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Serum adiponectin complexes and cardiovascular risk in children with chronic kidney disease

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Abstract

In contrast to the general population, patients with chronic kidney disease (CKD) experience increased total adiponectin levels despite an increased prevalence of cardiovascular disease. Adiponectin circulates as trimer, low molecular weight (LMW), and high molecular weight (HMW) complexes. The distribution and role of each subfraction in CKD is unknown. This cross-sectional analysis examined the association of serum adiponectin and its subfractions with known cardiovascular risk factors in 105 children (median age 12 years; 56% male) enrolled into the Chronic Kidney Disease in Children (CKiD) study, an observational cohort study of children with CKD stage 2–4. HMW accounted for 46% of total adiponectin, followed by LMW (34%) and trimer (20%). In multivariable analysis, LMW was independently associated with iothexol glomerular filtration rate (GFR) ($p=0.004$) and was higher in pubertal versus prepubertal children ($p=0.005$). HMW/LMW ratio was independently associated with age and iothexol GFR (all $p<0.001$). Unexpectedly, systolic blood pressure was positively correlated with HMW ($p=0.01$), and HMW/LMW ratio ($p=0.007$) and inversely correlated with LMW ($p=0.009$). Among subfractions, only LMW was significantly correlated with left ventricular mass (LVM) index ($p=0.05$). In multivariable analysis, decreased LMW was independently associated with higher LVM index [$\beta = -0.25$, 95% confidence interval (CI) -0.50 , -0.03 , $p=0.04$] after adjustment for body mass index (BMI), age, and blood pressure. The higher total adiponectin levels in children

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with CKD are associated with higher HMW and lower LMW. This imbalance may be an important biomarker for increased cardiovascular risk despite higher levels of total adiponectin in children with CKD.

Keywords

CKD; Adiponectin; Children; Cardiovascular disease

Introduction

Adiponectin is a product of adipose tissue involved in lipid and glucose metabolism regulation. Low circulating adiponectin is strongly associated with the known cardiovascular (CV) risk factors of dyslipidemia, insulin resistance, and inflammation. Some studies suggest that higher adiponectin levels confer a protective effect against CV disease (CVD). Adiponectin knockout mice develop hypertension and cardiac hypertrophy [1–3]. Several studies in children and adults have also indicated that lower adiponectin levels are associated with higher blood pressure (BP) and left ventricular hypertrophy (LVH) [4–12]. In chronic kidney disease (CKD), however, despite an increased risk of poor CV outcomes, blood levels of adiponectin are actually higher than typical physiological levels [13–15]. How these elevated circulatory adiponectin levels interact with CV risk factors is not clearly understood. Published studies evaluating the relationship between adiponectin and known CV risk factors in CKD patients showed inconsistent and sometimes contradictory results. One reason for the inconsistency may be that the majority of the literature describing adiponectin in CKD has evaluated the role of total circulatory adiponectin. However, adiponectin circulates in vivo as an octadecameric high molecular weight (HMW) form, a hexameric low molecular weight (LMW) form, and a trimeric form. The percent of HMW adiponectin and HMW/LMW ratio have been postulated to be the most accurate measurements of biological activity [16]. The relative importance of these different components of adiponectin has not been studied in patients with CKD.

The National Institutes of Health (NIH) recently established the Chronic Kidney Disease in Children (CKiD) study, a prospective observational study of children with mild to moderate CKD. Identification of the prevalence and evolution of traditional and novel CVD risk factors in progressive CKD is one of the primary aims of the CKiD study [17]. The aims of this cross-sectional study were to use a randomly selected subcohort of the CKiD population to: (1) describe adiponectin and its subfractions by age, pubertal status, sex, race, obesity, and degree of renal insufficiency; and (2) estimate the association of serum adiponectin and its subfractions with known CV risk factors, including elevated blood pressure (BP) and left ventricular mass (LVM) index in mild to moderate CKD.

Methods

Study population and design

The CKiD study is an observational cohort study of 586 children with CKD conducted at 46 pediatric nephrology centers in North America [17]. The study protocol has been reviewed and approved by the Institutional Review Boards of each participating center. Eligibility criteria for enrollment in CKiD include age 1–16 years, and estimated glomerular filtration rate (eGFR) as calculated by the Schwartz formula [18] of 30–90 ml/min/1.73m². The study presented here is a cross-sectional analysis of a subset of the CKiD population whose biobanked samples were approved for further analysis (year 2 of the CKiD study). There were 105 participants whose adiponectin fractions were determined and who had complete

demographic, clinical, and laboratory data. Among them, 87 children had echocardiography and the LVM Index was determined.

Adiponectin and its subfractions

The analysis was carried out at the Metabolic Phenotyping Core, The University of Texas Southwestern Medical Center, Dallas, Texas, using serum samples from the CKiD study. Original samples were collected locally, shipped, and stored at the NIH Biologic Repository at -80°F . Human adiponectin enzyme-linked immunosorbent assay (ELISA) kits (Millipore, Billerica, MA, USA) were used to determine total serum adiponectin. Subfractionation by gel filtration [19] was used to determine HMW, LMW, and trimer adiponectin fractions (Fig. 1).

In addition to adiponectin, fasting insulin levels (Human Insulin ELISA, Millipore, Billerica, MA, USA), tumor necrosis factor alpha (TNF- α), interleukin (IL)-6, and IL-10 (Multiplex Assay, Bio Rad Laboratories, Hercules, CA, USA) from the year two visit were determined using the same laboratory. The homeostasis model assessment of insulin resistance (HOMA-IR) was used as a measure of insulin resistance. HOMA-IR was calculated by dividing the product of serum insulin (mcU/ml) and glucose (mmol/ml) by a factor of 22.5.

Echocardiography

M-mode and Doppler echocardiography were performed at individual participating centers, but reading and analyses of echocardiographic data are performed by the Cardiovascular Core Imaging Research Laboratory at Cincinnati Children's Hospital Medical Center. To achieve standardization and uniformity of echocardiographic images across the centers, qualifying recordings were sent to each center and then certified at the Cardiovascular Core Imaging Research Laboratory. LVM was measured by two-dimensional directed M-mode echocardiography at rest according to the American Society of Echocardiography criteria [20]. LVM was indexed [mass divided by height raised to a power of 2.7 ($\text{g}/\text{m}^{2.7}$)] to account for body size [21]. LVH was defined as LVM index $\geq$ 95th percentile for healthy children and adolescents [22].

Other variables

Demographic and clinical information was collected at the same visit in which adiponectin measures were obtained. Weight, height, BP, GFR, and blood and urine collection were obtained on the day of the visit. The specific procedure for BP determination in the CKiD study is described elsewhere [23]. GFR was determined by plasma iothexol disappearance curves with four time points at 10, 30, 120, and 300 min after infusion of 5 ml of iothexol; details of the GFR assessment methods have been previously published [24]. Collected blood and urine samples were analyzed at the core CKiD laboratory (University of Rochester, Rochester, NY, USA). The analysis included measurement of fasting lipid and glucose levels from the same visit.

Statistical analysis

Continuous variables are presented as medians and interquartile ranges (IQR); categorical variables are presented as frequencies and percentages. Univariate and multivariable generalized linear regression models were fit to the data to examine associations between adiponectin/subfractions and CV risk factors. Variables with p values <0.2 in the univariate analyses were included in the multivariable analysis. Two sets of multivariable analyses were performed to determine: (1) predictors of adiponectin fractions (HMW, LMW, trimer, and HMW/LMW as dependent variables; and (2) predictors of LVM index (dependent variable) with adiponectin fractions as independent variables. All analyses were performed

using SAS statistical software (version 9.2 SAS Institute, Cary, NC, USA). All tests were two-sided, and a p value <0.05 was considered statistically significant. Reported p values were not adjusted for multiple testing.

Results

Cohort characteristics

Demographic and clinical characteristics of the 105 participants are summarized in Table 1. The majority was male and white and most had nonglomerular forms of CKD. Children with CKD tended to be short with preserved weight; 11% were obese. There was no significant difference in the demographic and clinical characteristics among children who had ($n=105$) and did not have ($n=437$) adiponectin fraction measured except for the higher prevalence of Caucasians and lower prevalence of African Americans in our subcohort.

Characteristics associated with adiponectin complexes

The IQR serum adiponectin level was 17.2 $\mu\text{g/ml}$ (12.3–22.2). HMW complex accounted for 46% (35–52) of total adiponectin, followed by LMW 34% (30–41) and trimer 20% (15–26). The correlations of adiponectin and its fractions are presented in Table 2. As with total adiponectin, HMW and HMW/LMW ratios were inversely correlated with age, body mass index (BMI), HOMA-IR and iohexol GFR, and positively correlated with high-density lipoprotein (HDL) cholesterol. LMW was positively correlated with iohexol GFR, and BMI. There were no significant associations of adiponectin complexes with LDL cholesterol, triglycerides, or markers of inflammation (TNF- α , IL-6, and IL-10). There were no significant differences with respect to the distribution of the different complexes according to gender and race (Fig. 2). Prepubertal and nonobese children had significantly higher HMW (Fig. 2). HMW/LMW ratio significantly increased with the decline of iohexol GFR (Fig. 3).

Results of multivariable analyses are presented in Table 3. HMW complex was associated with age, BMI Z score, and urine protein to creatinine ratio; iohexol GFR was not associated with HMW after adjusting for these factors. LMW complex was associated with iohexol GFR and was higher in pubertal versus prepubertal children. HMW/LMW ratio was associated with age and iohexol GFR.

Adiponectin complexes, blood pressure, and LVM index

Associations of adiponectin complexes with BP and LVM index are shown in Table 4. Systolic BP was positively correlated with HMW, HMW/LMW ratio and inversely correlated with LMW adiponectin. No significant associations between adiponectin and diastolic BP were observed. Among adiponectin subfractions, only LMW was significantly correlated with LVM index. Systolic (ρ 0.26, p 0.01) and diastolic (ρ 0.22, p 0.04) BP Z scores were positively correlated with LVM index. In the multivariable analysis, lower LMW (β -0.25, 95% CI -0.50 to -0.03, p 0.04) was associated with higher LVM index. Elevated LVM index was also significantly associated with higher BMI Z score (β 2.25, 95% CI 0.49–4.01, p 0.01) and younger age (β -0.68, 95% CI -1.23, -0.13, p 0.02). After adjustment in the multivariable analysis, neither systolic nor diastolic BPs were significantly associated with LVM.

Discussion

In this cross-sectional study, we examined serum adiponectin, an anti-inflammatory cytokine involved in the regulation of lipid and glucose metabolism in human diseases. To our knowledge, this is the first study to use the recently developed high-resolution gel

filtration assay [19] to analyze the adiponectin distribution across all three complexes, including a trimer, in a cohort of children with CKD. This method avoids the limitations of other assays where interactions between HMW and albumin-bound trimer can cause cross-reactivity when measuring the HMW complex [25]. In our subcohort, HMW was the most common complex accounting for approximately one half of the total adiponectin. These results are similar to the only published adiponectin subfraction study of healthy Japanese children aged 9–10 years [26]. The results also confirmed adult studies [27] and our previous findings [28] that the serum level of total adiponectin was increased in children with mild to moderate CKD compared with previously published normal values in children [29], and it was inversely correlated with kidney function. Adiponectin can also be found in the urine at levels inversely related to GFR [30]. The fact that adiponectin levels decrease significantly after renal transplantation [31, 32] suggests that decreased clearance leads to higher levels with progressive CKD. This increase was accompanied by a relative increase in HMW and decrease in LMW complexes in the circulation; the trimeric fraction remained unchanged. The mechanism of this shift in the adiponectin fraction balance might reflect a relatively low clearance of larger HMW oligomers compared with LMW clearance. A recent animal study using fluorescent-labeled recombinant adiponectin indicated that adiponectin is cleared primarily by the liver [33], with the kidney being the second most common site of clearance. What role the liver versus the kidney plays in the clearance and imbalance of adiponectin complexes in patients with decreased kidney function is unknown. Another possibility arises from the theory of a competitive nature between HMW and LMW fractions. Bouskila et al. [34] speculated that “the bivalent LMW form of adiponectin could act as an antagonist to adiponectin activity, preventing HMW-mediated receptor clustering on cell membrane.” Thus, with relatively high clearance of LMW complexes, it can be argued that in CKD, the lower concentration of LMW is not able to fully suppress HMW adiponectin. Shen et al. [35] confirmed that the proportion of HMW was increased in the dialysis group; this was accompanied by up-regulation of the adiponectin/receptor system, possibly as a counterregulatory response to uremic inflammation.

In healthy children, the onset of puberty is associated with the development of sex differences in total adiponectin. Specifically, total adiponectin levels significantly decline in healthy boys in parallel with pubertal development, subsequently leading to reduced adiponectin levels in adolescent boys compared with adolescent girls [36]. Bush et al. [37] also noted that adiponectin levels are lower among African American versus Caucasian children. Similar findings were reported by Lee et al. [38]. Conversely, in this study of children with CKD, no sex- or race-related differences in total, HMW, LMW, or trimer adiponectin levels were observed. As in the general pediatric population, prepubertal and lean children had higher total adiponectin mainly due to a larger percent of HMW complexes than in pubertal and obese children.

How the imbalance between HMW and LMW complexes is associated with CV risk factors in CKD patients remains unclear. Published adult studies evaluating the relationship between total adiponectin, insulin resistance, and inflammation in CKD showed inconsistent and sometimes contradictory results. Guebre-Egziabher et al. [14] found no relationship between adiponectin and insulin or C-reactive protein (CRP) in CKD stages 2–5. Similarly, Becker et al. [27] showed no significant relationship between adiponectin and CRP in nondiabetic adults with predialysis CKD. However, in contrast to a previous study, significant inverse associations of adiponectin with insulin were shown. Zoccali et al. [13] found no association between serum adiponectin and CRP, whereas Stenvinkel et al. [39] demonstrated significant negative associations between these two biomarkers in dialysis patients. Both of these studies showed significant positive associations between total adiponectin and HDL cholesterol and negative associations between adiponectin and triglycerides. A more recent study of predialysis CKD confirmed a positive correlation with

HDL cholesterol and a negative correlation with triglycerides [40]. In our study, only HMW was significantly associated (in a univariate analysis) with HOMA-IR and HDL cholesterol, confirming previous studies of the importance of the HMW isomer in insulin resistance and dyslipidemia. No association of any of the adiponectin complexes with markers of inflammation was evident in our patients, most likely due to a low degree of inflammation found in the CKiD cohort (data not shown). Interestingly, HMW was independently associated with protein/creatinine ratio in our multivariable analysis, similar to diabetes type 1 patients [41]. This brings up a question of the role of HMW as a marker of kidney dysfunction.

Unexpectedly, HMW adiponectin and HMW/LMW ratio were positively correlated with systolic BP, whereas LMW adiponectin was inversely correlated with systolic BP and LVM index. These associations were evident even after adjusting for age, sex, and height—factors known to affect the adiponectin level, BP, and LVM index. This contradiction adds to a number of previous publications showing conflicting results when analyzing adiponectin concentrations in relation to CV outcomes in CKD patients. A recent review from Sweden [42] summarized studies evaluating the role of adiponectin in CV outcomes in CKD and other patient groups. Among ten studies, five (three predialysis CKD and two hemodialysis) showed that low adiponectin levels predict worse clinical outcomes. Five more recent and better-powered studies (three coronary artery disease and congestive heart failure, and two CKD 3–4 and hemodialysis) showed that high levels were associated with worse overall and CV mortality. The authors speculated that a higher adiponectin level may induce protein energy wasting (PEW), a condition associated with malnutrition and inflammation. In this case, the association of higher adiponectin levels with poor outcomes fits well with the theory of reverse epidemiology in advanced CKD. Unfortunately, we could not test this hypothesis, as there were only three participants who met the criteria for PEW syndrome in our subcohort. A more plausible explanation of the positive relationship of HMW adiponectin with higher BP or LVM in CKD children is up-regulation of adiponectin receptors as a compensatory attempt to attenuate endothelial and vascular damage or cardiac hypertrophy. Finally, our findings can now be explained by the results of a recent study [43] in which the authors, using adiponectin transgenic mice, demonstrated that adiponectin potentially enhances catabolism of the sphingolipid ceramide and increases formation of its metabolite, sphingosine-1-phosphate (S1P). The latter is a growth factor known to be associated with cardiac and vascular hypertrophy.

Our study has important strengths, including the precise measurement of GFR by iothexol clearance; standardized demographic, clinical, laboratory, and echocardiographic measures through the CKiD framework; and the use of a unique assay to measure all three adiponectin complexes. This study is restricted only to children with CKD, a population with much less cumulative CV risk and morbidity compared with adults with CKD. Therefore, this evaluation of the associations of adiponectin complexes avoided multiple confounding variables present in adults. On the other hand, the results cannot be generalized to adults. A control group was also unavailable due to the nature of the parent CKiD study; however, a wide range of GFRs were represented by patients in our study, including those with minimal kidney dysfunction for comparison. This report is also limited by its cross-sectional nature, thus preventing the evaluation of causation between adiponectin fractions and CV risk. This shortcoming will be overcome as the participants in the CKiD study continue longitudinal follow-up with repeated measurements of adiponectin complexes. Finally, the results of this study need to be confirmed in a larger study, as our subcohort comprised only part of the CKiD cohort.

In conclusion, this preliminary cross-sectional analysis demonstrated that the increase in total adiponectin levels with progressive CKD in children is associated with increasing

HMW and decreasing LMW complexes. This imbalance may be an important biomarker for increased CV risk despite higher levels of total adiponectin in children with CKD.

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Fig. 1. Adiponectin complex distribution in serum of a child with chronic kidney disease (CKD). The relative intensity of adiponectin is plotted against the retention volume of each fraction. Three peaks are clearly separated, representing the high (HMW) and low LMW molecular weight and trimer complexes. The *bottom panel* shows the Western blot using adiponectin antibodies to filtration column fractions, separating out adiponectin complexes by molecular weight [19]

Fig. 2.
Distribution of adiponectin complexes according to gender, race, pubertal status and obesity.
Data presented as median (IQR)

Fig. 3. Distribution of HMW/LMW by iothexol GFR (n=105), overall p=0.049. Data presented as median (IQR)

Table 1

Demographic and clinical characteristics

Characteristic	CKiD cohort at visit 2 (n=437)	Studied population (n=105)	P value
Age, years (range)	12 (7–15)	12 (9–15)	0.29
Female	37%	44%	0.22
Race			
Caucasian	66%	78%	0.01
African American	18%	10%	0.04
Other	16%	12%	0.35
Pubertal	51%	51%	0.91
Weight percentile	48 (19–78)	37 (17–75)	0.16
Height percentile	25 (10–54)	28 (6–54)	0.48
BMI percentile	63 (35–90)	56 (33–82)	0.19
Obese, BMI >95th percentile	18%	11%	0.13
Glomerular CKD	22%	18%	0.43
Duration of CKD, years (range)	7.2 (4.0–11.2)	8.4 (4.5–12.5)	0.11
Iohexol-based GFR, ml/min/1.73 m ²	44 (33–57)	44 (34–57)	0.97
SBP percentile	60 (29–83)	61 (30–83)	
DBP percentile	60 (38–85)	60 (36–84)	
HDL cholesterol, mg/dl	48 (41–55)	47 (41–55)	
Non-HDL cholesterol, mg/dl	125 (106–144)	124 (106–149)	
Triglycerides, mg/dl	106 (74–141)	108 (74–171)	
Urine protein/creatinine ratio ^a	0.47 (0.2–1.2)	0.48 (0.18–1.09)	

Data presented as median (interquartile range) or percent. Pubertal defined as Tanner stage > 1. Comparisons were made using Fisher's exact test for binary variables and nonparametric (Wilcoxon) rank sum test for continuous variables.

BMI body mass index, *CKD* chronic kidney disease, *GFR* glomerular filtration rate, *SBP* and *DBP* systolic and diastolic blood pressure

^aFirst morning void sample.

Table 2

Spearman correlation of adiponectin and its fractions

Variable	P value	95%CL	P value
Total adiponectin, µg/ml			
Age, years	−0.39	(−0.54 to −0.21)	<0.001
BMI Z score	−0.36	(−0.52 to −0.17)	<0.001
HOMA-IR	−0.21	(−0.39 to −0.02)	0.033
HDL-C, (mg/dl)	0.36	(0.18 to 0.52)	<0.001
Urine Pr/Cr	0.18	(−0.02 to 0.36)	0.071
Iohexol GFR, (ml/min/1.73m ²)	−0.31	(−0.47 to −0.11)	0.002
HMW, %			
Age, years	−0.34	(−0.50 to −0.16)	<.001
BMI Z score	−0.30	(−0.47 to −0.11)	0.002
HOMA IR	−0.20	(−0.38 to −0.00)	0.044
HDL-C, (mg/dl)	0.21	(0.02 to 0.39)	0.030
Urine Pr/Cr	0.17	(−0.03 to 0.35)	0.087
Iohexol GFR, (ml/min/1.73m ²)	−0.23	(−0.41 to −0.03)	0.023
LMW, %			
Age, years	0.14	(−0.05 to 0.32)	0.151
BMI Z score	0.24	(0.05 to 0.42)	0.015
HOMA IR	0.14	(−0.06 to 0.32)	0.171
HDL-C, (mg/dl)	−0.16	(−0.35 to 0.03)	0.097
Urine Pr/Cr	−0.04	(−0.24 to 0.15)	0.663
Iohexol GFR, (ml/min/1.73m ²)	0.24	(0.05 to 0.42)	0.016
Trimer, %			
Age, years	0.29	(0.10–0.45)	0.003
BMI Z score	0.30	(0.10–0.47)	0.003
HOMA IR	0.20	(0.00–0.38)	0.046
HDL-C, (mg/dl)	−0.13	(−0.31–0.07)	0.205
Urine Pr/Cr	−0.16	(−0.34–0.04)	0.111
Iohexol GFR, (ml/min/1.73m ²)	0.04	(−0.16–0.23)	0.718
HMW / LMW			
Age, years	−0.31	(−0.47 to −0.12)	0.001
BMI Z score	−0.31	(−0.48 to −0.12)	0.002
HOMA IR	−0.21	(−0.39 to −0.02)	0.030
HDL-C (mg/dl)	0.23	(0.04 to 0.41)	0.018
Urine Pr/Cr	0.13	(−0.06 to 0.32)	0.185
Iohexol GFR, (ml/min/1.73m ²)	−0.25	(−0.42 to −0.05)	0.013

BMI body mass index, *HOMA-IR* homeostasis model assessment of insulin resistance, *HDL-C* high-density lipoprotein cholesterol, *Pr/Cr* protein/creatinine, *GFR* glomerular filtration rate, *HMW* high molecular weight, *LMW* low molecular weight, *CL* confidence limits; 95% CL were generated using Fisher's transformation

Table 3

Multivariable analyses of adiponectin fractions

Variable	R ²	Estimate	95% CI	P value
HMW, %	0.33			
Age, years		−1.30	−1.91 to −0.70	<0.001
BMI Z score		−5.17	−7.28 to −3.06	<0.001
Log (urine Pr/Cr)		4.30	2.28–6.32	<0.001
LMW, %	0.15			
Ioexol GFR, ml/min/1.73 m ²		0.12	0.04–0.20	0.004
Pubertal		4.24	1.34–7.14	0.005
HMW/LMW	0.22			
Age, years		−0.07	−0.11 to −0.30	<0.001
Ioexol GFR, ml/min/1.73 m ²		−0.015	−0.02 to −0.01	<0.001

CI confidence interval, HMW high molecular weight, BMI body mass index, LMW low molecular weight, GFR glomerular filtration rate

Table 4

Spearman correlation of adiponectin with blood pressure (BP) and left ventricular mass (LVM) index

Variable	<i>P</i> value	95%CL ^a	<i>P</i> value
LVM Index, g/m ^{2.7}			
Total adiponectin, µg/ml	0.10	(−0.11 to 0.31)	0.335
HMW, %	0.15	(−0.07 to 0.34)	0.179
LMW, %	−0.21	(−0.40 to 0.00)	0.049
Trimer, %	−0.03	(−0.24 to 0.19)	0.813
HMW/LMW	0.19	(−0.02 to 0.38)	0.080
SBP Z score			
Total adiponectin, ng/ml	0.17	(−0.04 to 0.35)	0.104
HMW, %	0.24	(0.04–0.42)	0.018
LMW, %	−0.26	(−0.44 to −0.07)	0.009
Trimer, %	−0.08	(−0.27 to −0.12)	0.446
HMW/LMW	0.26	(0.06–0.44)	0.010
DBP Z score			
Total adiponectin, µg/ml	0.15	(−0.06 to 0.33)	0.154
HMW, %	0.17	(−0.04 to 0.35)	0.104
LMW, %	−0.14	(−0.33 to 0.06)	0.169
Trimer, %	−0.09	(−0.28 to 0.11)	0.397
HMW/LMW	0.16	(−0.04 to 0.35)	0.110

HMW high molecular weight, BMI body mass index, LMW low molecular weight, SBP and DBP systolic and diastolic blood pressure, GFR glomerular filtration rate

^a CL confidence limits; 95% CL were generated using Fisher's transformation