

HIGH MOLECULAR WEIGHT ADIPONECTIN LEVELS ARE NEITHER INFLUENCED BY ADIPONECTIN POLYMORPHISMS NOR ASSOCIATED WITH INSULIN RESISTANCE IN MIXED-ANCESTRY HYPERGLYCEMIC SUBJECTS FROM SOUTH AFRICA

NA NIVOE ADIPONEKTINA VELIKE MOLEKULSKE TEŽINE NE UTIČU POLIMORFIZMI ADIPONEKTINA, NITI SU POVEZANI SA INSULINSKOM REZISTENCIJOM KOD JUŽNOAFRIČKIH ISPITANIKA MEŠOVITE RASE SA HIPERGLIKEMIJOM

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Summary

Background: High molecular weight (HMW) adiponectin has antiatherogenic, antiinflammatory and antidiabetic properties and these effects have been linked to its effect on high density lipoprotein cholesterol (HDL-c). Single nucleotide polymorphisms (SNPs) in the adiponectin gene influence adiponectin levels. We examined the relationship between HMW-adiponectin levels and cardiometabolic traits in normo- and hyperglycemic mixed ancestry South Africans and correlated these levels to two common polymorphisms.

Methods: HMW-adiponectin was determined in 101 subjects from the Cape Town Bellville South community-based study on a mixed ancestry population. Comparisons were made between individuals with normo- and hyperglycemia. Two common SNPs, ADIPOQ SNPs rs17300539 and rs266729, known to affect adiponectin levels were also tested for. Levels of HMW-adiponectin were then correlated with cardiometabolic traits in all groups.

Results: Levels of HMW-adiponectin were not significantly different in the normo- and hyperglycemic groups (median 11.6 vs. 10.5 µg/mL, $p=0.3060$) and in men and women (8.44 vs. 11.34 µg/mL, $p=0.67$). ADIPOQ SNPs rs17300539 and rs266729 did not influence levels of

Kratak sadržaj

Uvod: Adiponektin velike molekulske težine (VMT) ima antiaterogena, antiinflamatorna i antidijabetska svojstva i ovi efekti su dovedeni u vezu s njegovim dejstvom na HDL holesterol (HDL-h). Polimorfizmi pojedinačnih nukleotida (SNP) u genu za adiponektin utiču na nivoje adiponektina. Ispitali smo odnos između nivoa adiponektina VMT i kardiometaboličkih odlika kod Južnoafrikanaca mešovite rase sa normoglikemijom i hiperglikemijom i napravili korelaciju između ovih nivoa i dva česta polimorfizma.

Metode: Adiponektin VMT određen je kod 101 ispitanika mešovite rase iz zajednice Južni Belvil u Kejptaunu koja je kao populacija obuhvaćena studijom. Poređenja su izvršena između pojedinaca sa normoglikemijom i hiperglikemijom. Dva uobičajena SNP-a, polimorfizmi pojedinačnih nukleotida ADIPOQ rs17300539 i rs266729, za koje se zna da utiču na nivoje adiponektina, takođe su testirani. Nivoje adiponektina VMT su zatim dovedeni u korelaciju sa kardiometaboličkim odlikama u svim grupama.

Rezultati: Nivoje adiponektina VMT nisu se značajno razlikovali između grupa sa normoglikemijom i hiperglikemijom (medijana 11,6 vs. 10,5 µg/mL, $p=0,3060$) niti između muškaraca i žena (8,44 vs. 11,34 µg/mL, $p=0,67$). Polimorfizmi pojedinačnih nukleotida ADIPOQ rs17300539 i

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HMW-adiponectin. Robust correlation analyses revealed a significant positive correlation between HMW-adiponectin and HDL-c ($r=0.45$; 95%CI: 0.27–0.59), similarly in normo- and hyperglycemic participants ($p>0.99$). This association was substantially attenuated in robust linear regressions adjusted for age, gender and adiposity.

Conclusions: Adiponectin levels in this population were not determined by the commonest SNPs of the adiponectin gene, were unaffected by glycemic status; but were significantly correlated with HDL-c levels. Previous studies have attributed some of the beneficial effects of adiponectin to its effect on HDL-c.

Keywords: HMW-adiponectin, hyperglycemia, cardio-metabolic traits, polymorphisms

Introduction

Adiponectin is the most common adipokine secreted by adipose tissue and increased visceral fat accumulation inhibits its secretion (1–3). It plays an important role in regulating glucose levels and fatty acid oxidation by enhancing insulin sensitivity and decreasing free fatty acid production (2, 4). It is known to have antiatherogenic, antiinflammatory and antidiabetic properties (5, 6). Higher circulating adiponectin concentrations are associated with reduced plasma glucose and serum triglyceride levels and increased high density lipoprotein cholesterol concentrations, decreased blood pressure and a lower risk of obesity and type 2 diabetes (2, 4, 6–8). It is thought that the lower adiponectin levels observed in obesity and diabetes may be due to inhibitory effects of inflammatory cytokines such as tumour necrosis factor (TNF)- α , interleukin (IL)-6 and IL-8. Additionally, adiponectin stimulates the production of nitrous oxide and inhibits the expression of adhesion molecules (2, 3, 5, 9). As such, adiponectin levels were suggested as a therapeutic target for lowering the risk of developing metabolic syndrome, type 2 diabetes and cardiovascular disease (10). Subsequently, several studies have described a paradox where raised adiponectin levels are associated with increased mortality especially in subjects with pre-existing cardiovascular disease (11–18).

There are conflicting reports on the association between body mass index (BMI), waist circumference, insulin resistance, duration of diabetes and adiponectin levels in Africans. The inconsistencies may be attributed to the different assays used to measure adiponectin levels, renal status, molecular forms of adiponectin and the influence of polymorphisms in the adiponectin gene (19–22). It is now known that adiponectin is secreted in low molecular weight (trimer), medium molecular weight (hexamer) and high molecular weight (HMW) (12–18mer) forms (2–5, 23). The HMW form has been found to be the active form and also has increased affinity to collagen and is thus able to bind exposed collagen in damaged vasculature, decrease apoptosis of endothelial cells

rs266729 nisu uticali na nivoe adiponektina VMT. Robusne korelacione analize pokazale su da postoji značajna pozitivna korelacija između adiponektina VMT i HDL-h ($r=0.45$; 95%CI: 0,27–0,59) slična kod ispitanika sa normoglikemijom i hiperglikemijom ($p>0.99$). Ova povezanost je znatno oslabila nakon robusnih linearnih regresija prilagođenih prema starosti, polu i adipozitetu.

Zaključak: Nivoi adiponektina u ovoj populaciji nisu bili određeni najčešćim polimorfizmima pojedinačnih nukleotida gena za adiponektin, niti je na njih uticao glikemijski status, ali su bili u značajnoj korelaciji sa nivoima HDL-h. Prethodne studije su neke od povoljnih efekata adiponektina pripisale njegovom dejstvu na HDL-h.

Ključne reči: adiponektin VMT, hiperglikemija, kardio-metaboličke odlike, polimorfizmi

and increase reverse cholesterol transport due to its effect on high density lipoprotein cholesterol (HDL-c) (6, 8). Moreover, it is believed that between 30–70% of the variability in adiponectin levels is influenced by genetic factors (4, 8, 24). The adiponectin (*ADIPOQ*) gene is located on chromosome 3q27, a susceptibility locus for metabolic syndrome and its components (2, 6, 25). Numerous functional single nucleotide polymorphisms (SNPs) and missense mutations have been identified in various populations which may affect adiponectin levels and impact on insulin sensitivity and risk of cardiovascular disease (23). In this regard, two adiponectin polymorphisms, rs17300539 (11391 G>A) and rs266729 (11377 C>G) have been shown to be associated with risk for type 2 diabetes by modulating adiponectin levels and activity (23). Both these SNPs are in the promoter region of the adiponectin gene and affect adiponectin levels and thus the risk of metabolic syndrome, cardiovascular disease and type 2 diabetes (26). In a meta-analysis by Han et al. (27) rs266729 was associated with lower adiponectin levels and type 2 diabetes risk in Whites, whereas rs17300539 was associated with higher adiponectin levels and no increased risk of type 2 diabetes unless there is a family history of the disease. However, the association between the adiponectin gene polymorphisms and adiponectin levels varies across different populations and some studies have found that rs17300539 is indeed associated with increased risk of metabolic syndrome and type 2 diabetes (26). Kaftan et al. (28) described that both homozygous and heterozygous mutations of rs 266729 are associated with an increased risk of type 2 diabetes in Iraqis, but that conflicting results have been found in other populations. These conflicting results of genetic studies may be due to differences in age, genetic and ethnic backgrounds of the study populations (26, 28).

Not much is known about the effect of BMI, insulin resistance, glycemia and obesity on the HMW form of adiponectin and how these are affected by commonly reported polymorphisms in the adiponectin gene in African populations. In this study, we examined the relationship between HMW-adiponectin

levels and cardiometabolic traits in normo- and hyperglycemic subjects of a mixed ancestry South African population. We further investigated whether these levels were influenced by two commonly reported adiponectin polymorphisms, namely rs17300539 and rs266729.

Materials and Methods

Study setting and population

The study setting has been described in details elsewhere (29–31). Briefly, participants were members of a cohort study conducted in Bellville South, Cape Town, a mixed ancestry township formed in the late 1950s. Eligible participants were invited to take part in a community based survey. All participants were adults of mixed ancestry race.

Anthropometric measurements

All consenting participants received a standardized interview and physical examination during which blood pressure was measured according to WHO guidelines (32) using a semiautomated digital blood pressure monitor (Rossmax PA, USA). Other clinical measurements included the body weight, waist and hip circumferences. Participants underwent a standard two hour 75 g oral glucose tolerance test (OGTT) as prescribed by the World Health Organization (WHO), with fasting and 2-hour plasma glucose being determined. Impaired glucose tolerance (IGT) and impaired fasting glucose (IFG) were diagnosed based on WHO criteria (33). Normoglycemia was defined as normal fasting glucose levels and normal response to 75 g OGTT, whereas hyperglycemia was defined as known diabetes, screen detected diabetes, IGT and/or IFG.

Laboratory measurements

Fasting whole blood and serum samples were collected and processed for further analysis. Separated serum was stored at -70°C . Plasma glucose was measured by enzymatic hexokinase method (Cobas 6000, Roche Diagnostics). Glycated hemoglobin (HbA1c) was assessed by turbidimetric inhibition immunoassay (Cobas 6000, Roche Diagnostics). This method is National Glycohemoglobin Standardization Programme (NGSP) certified according to Roche Diagnostics. Total cholesterol, HDL-c and triglycerides (TG) were estimated by enzymatic colorimetric methods (Cobas 6000, Roche Diagnostics). Insulin was determined by a microparticle enzyme immunoassay (AxSYM, Abbott). C-reactive protein (CRP) was measured by a high-sensitivity CRP assay, based on the highly sensitive Near Infrared Particle Immunoassay rate methodology (Immage $\hat{\alpha}$ Immunochemistry System; Beckman Coulter).

Levels of HMW-adiponectin were measured in duplicate on serum by a sandwich ELISA (DRG $\text{\textcircled{R}}$ Adiponectin Human (HMW) ELISA). The assay is specific for HMW-adiponectin and has no interference from medium or low molecular weight adiponectin. The limit of sensitivity of the assay is $0.5\ \mu\text{g}/\text{mL}$ and the appropriate range is $1.56\text{--}200\ \mu\text{g}/\text{mL}$. Serum samples were stored and are stable at -70°C .

Genotyping

Genomic DNA was extracted from whole blood samples collected in an EDTA tube. Single nucleotide polymorphisms, *ADIPOQ* rs266729 and *ADIPOQ* rs17300539, were genotyped using high throughput real-time polymerase chain reaction (RT-PCR) in two independent laboratories on the ABI Prism 7900HT platform (Applied Biosystems, USA) and a BioRad Optica (BioRad, USA) using Taqman genotyping assay (Applied Biosystems, USA). Direct sequencing was used for analytical validation of high throughput genotyping against direct sequencing as the gold standard.

Definitions and calculations

The BMI was calculated as weight per square meter (kg/m^2) and waist-hip-ratio (WHR) as waist/hip circumferences (cm). Diabetes was based on a history of doctor-diagnosis, fasting blood glucose concentration $\geq 7.0\ \text{mmol}/\text{L}$ and/or 2-hour post-OGTT plasma glucose $\geq 11.1\ \text{mmol}/\text{L}$ (33). The homeostatic model assessment of insulin resistance (HOMA-IR) was calculated according to the formula: $\text{HOMA-IR} = [\text{fasting insulin concentration (mIU/L)} \times \text{fasting plasma glucose (mmol/L)}] / 22.5$ (34). Low density lipoprotein cholesterol (LDL-c) was calculated using Friedewald's formula (35).

Ethics

The study was approved by the Cape Peninsula University of Technology Faculty of Health and Wellness Sciences ethics committee (Reference Number: CPUT/HW-REC 2008/002, CPUT/HW-REC 2010, NHREC: REC-230 408-014 and N14/01/003) and the University of Stellenbosch (N09/03/090). The study was conducted according to the Code of Ethics of the World Medical Association (Declaration of Helsinki). All participants signed written informed consent after all the procedures had been fully explained in the language of their choice.

Statistical methods

The statistical software programmes, STATISTICA 12 (StatSoft, Inc) and R (version 3.0.3 [2014-03-04], The R Foundation for statistical computing,

Vienna, Austria) were used for statistical analyses. The Shapiro-Wilk W test was used to determine the distribution of HMW-adiponectin. Due to the skewed distribution, nonparametric tests were used and the median and lower and upper quartiles (25Q, 75Q) were used for descriptive statistics of the general characteristics of the study group. The Mann-Whitney U test was used to compare subgroups. Robust correlations were used to investigate the continuous associations between continuous variables and adiponectin levels, and the potential effects of extraneous factors adjusted for in robust linear regression models. SNPs were tested for departure from Hardy-Weinberg Equilibrium (HWE) expectation via a chi square goodness of fit test. Results corresponding to p-values below 5% are described as significant. We did not adjust for multiple testing.

Results

A total of 101 mixed ancestry subjects with a median age of 58 years were analysed: 38 had normoglycemia and 63 had hyperglycemia. The demographic data of our cohort is shown in *Table I* with normoglycemia compared to hyperglycemia. Significant differences between the two groups were found for HOMA-IR, HbA1c, triglycerides, HDL-c, BMI and WHR. Only 14 of the subjects were males and separate analysis found no significant gender difference in HMW-adiponectin levels (median 8.44 $\mu\text{g/mL}$ in males and 11.34 $\mu\text{g/mL}$ in females; $p=0.67$) and therefore we did not further stratify according to gender. There was no significant difference in HMW-adiponectin levels between the normoglycemic and hyperglycemic groups (median 11.6 vs. 10.5 $\mu\text{g/mL}$; $p=0.306$).

ADIPOQ rs17300539 was in HWE ($p=0.8535$), whilst *ADIPOQ* rs266729 was not ($p=0.0201$). The HMW-adiponectin levels were higher in individuals with heterozygous *ADIPOQ*-2-17300539 AG when compared to homozygous GG, respectively [median (25th-75th percentile): 12.97 (9.22-20.12) and 11.57 (4.99-16.40); $p=0.4547$], however, only three individuals carried the A allele.

Table II shows correlation of HMW-adiponectin with the variables for the group as a whole and separately for the normo- and hyperglycemia groups. Using Spearman Rank Order correlations, HMW-adiponectin was found to correlate significantly with HDL-c and age and inversely with triglycerides, BMI and WHR in the total group. In the normoglycemic group, HMW-adiponectin correlated significantly with HDL-c and inversely with HOMA-IR, triglycerides, total cholesterol, BMI and WHR. In the hyperglycemic group, the only significant correlation was with HDL-c.

Using robust correlation statistics to eliminate the effect of outliers and account for possible non-lin-

ear correlation, we found that HDL-c was significantly correlated with HMW-adiponectin in overall, normoglycemic and hyperglycemic groups. BMI correlated inversely in the normal group but not in hyperglycemia. Waist circumference had inverse significant correlation in the overall sample likely driven by a negative correlation in the normoglycemic group and a non-significant correlation in the hyperglycemic group. A similar pattern was observed with WHR. The correlation with triglycerides and LDL-c was only in the normoglycemic group, with evidence that the correlation coefficients were significantly different across glycemic status for LDL-c ($p<0.001$) (*Table III*).

In robust linear regressions adjusted for age, gender and BMI, none of the covariates was significantly associated with adiponectin levels. For HOMA-IR, LDL-c and to lesser extent triglycerides, there was suggestion of significant interaction with status for glycemia, on their effects on adiponectin levels (*Table IV*).

Discussion

In this study, we investigated the relationship between two common polymorphisms reported in the adiponectin gene and HMW-adiponectin in mixed ancestry South Africans, and further examined its relationship with insulin resistance and cardiometabolic traits in normo- and hyperglycemic subjects. Most studies have established total adiponectin levels as a marker of insulin resistance. However, this association has not been observed consistently in African populations. This has been attributed to variability in adiponectin's secretion due to genetic differences. Ebinuma et al. developed a novel ELISA to determine HMW-adiponectin levels in 2006 which was followed by the development of ELISA assays to specifically determine this fraction of adiponectin (36). A probable reason for the limited studies on HMW-adiponectin from Africa is that until recently there was no reliable assay and even when available, the cost is prohibitive.

We found no difference in HMW-adiponectin levels between the normo- and hyperglycemic groups, despite the higher degree of insulin resistance in the latter group. Epidemiological studies demonstrate an association between lower adiponectin and the prevalence and incidence of insulin resistance, and type 2 diabetes in various populations (37). There are indications that this progression to diabetes associated with low adiponectin levels is modulated by insulin resistance (11,386). A recent meta-analysis emphasized the substantial inverse association between total plasma adiponectin levels and the incidence of type 2 diabetes, which was clearly consistent in various populations (37).

A study from the United States found paradoxically high total adiponectin (PHA) levels in obese metabolically healthy Afro-Americans and also

Table 1 Demographic data of study cohort according to glycemic status.

	Total (N 101)	Normal (N 38)	Hyperglycemia (N 63)	
	Median (25 th –75 th percentiles)	Median (25 th –75 th percentiles)	Median (25 th –75 th percentiles)	P-value
HMW-adiponectin (µg/mL)	11.1 (6.3–16.4)	11.6 (6.3–16.8)	10.5 (5.2–16.4)	0.31
Age (years)	58 (50–64)	59 (47–64)	57 (52–64)	0.77
Fasting blood glucose (mmol/L)	5.9 (5.0–8.0)	5.1 (4.7–5.3)	7.4 (6.0–10.0)	< 0.0001
2-hour glucose (mmol/L)	7.6 (5.7–9.0)	5.7 (5.4–6.2)	8.9 (8.1–14.9)	< 0.0001
Fasting insulin (mU/L)	11.4 (6.1–17.0)	36.7 (27.8–61.4)	57.3 (24.9–129.3)	0.06
HOMA-IR	3.5 (1.6–5.2)	2.3 (1.2–3.4)	3.9 (1.8–7.0)	0.0021
HbA1c (%)	6.1 (5.7–6.8)	5.7 (5.6–6.0)	6.3 (6.1–8.1)	< 0.0001
C-reactive protein (mg/L)	5.4 (1.9–10.4)	4.2 (1.2–10.7)	5.9 (2.3–10.4)	0.28
Triglycerides (mmol/L)	1.4 (1.1–1.9)	1.1 (0.8–1.5)	1.7 (1.3–2.1)	< 0.0001
HDL-cholesterol (mmol/L)	1.2 (1.1–1.5)	1.4 (1.2–1.7)	1.1 (1.0–1.4)	0.0006
LDL-cholesterol (mmol/L)	3.8 (3.1–4.5)	3.7 (3.2–4.4)	3.9 (3.1–4.5)	0.55
Total cholesterol (mmol/L)	5.8 (5.1–6.6)	5.8 (5.0–6.5)	5.9 (5.2–6.7)	0.52
Height (m)	1.6(1.5–1.6)	1.6 (1.5–1.6)	1.6 (1.5–1.6)	0.02
Weight (kg)	80.3 (72.4–88.0)	71.5 (59.1–85.0)	81.5 (75.0–89.5)	0.01
Body mass index (kg/m ²)	32.3 (28.2–36.5)	30.6 (24.6–34.6)	32.6 (29.1–38.4)	0.04
Waist circumference (cm)	100.0 (93.5–108.0)	93.6 (84.0–102.3)	102.0 (96.5–112.3)	0.0007
Hip circumference (cm)	113.0 (104.0–120.0)	107.7 (99.0–117.5)	115.0 (105.0–121.0)	0.06
Waist-to-hip ratio	0.88 (0.84–0.93)	0.87 (0.81–0.90)	0.89 (0.85–0.96)	0.01
Systolic blood pressure (mmHg)	130 (119–140)	126 (115–135)	132 (123–142)	0.15
Diastolic blood pressure (mmHg)	80 (71–86)	81 (75–87)	79 (69–86)	0.31

HMW: high molecular weight, HOMA-IR: homeostatic model assessment of insulin resistance

Table II Correlation between HMW-adiponectin and parameters tested.

	Total N101		Normal N38		Hyperglycemia	
	R	P-value	R	P-value	R	P-value
Age (years)	0.1991	0.05	0.2743	0.10	0.1439	0.26
Fasting blood glucose (mmol/L)	-0.0452	0.65	0.1134	0.50	-0.0591	0.65
2-hour glucose (mmol/L)	0.0492	0.67	-0.0174	0.92	0.1894	0.24
Fasting insulin (mU/L)	-0.1581	0.12	-0.4148	0.01	0.0028	0.98
HOMA-IR	-0.1510	0.13	-0.4212	0.01	-0.0768	0.55
% HbA1c	-0.1456	0.15	-0.0906	0.59	-0.1332	0.30
C-reactive protein (mg/L)	-0.0913	0.36	-0.1845	0.27	0.0113	0.93
Triglycerides (mmol/L)	-0.2616	0.01	-0.5130	<0.05	-0.1208	0.35
HDL-cholesterol (mmol/L)	0.3361	<0.05	0.3525	0.03	0.2758	0.03
LDL-cholesterol (mmol/L)	0.1075	0.29	0.2951	0.08	0.0080	0.95
Cholesterol (mmol/L)	0.1802	0.07	0.3810	0.02	0.0777	0.55
Height (m)	-0.0730	0.47	0.2855	0.09	-0.2026	0.11
Weight (kg)	-0.1812	0.15	-0.4379	0.06	-0.0655	0.67
Body mass index (kg/m ²)	-0.2262	0.02	-0.4072	0.01	-0.0677	0.60
Waist circumference (cm)	-0.2267	0.02	-0.4398	0.01	-0.0904	0.48
Hip circumference (cm)	-0.0992	0.32	-0.3527	0.03	0.0764	0.55
Waist-to-hip ratio	-0.2279	0.02	-0.3397	0.04	-0.1607	0.21
Systolic blood pressure (mmHg)	-0.0496	0.62	0.0439	0.80	-0.0347	0.79
Diastolic blood pressure (mmHg)	-0.0640	0.53	0.0626	0.71	-0.1597	0.21

HOMA-IR: homeostatic model assessment of insulin resistance

reported gender differences despite having higher BMIs and waist circumferences (39). We did not observe gender differences despite a higher degree of obesity in females and speculate PHA as a possible cause, though numerous other studies have described higher adiponectin levels in females (2, 25, 40). However, Moriyama et al. (41) also found no gender difference in their study. Another possible factor that may have contributed in our study may be the extremely low number of males in our cohort (only 14 out of 101).

Recent studies have also described the so-called »adiponectin paradox« where raised adiponectin levels are associated with increased mortality, espe-

cially in older individuals with pre-existing cardiovascular disease (11–18). The reason for this is still unknown, but adiponectin resistance at receptor level, increased adiponectin levels in response to raised N-terminal fragment of pro-brain natriuretic peptide (NT-proBNP), and an increase in proinflammatory cytokines have been postulated (12, 16–18, 42).

It is difficult to compare our results with these studies, as to our knowledge only two studies have reported HMW-adiponectin in South African populations. In one, levels of HMW- and total adiponectin were determined in HIV-infected women both on and off antiretroviral therapy and it was determined that levels of HMW-adiponectin were lower in the treat-

Table III Robust Correlation of HMW-adiponectin ($\mu\text{g/mL}$) with biochemical and anthropometric parameters.

Correlates	Overall N=96	Normal N=36	Hyperglycemia N=60	P
Age (years)	0.20 (0.00 to 0.38)	0.22 (-12 to 0.51)	0.15 (-0.11 to 0.39)	0.76
Fasting blood glucose (mmol/L)	0.01 (-0.19 to 0.21)	0.05 (-0.28 to 0.38)	-0.01 (-0.26 to 0.25)	0.78
Fasting insulin (mU/L)	-0.12 (-0.32 to 0.08)	-0.39 (-0.64 to 0.07)	0.06 (-0.20 to 0.31)	0.03
HOMA-IR	-0.09 (-0.28 to 0.12)	-0.35 (-0.61 to -0.03)	0.02 (-0.23 to 0.28)	0.07
% HbA1c	-0.08 (-0.28 to 0.12)	-0.14 (-0.44 to 0.20)	-0.09 (-0.34 to 0.16)	0.84
Triglycerides (mmol/L)	-0.21 (-0.14 to 0.07)	-0.46 (-0.68 to -0.15)	-0.07 (-0.32 to 0.19)	0.05
HDL-cholesterol (mmol/L)	0.45 (0.27 to 0.59)	0.42 (0.11 to 0.66)	0.42 (0.19 to 0.61)	>0.99
LDL-cholesterol (mmol/L)	0.19 (-0.01 to 0.37)	0.53 (0.24 to 0.73)	0.05 (-0.30 to 0.20)	<0.001
Body mass index (kg/m^2)	-0.17 (-0.36 to 0.03)	-0.33 (-0.59 to 0.00)	0.005 (-0.25 to 0.26)	0.11
Waist circumference (cm)	-0.23 (-0.41 to -0.03)	-0.39 (-0.64 to 0.07)	-0.04 (-0.29 to 0.22)	0.09
Hip circumference (cm)	-0.10 (-0.30 to 0.10)	-0.22 (-0.51 to 0.11)	0.04 (-0.22 to 0.29)	0.23
Waist-to-hip ratio	-0.21 (-0.40 to -0.01)	-0.39 (-0.64 to 0.07)	-0.08 (-0.33 to 0.17)	0.13
Systolic blood pressure (mmHg)	0.03 (-0.17 to 0.23)	0.16 (-0.17 to 0.47)	-0.03 (-0.28 to 0.22)	0.37
Diastolic blood pressure (mmHg)	0.01 (-0.19 to 0.21)	0.20 (-0.14 to 0.50)	0.11 (-0.35 to 0.15)	0.15

HOMA-IR: homeostatic model assessment of insulin resistance

ment group and inversely correlated with waist circumferences, insulin and HOMA-IR (43). Another study examined levels of HMW- and total adiponectin and other markers of endothelial dysfunction in Black and White South Africans with rheumatoid arthritis. They found no ethnic differences and significant associations with glucose and lipid profile (44). Whilst Meilleur et al. (45) and Sobngwi et al. (21) showed a significant association between total adiponectin levels and either insulin resistance or obesity in West Africans, Ferris et al. (20) from South Africa and Orluwene and Kasia (46) from Nigeria did not observe this relationship. Similarly, Ntyintyane et al. (47) from South Africa also observed no relationship. In contrast to observations made in this study, numerous Japanese studies have reported an association between HMW-adiponectin and insulin resistance (41, 48–52) and found it to be a better predictor of insulin resistance, type 2 diabetes progression, metabolic syndrome and coronary artery disease prediction in patients with type 2 diabetes (48–50). As our

study was cross-sectional, it is difficult to evaluate the prognostic value of our findings.

As variability in adiponectin levels in different ethnic groups has also been attributed to polymorphisms in the adiponectin gene, we investigated the role of two commonly reported polymorphisms and found that these did not affect HMW-adiponectin levels nor did they have an association with insulin resistance or components of the metabolic syndrome. Karmelic et al. studied the same polymorphisms as in this study and found that there was a significant decrease in total adiponectin with metabolic syndrome traits and that levels correlated with central obesity and triglyceride levels (24). A study by Lin et al. (53) described ethnic differences in the incidence of polymorphisms and found that rs266729 decreased adiponectin levels and increased the risk of metabolic syndrome in Chinese. Similarly Vasseur et al. (23) also described an increased incidence of type 2 diabetes with the rs266729 polymorphism and an association between rs266729 and coronary artery

Table IV Robust linear regressions (age, gender, BMI adjusted) showing the effects of covariates on HMW-adiponectin levels.

Variables	Overall N=96		P interaction with glycemic status
	(se)	p-value	
Age (years)	0.070 (0.098)	0.473	0.760
Sex (women)	2.031 (3.413)	0.553	0.424
Fasting blood glucose (mmol/L)	-0.179 (0.327)	0.586	0.658
Fasting insulin (mU/L)	-0.004 (0.087)	0.961	0.156
HOMA-IR	-0.060 (0.309)	0.845	0.032
% HbA1c	-0.588 (0.391)	0.136	0.435
Triglyceride (mmol/L)	-1.243 (1.330)	0.353	0.086
HDL-cholesterol (mmol/L)	5.836 (3.520)	0.101	0.966
LDL-cholesterol (mmol/L)	0.654 (1.191)	0.584	0.058
Body mass index (kg/m ²)	-0.225 (0.167)	0.182	0.515
Waist circumference (cm)	-0.058 (0.126)	0.648	0.109
Hip circumference (cm)	0.041 (0.075)	0.584	0.357
Waist-to-hip ratio	-15.861 (10.867)	0.148	0.187
Systolic blood pressure (mmHg)	-0.001 (0.050)	0.982	0.953
Diastolic blood pressure (mmHg)	0.014 (0.090)	0.877	0.839
Normoglycemia	1.158 (1.689)	0.495	-

HOMA-IR: homeostatic model assessment of insulin resistance

disease was reported by Fisman and Teenbaum (10). Furthermore, in a meta-analysis Han et al. (27) concluded that rs17300539 was not associated with an increased risk of type 2 diabetes, but that rs266729 decreased total adiponectin levels and increased the risk of diabetes. In the Erasmus Rucphen study in Netherlands, rs17300539 was found to be associated with plasma insulin levels and HOMA-IR due to functional variation of the adiponectin protein which affected insulin sensitivity independently of adiponectin levels (54). Kaftan et al. (28) found that rs266729 was associated with type 2 diabetes and also had an effect on HDL-c in an Iraqi population; however, they did not perform serum adiponectin levels. In contrast to these observations, Gao et al. (55) found no association between rs266729 polymorphism and metabolic syndrome in a Chinese popula-

tion which they felt was due to their small study population and additionally they did not correlate their results with serum adiponectin levels.

Several studies have postulated that the most important beneficial effect of adiponectin may be due to its effect on HDL-c and the reverse cholesterol transport (8). A sub-study of The Nurses' Health Study in United States found that HMW-adiponectin was protective and that this effect was mainly due to increased HDL-c, with these effects being stronger in younger women (56). While another study on the same Nurses' Health Study found that HMW-adiponectin was associated with an increased risk in women who developed type 2 diabetes (57), the British Women's Heart and Health study found no association between HMW-adiponectin and coronary heart

disease, although levels were inversely associated with WHR, fasting insulin, glucose, HOMA-IR, triglycerides and HDL-c (58). Moriyama et al. (41) determined that HMW-adiponectin was associated with the metabolic syndrome mainly due to its effect on HDL-c by influencing its size. In a South African study on total adiponectin levels, Ferris et al. (20) found that although total adiponectin did not correlate with insulin resistance after correcting for BMI and ethnicity, levels did have a role in determining HDL-c levels. A previous study where adipocytes were incubated with HDL-c found that the HDL upregulated adiponectin expression (59). As both HDL and adiponectin activate AMP-kinase and decrease insulin levels, adiponectin may mediate the insulin sensitizing effects of HDL-c (60).

There are numerous limitations that require consideration. We had a small sample size with very few male participants so could not divide our adiponectin levels into quartiles or reliably determine gender differences. Because of the high prevalence of obesity in this population group the controls as well as subjects with hyperglycemia were obese which may have adversely affected the HMW-adiponectin levels in normoglycemic subjects. Our population was older and according to Pischon, adiponectin level is a better predictor in younger subjects. In contrast to findings by Pischon (56), a meta-analysis by Wu et al. (14) found that increased adiponectin levels may in fact be associated with increased mortality in older subjects. The cross-sectional nature of our study is a further limitation. Despite these limitations, a unique and major contribution to previous studies from Africa was the measurement of HMW-adiponectin, known to be the metabolically active form of adiponectin. Additionally, we used robust correlation and robust regressions which avoid the effect of outliers and the issues with the distribution of HMW-adiponectin and correlates.

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Conclusion

In our cohort of 101 adult South Africans, we found no significant difference in HMW-adiponectin levels between normo- and hyperglycemic subjects. Furthermore, levels of adiponectin were not determined by common adiponectin polymorphisms known to influence adiponectin levels. We did find some correlations between HMW-adiponectin and traits of the metabolic syndrome. However, the only correlation that remained statistically significant in the group as a whole and in both groups was with HDL-c. This is in keeping with literature which describes that one of the most important reasons for adiponectin's beneficial effect may be due to its effect on HDL-c function and size and the reverse cholesterol transport system. Further follow-up studies are needed on a larger population with a more equal gender distribution with lower BMI.

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Conflict of interest statement

The authors stated that they have no conflicts of interest regarding the publication of this article. No competing financial interests exist for any of the authors.

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