined as aged 55 years or older. All elderly had between 0 and 4 comorbidities, with a median of 1, no significant difference in the number of comorbidities was found ($p = 0.179$).

Serum samples were obtained from 26 children with informed consent of their parents. Their ages were between 8 and 16 years old, their median BMI was 18.5 kg/m^2.

Serum was stored at −70 °C before analysis.

2.2. Analytical methods

Serum adiponectin was measured in all available samples using both a total adiponectin RIA (Millipore, Germany) with an intra-assay variation (CV%) of 3.3% and a HMW adiponectin automated CLEIA (Lumipulse, Fujirebio, Japan; IH7 monoclonal antibodies) with a CV% 2.3% based on duplicate measurements of the 35 highest and lowest total and HMW adiponectin measurements [29,30].

2.3. Data analysis

Passing & Bablok regression analysis was used to compare the methods. A Pearson's correlation coefficient was calculated between both methods. To compare outcome data concerning clinical and laboratory outcomes students *t*-test and one-way ANOVA tests were used as appropriate. Statistical calculations were performed with MedCalc® version 15.11 and IBM SPSS Statistics® version 22. Graphs were made with GraphPad Prism® version 6.

3. Results

A total of 126 patients were included in the analysis. Due to a technical problem, one sample was lost in the group of elderly with BMI 30–35 kg/m^2.

The range of total adiponectin concentrations was between 3.4 mg/L and 42.4 mg/L, the range of HMW adiponectin concentrations was between 1.4 mg/L and 20.6 mg/L. As expected, median levels of total and HMW adiponectin were highest in the group of elderly with BMI <21 kg/m^2 and in the dialysis group (Table 1).

The Passing & Bablok regression analysis resulted in HMW adiponectin$_{LUMIPULSE} = 0.5 * \text{total adiponectin}_{MILLIPORE} - 0.9$ (Fig. 1), albeit with a significant deviation from linearity ($p < 0.01$). Pearson's correlation was $R = 0.987$ ($p = 0.000$). Fig. 2 shows the ratio of HMW adiponectin$_{LUMIPULSE}$/total adiponectin$_{MILLIPORE}$ (HMW/total adiponectin ratio) versus the total adiponectin$_{MILLIPORE}$. As stated above, it can be seen clearly that although the two methods apparently show a good relationship, the HMW/total adiponectin ratio is lower at lower concentrations of total adiponectin. This trend was seen in all patient groups, the HMW/total adiponectin ratio did not differ significantly between the groups ($p = 0.190$) (Fig. 1, Table 1).

4. Discussion

Adiponectin circulates in human blood in multiple isoforms. Total and HMW have previously been seen as the most biologically active, functioning in many vascular, endocrine and inflammatory processes. Reports in the past have suggested that HMW and total adiponectin are biologically active independent of each other, with variable ratios between the two [4–7,31]. Although in general the roles for HMW and total adiponectin seem reasonably clear, in detail they still remain inconclusive.

In this report we compared the HMW adiponectin assay of Lumipulse® to the total adiponectin assay of Millipore®. At a first glance the method comparison showed an impressive relationship between HMW and total adiponectin, with a slope of 0.5 and R of 0.987 ($p = 0.000$). This result is in good agreement with the results reported by Almeda-Valdes et al. who conclude that "total and HMW adiponectin have similar utility for the identification of insulin resistance" [3]. However, on closer examination, in our study the HMW/total adiponectin ratio changes with total adiponectin concentrations, which is in line

Table 1
Range and median of total adiponectin$_{MILLIPORE}$, HMW adiponectin$_{LUMIPULSE}$ and HMW/total adiponectin ratios per patient group.

	N	Total adiponectin$_{MILLIPORE}$ (mg/L)		HMW adiponectin$_{LUMIPULSE}$ (mg/L)		HMW/total adiponectin ratio (%)	
		Range	Median	Range	Median	Range	Median
Hemodialysis	22	4.5–42.4	14.0	1.5–20.6	5.9	33–51	44
Healthy individuals	29	4.8–19.1	9.6	1.8–9.7	4.5	31–51	43
Elderly BMI 30–35 kg/m^2	24	3.5–28.0	10.2	1.3–15.0	4.3	29–54	41
Elderly BMI <21 kg/m^2	25	6.4–38.6	13.0	2.1–17.4	5.4	32–52	41
Children	26	4.1–26.0	12.4	1.4–12.4	4.9	32–48	40
Total	126	3.5–42.4	11.7	1.3–20.6	4.9	29–54	41

N = number of included cases; HMW/total adiponectin ratio: no significant difference between groups was found ($p = 0.190$).