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Original Article

Correlations of serum high-molecular-weight (HMW) adiponectin level with various metabolic parameters in special health checkup programs

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Abstract

***Objective*:** Adiponectin (ADPN) is secreted from adipocytes and is shown to reduce blood glucose level, atherosclerosis and tumor growth in *in vitro* and animal experiments. Also, succination of ADPN reportedly contribute to the decrease in high-molecular-weight (HMW) ADPN level in diabetes. In the present study, we investigated the relationships of serum HMW-ADPN level with various metabolic parameters in 403 Japanese subjects, who participated in special health checkup programs.

***Methods*:** Serum HMW-ADPN levels were analyzed in 232 men and 171 women, aged 29 to 92 years, who participated in our special health checkup program for "renal and metabolic syndrome", "locomotive organs" or "anti-aging" during a 3-year period and met the inclusion criteria. Visceral fat area (VFA) and subcutaneous fat area (SFA) were measured by computed tomography at the umbilical level. Serum HMW-ADPN concentration was measured by chemiluminescent enzyme immunoassay (CLEIA).

***Results*:** Medians of serum HMW-ADPN level were 2.44 μg/mL in men and 5.20 μg/mL in women. HMW-ADPN in both sex were positively correlated with HDL-cholesterol and age, and were negatively correlated with BMI, waist circumference, VFA, SFA, red blood cell count (RBC), HOMA-IR, serum triglycerides, C-reactive protein, etc. Stepwise multiple regression analyses revealed that log[HMW-ADPN] was independently correlated with HDL-cholesterol, RBC, VFA, age, gender and log[HOMA-IR] in this order ($F \geq 7.0$, $p < 0.0001$, $R^2 = 0.491$).

***Conclusion*:** It is suggested that serum HMW-ADPN level by CLEIA to be positively correlated with HDL-C, age and female gender, and negatively correlated with VFA, HOMA-IR and RBC in participants in health checkup programs.

***KEY WORDS*:** Adiponectin, high-molecular-weight form, visceral fat area, insulin resistance.

Introduction

Adiponectin (ADPN) was discovered by researchers at Osaka University (Osaka, Japan) in 1996 as the most abundant transcript in adipose tissue [1]. It was found to be the same substance as a 28-kD gelatin-binding protein (GBP28) extracted from human serum by the Faculty of Pharmaceutical Sciences, Showa University (Tokyo, Japan) in the same year [2]. In *in vitro* experiments, ADPN was shown to inhibit signals triggering inflammation in vascular endothelial cells and the growth of vascular smooth muscle cells [3]. Animal studies also revealed that ADPN attaches to injured blood vessel walls. Yamauchi *et al.* [4] and Berg *et al.* [5] reported separately, using various types of obese mice and diabetic mice, that the administration of ADPN improves both insulin resistance and blood glucose level. Another study reported that circulating ADPN levels decreased in rhesus monkeys with diabetes mellitus (DM) as insulin resistance worsened [6]. Studies using ADPN-knockout mice [7,8] revealed that a high-fat diet induced insulin resistance and atherosclerosis in such mice. Furthermore, ADPN was found to inhibit hepatic fibrosis [9] and growth of cancer cells [10], and provide survival benefits [11] in animal studies. Arai *et al.* reported that the centenarians are characterized to be more sensitive to insulin, to have high blood ADPN levels, and to be less likely to have DM and/or metabolic syndrome (MetS) [12].

Gel filtration analyses have revealed that ADPN does not exist as a monomer in serum, and that high-molecular-weight (HMW) forms of ADPN, such as a dodecamer (4×3mer) and octadecamer (6×3mer), are more closely associated with the onset of coronary artery disease (CAD) and weight reduction [13], and play the role of insulin sensitizers [14]. Several *in vitro*

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studies have reported that HMW-ADPN mainly activates AMP kinase [15], prevents apoptosis of vascular endothelial cells [13] and has cytostatic effects [16]; and therefore HMW-form of ADPN is considered to be an active form.

Recently, it is reported that succination of ADPN blocks its incorporation into trimetric and HMW-, secreted form of ADPN [17]. They proposed that succination of proteins is a biomarker of mitochondorial stress, which is increased in diabetes, and that succination of ADPN may contribute to the decrease in plasma HMW-ADPN level in diabetes [17].

In the present study targeting a total of 403 Japanese men and women (aged 29 to 92 years), who participated in the special health checkup programs at our university hospital, relationships of HMW-ADPN level with various metabolic parameters, including visceral fat area (VFA) and subcutaneous fat area (SFA) measured by computed tomography (CT) and homeostasis model assessments of insulin resistance (HOMA-IR) and β-cell function (HOMA-β) [18] were assessed.

Subjects and Methods

Subjects in this study

This study targeted 502 Japanese subjects who had first participated in the special health checkup program for "renal and metabolic syndrome", "locomotive organs" or "anti-aging" at the Center for Preventive Medicine, Keio University Hospital (Tokyo) during a 3-year period from August 2012 to July 2015, whose serum HMW-ADPN level was measured. Out of these candidates, subjects who had suffered from malignant disease, endocrine disease, inflammatory disease, severe hepatic, renal or hematological disease, or who were on corticosteroids were excluded. To evaluate HOMA-IR values appropriately, we also excluded participants with moderate to severe DM or on medication for DM from the analyses, because in subjects with DM, HOMA-IR is not reliable: FPG is abnormally high and/or insulin level may be elevated by medication. The exclusion criteria by laboratory data were as follows: white blood cell count (WBC) ≥ 10 x 10^3/μL, hemoglobin < 10 g/dL, platelet count ≥ 50 x 10^4/μL, serum alanine amino transferase (ALT) ≥ 100 IU/L, creatinine ≥ 1.6 mg/dL, C-reactive protein (CRP) ≥ 2.2 mg/dL, or fasting plasma glucose (FPG) ≥ 140 mg/dL. Finally, the analyses included 232 men (age, 29-92 years; mean, 59.0 years) and 171 women (age, 32-85 years; mean, 62.5 years) in this study.

The diagnosis of MetS was made based on the reports in 2005 by the Japanese Society of Internal Medicine [19]. In addition to waist circumference (WC) at the umbilical level ≥ 85 cm in men or ≥ 90 cm in women, the presence of at least 2 of the following 3 abnormalities indicated MetS: Serum triglycerides (TG) ≥ 150 mg/dL and/or high-density lipoprotein cholesterol (HDL-C) < 40 mg/dL; Systolic blood pressure (SBP) ≥ 130 mmHg and/or diastolic blood pressure (DBP) ≥ 85 mmHg; FPG ≥ 110 mg/dL.

We also divided male and female participants, respectively, into 4 age groups: age ≤ 45, 46-60, 61-75 and >75 and compared the HMW-ADPN levels, WC, and correlations of HMW-ADPN with VFA and HOMA-IR.

This study was conducted according to the principles expressed in the Declaration of Helsinki. Informed consent was obtained from each participant after full explanation of the purpose, nature and risk of all procedures used. The study protocol was approved by the Ethical Committee, Keio University School of Medicine, Tokyo (approval number 20130039).

Measurements

Height, weight, blood pressures and laboratory data were measured around 9:00 am after an overnight fast. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. SBP, DBP and pulse rate were measured twice using an automatic electronic sphygmomanometer (BP-900; Tanita, Inc., Tokyo, Japan) with the subject seated after resting for at least 5 min, as described previously [20-22].

Peripheral blood cell count, plasma glucose, HbA1c, serum TG, HDL-C, hepatic enzymes and creatinine levels were assayed by routine automated laboratory methods, as described previously [20-22]. Serum C-reactive protein (CRP) level was measured by nephelometry, a latex particle-enhanced immunoassay (N Latex CRP II, Dade Behring, Tokyo, Japan) with both intra- and inter-assay coefficients of variance (CVs) < 5.0%. Serum insulin concentration was measured by an enzyme immunoassay (IRI), using a commercially available kit (Tosoh, Tokyo, Japan), with intra- and inter-assay CVs of 2.9–4.6% and 4.5–7.0%, respectively [20-22]. Serum HMW-ADPN concentration was measured by chemiluminescent enzyme immunoassay (CLEIA) [22, 23] using the "cartridge type CLEIA reagent specific for HMW-ADPN measurement" in combination with Lumipulse® *f* (Fujirebio Inc., Tokyo, Japan). The antibody used was the same IH7, as used in enzyme-linked immunosorbent assay (ELISA), which is shown to react specifically for HMW-ADPN [24, 25].

VFA, SFA and WC were measured in a single slice taken at the umbilical level by a CT device Aquilion CXL (Toshiba Medical Systems Corporation, Tochigi, Japan). HOMA-IR and HOMA-β were calculated from FPG (mg/dL) and fasting IRI (μU/mL) levels by using the following formulae of Matthews, et al. [18]: HOMA-IR FPG x IRI / 405, and HOMA-β 360 x IRI / (FPG - 63) (%).

Statistical analyses

We used the IBM SPSS® Statistics version 22 (IBM Japan, Ltd., Tokyo, Japan) and the StatView® program for Windows version 5.0-J (SAS Institute, Inc., Cary, California, USA) for statistical analyses. All normally distributed data are expressed as mean ± SD, while non-normal data are expressed as median (interquartile range, IQR). *P* values of less than 0.05 were considered as statistically significant, though Bonferroni correction was used to evaluate the significance of multiple parameters in univariate and multivariate regression analyses. To assess the significance of difference between the non-normal HMW-ADPN data, we used the non-parametric Mann-hitney *U*-test for two groups.

Because the WC, VFA/SFA ratio, HOMA-IR, HOMA-β, serum TG, ALT, IRI and HMW-ADPN levels approximated a normal distribution after logarithmic transformation, their log-transformed values were used for univariate and multivariate regression analyses in this study.

Results

1. Medians of HMW-ADPN level and Comparison of the levels between MetS and non-MetS

Median levels of serum HMW-ADPN were 2.44 μg/mL in men and 5.20 μg/mL in women. The serum HMW-ADPN level in male subjects with MetS (median, 1.89; IQR, 1.38 – 2.76 μg/mL; $n = 51$) was significantly lower than those without

MetS (median, 2.77; IQR, 1.80–4.16 μg/mL; $n = 181$, $p < 0.001$). Although the number of female subjects with MetS was only 12, HMW-ADPN level in female subjects with MetS (median, 2.59; IQR, 1.64 – 4.40 μg/mL) was also significantly lower than those without MetS (median, 5.70; IQR, 3.49 – 8.34 μg/mL; $n = 159$, $p < 0.001$).

2. Simple and multiple regressions with HMW-ADPN as a dependent variable

As shown in ***Tables 1 and 2***, serum HMW-ADPN levels were positively correlated with HDL-C and age, and were negatively correlated with BMI, WC, VFA, SFA, WBC, red blood cell count (RBC), hemoglobin, HbA1c, HOMA-IR, HOMA-β, serum IRI , TG, ALT, CRP, etc. in both men and women. RBC was negatively correlated with age in both men ($r = -0.425$) and women ($r = -0.251$), and after adjustment for RBC correlations of age with log[HMW-ADPN] were still significant in males but not in females. Even after adjustment for age and BMI, the correlations of log[HMW-ADPN] with VFA, log[IRI], log[HOMA-IR], log[TG] and HDL-C were significant in both men and women.

3. Stepwise multiple regressions with HMW-ADPN as a dependent variable

Stepwise multiple regression analyses revealed that log [HMW-ADPN] was independently correlated with HDL-C, RBC, VFA, age, gender and log[HOMA-IR] in this order (***Table 3***, $F \geq 7.0$, $p < 0.0001$, $R^2 = 0.491$). Even when log[WC] was entered instead of BMI, the same results were obtained except for each F value.

4. HMW-ADPN levels when divided by 4 age groups

We further investigated the HMW-ADPN levels when divided into 4 age groups. HMW-ADPN level linearly increased with age in male participants (upper panel in ***Table 4***). Although HMW-ADPN level in female participants also tended to increase with age, this relationship was not significant (lower panel in ***Table 4***). Furthermore, negative correlation of HMW-ADPN with VFA was strongest ($r = -0.506$, $p = 0.0007$) in the oldest group of age more than 75 in the male participants.

Discussion

In the previous studies, we have investigated the significance of HMW-ADPN levels measured by ELISA [21, 26] and CLEIA [22] in health checkups of teachers and other workers, aged 40 to 65, in our university. Although HOMA-IR was included in these studies, VFA and SFA were not. In the present study of comprehensive health checkup, VFA and SFA were routinely measured in all the participants. There have been several reports of negative correlation of VFA with total ADPN level [27, 28]. Also, anemia [29), 30)], kidney disease [29), 30)]

Table 1. Relationships between HMW-ADPN level and various metabolic parameters in 232 Japanese male participants in the special health checkup programs.

Parameters		median (IQR) or mean ± SD	vs. HMW-ADPN (log)		Adjusted for RBC		Adjusted for age & BMI	
			r	p	r'	p	r'	p
Age	(year)	59.0 ± 15.0	0.375	< 0.0001	0.250	0.0001	-	-
BMI	(kg/m^2)	24.2 ± 3.5	-0.367	< 0.0001	-0.304	< 0.0001	-	-
Waist circumference (log)	(cm)	83.9 (10.9)	-0.374	< 0.0001	-0.314	< 0.0001	-0.168	0.0175
Visceral fat area	(cm^2)	107.8 ± 50.0	-0.307	< 0.0001	-0.295	< 0.0001	-0.272	< 0.0001
Subcutaneous fat area	(cm^2)	151.2 ± 74.4	-0.375	< 0.0001	-0.314	< 0.0001	-0.075	0.2588
VFA/SFA (log)	(-)	0.70 (0.46)	0.065	0.3210	0.000	0.9988	-0.155	0.0187
Systolic blood pressure	(mm Hg)	123.4 ± 17.5	-0.012	0.8515	-0.044	0.5062	-0.062	0.3486
Diastolic blood pressure	(mm Hg)	77.8 ± 11.1	-0.128	0.0517	-0.074	0.2624	0.021	0.7564
White blood cell	(10^3/μL)	5.21 ± 1.31	-0.320	< 0.0001	-0.216	0.0010	-0.260	< 0.0001
Red blood cell (RBC)	(10^6/μL)	4.68 ± 0.45	-0.392	< 0.0001	-	-	-0.240	0.0002
Hemoglobin	(mg/dL)	14.6 ± 1.2	-0.386	< 0.0001	-0.107	0.1037	-0.223	0.0007
Fasting plasma glucose	(mg/dL)	104.8 ± 9.8	-0.098	0.1351	-0.144	0.0287	-0.073	0.2685
Hemoglobin A1c	(%)	5.6 ± 0.4	-0.144	0.0280	-0.184	0.0050	-0.158	0.0165
Insulin (log)	(μU/mL)	5.0 (4.0)	-0.410	< 0.0001	-0.324	< 0.0001	-0.271	< 0.0001
HOMA-IR (log)	(-)	1.4 (1.2)	-0.403	< 0.0001	-0.327	< 0.0001	-0.271	< 0.0001
HOMA-β (log)	(%)	48.0 (39.1)	-0.395	< 0.0001	-0.291	< 0.0001	-0.243	0.0002
Triglycerides (log)	(mg/dL)	102.0 (78.5)	-0.336	< 0.0001	-0.295	< 0.0001	-0.249	0.0001
HDL-cholesterol	(mg/dL)	52.5 ± 13.0	0.321	< 0.0001	0.281	< 0.0001	0.243	0.0002
ALT (log)	(IU/L)	20 (15)	-0.311	< 0.0001	-0.232	0.0004	-0.139	0.0346
Creatinine	(mg/dL)	0.91 ± 0.17	0.139	0.0338	0.111	0.0914	0.047	0.4796
CRP (log)	(mg/dL)	0.04 (0.06)	-0.302	0.0001	-0.239	0.0002	-0.243	0.0002

Values are median (interquartile range: IQR) for data with non-normal distribution, or mean ± standard deviation (SD) for data with normal distribution. r, correlation coefficient; r', standardized correlation coefficient; HMW-ADPN, high-molecular-weight adiponectin; BMI, body mass index; VFA, visceral fat area; SFA, subcutaneous fat area; HOMA-IR, homeostasis model assessment-insulin resistance; HDL, high-density-lipoprotein; ALT, alanine amino-transferase; CRP, C-reactive protein.

Table 2. Relationships between HMW-ADPN level and various metabolic parameters in 171 Japanese female participants in the special health checkup programs.

Parameters		median (IQR) or mean ± SD	vs. HMW-ADPN (log)		Adjusted for RBC		Adjusted for age & BMI	
			r	*p*	*r'*	*p*	*r'*	*p*
Age	(year)	62.5 ± 12.3	0.180	0.0186	0.123	0.1107	-	-
BMI	(kg/m^2)	22.0 ± 3.7	-0.369	< 0.0001	-0.339	< 0.0001	-	-
Waist circumference (log)	(cm)	78.7 (14.2)	-0.366	< 0.0001	-0.338	< 0.0001	-0.156	0.0586
Visceral fat area	(cm^2)	69.9 ± 40.0	-0.459	< 0.0001	-0.431	< 0.0001	-0.407	< 0.0001
Subcutaneous fat area	(cm^2)	167.3 ± 84.1	-0.356	< 0.0001	-0.322	< 0.0001	-0.103	0.1820
VFA/SFA (log)	(-)	0.41 (0.21)	-0.127	0.0992	-0.126	0.1015	-0.181	0.0184
Systolic blood pressure	(mm Hg)	119.6 ± 23.0	-0.012	0.8728	0.026	0.7391	0.054	0.4858
Diastolic blood pressure	(mm Hg)	74.9 ± 12.2	-0.067	0.3835	0.004	0.9555	0.033	0.6691
White blood cell	(10^3/μL)	4.73 ± 1.18	-0.276	0.0002	-0.225	0.0031	-0.208	0.0066
Red blood cell (RBC)	(10^6/μL)	4.29 ± 0.36	-0.258	0.0006	-	-	-0.166	0.0312
Hemoglobin	(mg/dL)	13.1 ± 1.0	-0.247	0.0011	-0.062	0.4218	-0.170	0.0276
Fasting plasma glucose	(mg/dL)	100.9 ± 9.9	-0.299	< 0.0001	-0.270	0.0004	-0.224	0.0034
Hemoglobin A1c	(%)	5.6 ± 0.4	-0.204	0.0074	-0.170	0.0263	-0.172	0.0256
Insulin (log)	(μU/mL)	4.0 (4.0)	-0.434	< 0.0001	-0.385	< 0.0001	-0.305	< 0.0001
HOMA-IR (log)	(-)	1.1 (1.0)	-0.447	< 0.0001	-0.399	< 0.0001	-0.323	< 0.0001
HOMA-β (log)	(%)	46.0 (28.2)	-0.344	< 0.0001	-0.294	0.0001	-0.205	0.0075
Triglycerides (log)	(mg/dL)	77.0 (48.0)	-0.373	< 0.0001	-0.331	< 0.0001	-0.281	0.0002
HDL-cholesterol	(mg/dL)	66.3 ± 14.4	0.400	<< 0.0001	0.401	< 0.0001	0.338	< 0.0001
ALT (log)	(IU/L)	16 (10)	-0.212	0.0052	-0.172	0.0252	-0.062	0.4250
Creatinine	(mg/dL)	0.64 ± 0.12	0.045	0.5586	0.030	0.7013	0.027	0.7300
CRP (log)	(mg/dL)	0.02 (0.05)	-0.215	0.0046	-0.163	0.0336	-0.066	0.3971

Values are median (interquartile range: IQR) for data with non-normal distribution, or mean ± standard deviation (SD) for data with normal distribution. *r*, correlation coefficient; *r'*, standardized correlation coefficient; HMW-ADPN, high-molecular-weight adiponectin; BMI, body mass index; VFA, visceral fat area; SFA, subcutaneous fat area; HOMA-IR, homeostasis model assessment-insulin resistance; HDL, high-density-lipoprotein; ALT, alanine amino-transferase; CRP, C-reactive protein.

Table 3. Stepwise multiple regression with log[HMW-ADPN] as a dependent variable in 403 Japanese participants in the special health checkup programs

Variable	Standardized regression coefficient	*p* value	*F* value	Change in R^2
HDL-cholesterol	0.210	< 0.0001	23.7	23.9 %
Red blood cell count	-0.123	< 0.0001	7.7	11.3 %
Visceral fat area	-0.221	< 0.0001	19.7	5.3 %
Age	0.251	< 0.0001	38.6	5.7 %
Gender	0.171	< 0.0001	15.0	1.4 %
HOMA-IR (log)	-0.161	< 0.0001	12.4	1.5 %

Gender: male = 0, female = 1. $R^2 = (0.701)^2 = 0.491$. Body mass index, log [ALT], creatinine and log [C-reactive protein] were also permitted to enter the regression mode. HMW-ADPN, high-molecular-weight adiponectin; ALT, alanine amino-transferase; HDL, high-density-lipoprotein; HOMA-IR, homeostasis model assessment-insulin resistance.

Table 4. Serum HMW-ADPN levels, waist circumference (WC), and correlations of HMW-ADPN with visceral fat area (VFA) and HOMA-IR, when divided into 4 age groups

Groups in Males		1		2		3		4		F	p
Age	(years)	29 ~ 45		46 ~ 60		61 ~ 75		76 ~ 92			
n		48		79		65		40			
HMW-ADPN	(μg/ml)	1.95 (1.76)		2.35 (1.91)		2.92 (3.19)**		3.38 (3.10)**		11.93	< 0.0001
WC	(cm)	85.9 (15.4)		85.5 (12.3)		82.4 (11.1)		82.9 (8.0)		3.05	0.0298
		r	*p*	*r*	*p*	*r*	*p*	*r*	*p*		
Correlation with VFA		-0.383	0.0069	-0.378	0.0005	-0.312	0.0112	-0.506	0.0007		
Correlation with HOMA-IR		-0.289	0.0459	-0.446	<0.0001	-0.334	0.0062	-0.442	0.0039		
Groups in Females		1		2		3		4		F	p
Age	(years)	32 ~ 45		46 ~ 60		61 ~ 75		76 ~ 85			
n		14		56		69		32			
HMW-ADPN	(μg/ml)	5.82 (5.08)		5.11 (4.45)		5.12 (5.04)**		5.73 (5.64)		1.36	0.2572
WC	(cm)	65.7 (9.1)		80.5 (17.7)		79.3 (16.1)		79.5 (9.6)*		5.40	0.0015
		r	*p*	*r*	*p*	*r*	*p*	*r*	*p*		
Correlation with VFA		-0.328	0.2585	-0.433	0.0007	-0.635	<0.0001	-0.494	0.0035		
Correlation with HOMA-IR		-0.475	0.0864	-0.291	0.0293	-0.595	<0.0001	-0.511	0.0024		

Values are median (interquartile range) for data with non-normal distribution, and correlation coefficients (*r*) and *p* values. *F* and *p* values by analysis of variance (ANOVA) using logarithmic values. *$P < 0.05$ and **$p < 0.001$ vs. Group 1 by post-hoc Tukey test. HMW-ADPN, high-molecular-weight adiponectin; HOMA-IR, homeostasis model assessment-insulin resistance.

and high BNP [30] were reportedly associated with elevation of serum HMW-ADPN levels in patients with DM [29] and CAD [30]. In this study, relationships among HOMA-IR, VFA, complete blood cell counts and HMW-ADPN measured by CLEIA were investigated simultaneously in subjects with wide range of age from 29 to 92 years.

HMW-form of adiponectin

HMW-form of ADPN is considered as an active form of ADPN *in vitro* [15, 16]. Clinical studies [13, 14] also suggest that HMW-ADPN is more useful than total ADPN particularly in type 2 diabetic patients. For example, Pajvani et al. [14] reported that the HMW/total ADPN ratio was significantly more useful for monitoring improvements in insulin sensitivity in response to thiazolidinedione in type 2 DM. Hara et al. [31] reported that the HMW/total ADPN ratio had better power for predicting insulin resistance and MetS than the plasma total ADPN level. Aso et al. [32] also reported that the HMW/total ADPN ratio was more useful for evaluating CADs in type 2 diabetic patients than simply measuring the serum total ADPN level. We conducted a cross-sectional study in healthy Japanese male subjects without any medication, and reported that HMW-ADPN measured by ELISA was as effective as the HMW/total ADPN ratio for predicting insulin resistance and/or MetS [21]. Actually, negative correlations of HMW-ADPN with both HOMA-IR and MetS score were stronger than those of HMW/Total ratio (Table 2 in Ref. 21) at least in subjects without medication for DM. Also, dividing into 4 groups by HMW-ADPN level resulted in better discrimination of both HOMA-IR and MetS score than by HMW/Total ratio (B vs. C in Figs. 2 and 3 in Ref. 21). Therefore, we decided to measure only HMW-ADPN level instead of measuring both HMW- and total ADPN levels thereafter.

HMW-ADPN levels measured by CLEIA

From the year 2010, CLEIA [23] has been used also in the SRL laboratories, which method was shown to be faster and more accurate than the conventional ELISA. Due to changes in the calibrator concentration, the HMW-ADPN levels measured by CLEIA were approximately a half of those measured by the conventional ELISA [23]: HMW-ADPN (CLEIA) = HMW-ADPN (ELISA) x 0.576 + 0.318. However, the correlation with the levels measured by ELISA was very high ($r = 0.984$, $n = 297$). The intra-assay CV was 1.0% to 2.2%, and the inter-assay CV was 1.7% to 3.3% by CLEIA [23], and they were 2.4% to 3.0%, and 4.2% to 5.1% by ELISA [25]. Therefore, the CLEIA is shown to be more accurate and faster than the conventional ELISA.

Limitations of this study

The limitations of this study include selection biases for applying the results to general findings: (1) Because participants in this study were selected from special health checkup programs in our University hospital, they may have a relatively high consciousness on health, and the elderly participants may be regarded as those who had lived without developing DM, cardiovascular and/or malignant diseases. (2) The present study excluded participants with moderate to severe DM, because in subjects with DM, HOMA-IR is not reliable. Previous studies, including ours[33], suggested that administration of PPAR gamma agonists, both pioglitazone [33] and rosiglitazone [34], increase total ADPN concentration approximately 2-fold and serum HMW-ADPN concentration 3-fold. In this study, log[HMW-ADPN] in male subjects with medication on DM (n = 29) tended to be higher (0.433±0.300 μg/mL vs. 0.411±0.286 μg/mL) despite having DM, though it is not significant.

Relations with glycative stress and succination of ADPN

The present study suggested that the reduction in serum HMW-ADPN level induce insulin resistance, which will cause post-prandial hyperglycemia. Therefore, these cascade may influence upstream of glycative stress pathways. However, we could not check the effects on production of advanced glycation endproducts (AGEs) in this study. Further studies will be needed to clarify the effects of ADPN level on the downstream of glycative stress pathways. As we have described in the Introduction, succination of ADPN, which is increased in DM, is reported to block the formation of HMW-, secreted form of ADPN [17]. It is obvious that obesity/MetS reduces serum ADPN level, and resulting hyperglycemia seems to reduce the HMW-ADPN level further. Because this study was of cross-sectional study design, no cause-effect relationship can be assessed. In the near future, longitudinal studies will further facilitate the understanding of these important issues.

Conclusion

It is suggested that serum HMW-ADPN level measured by CLEIA to be independently and positively correlated with HDL-C, age and female gender, and negatively correlated with VFA, HOMA-IR and RBC in participants in health checkup programs.

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Conflict of Interest Statement

The authors state that performance of this study entailed no issues representing a conflict of interest.

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