The negative association of lower body fat mass with cardiometabolic disease risk factors is partially mediated by adiponectin

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Abstract

Gluteofemoral fat correlates negatively with a number of cardiometabolic disease risk factors, but the mechanisms involved in these relationships are unknown. The aim of this study was to test the hypothesis that gluteofemoral fat attenuates the risk of cardiometabolic disease by increasing blood adiponectin levels. This was a cross-sectional study in which arm, leg, gluteofemoral, abdominal s.c. and visceral fat levels were measured by dual-energy X-ray absorptiometry in 648 African females. Fasting serum adiponectin, lipid, insulin and plasma glucose levels and blood pressure were measured. Relationships between variables were analysed using multivariable linear regression and structural equation modelling. Adiponectin correlated positively ($\beta = 0.45, P < 0.0001$) with gluteofemoral fat in a multivariable regression model that included age, height, and arm, s.c. and visceral fat levels. In further regression models, there was a negative correlation of gluteofemoral fat with fasting glucose ($\beta = -0.28; P < 0.0001$) and triglyceride levels ($\beta = -0.29; P < 0.0001$) and insulin resistance (HOMA; $\beta = -0.26; P < 0.0001$). Structural equation modelling demonstrated that adiponectin mediated 20.7% ($P < 0.01$) of the association of gluteofemoral fat with insulin resistance and 16.1% ($P < 0.01$) of the association with triglyceride levels but only 6.67% ($P = 0.31$) of the association with glucose levels. These results demonstrate that gluteofemoral and leg fat are positively associated with adiponectin levels and that the negative association of lower body fat with insulin resistance and triglyceride levels may partially be mediated by this adipokine. Further studies are required to determine other factors that mediate the effect of lower body fat on cardiometabolic disease risk factors.

Introduction

Adipose tissue is a functioning endocrine organ that produces a number of circulating hormones, including adiponectin. Unlike other adipokines, adiponectin levels fall with increasing total body fat mass (1, 2). In addition, several studies have shown that low levels of adiponectin are associated with a heightened risk for hypertension (3, 4), insulin resistance, diabetes (5) and inflammation (6), while higher levels are cardioprotective (5, 7).

Regional fat distribution rather than total body adiposity (8) has been associated with differing levels of...
cardiometabolic disease (CMD) risk. Thus, studies have shown that an accumulation of visceral adipose tissue increases the risk of CMD (9), while the deposition of gluteofemoral (for which hip circumference is used as a proxy marker) and leg fat appears to be protective (10). In addition, a recent study has shown that increased visceral fat is associated with lower levels of adiponectin, while increasing levels of gluteofemoral fat are linked to higher adiponectin levels (11). Studies have shown that leg fat is also positively associated with serum adiponectin levels (12, 13, 14). It is therefore possible that the competing effects of visceral and lower body adipose tissue determine blood adiponectin levels, and the differential effects of these two body fat depots on CMD risk may be mediated by their opposing effects on adiponectin. The principal aims of this study were therefore to determine whether a negative relationship exists between gluteofemoral fat and CMD risk factors and whether serum adiponectin levels are positively correlated with this fat depot. A further aim was to determine if any association of gluteofemoral and visceral fat, or any other body fat depot, with CMD risk factors is mediated by adiponectin.

This study was conducted in a population of sub-Saharan African midlife women with a known high prevalence of CMD risk factors and obesity (15) and for whom the anthropometric determinants of adiponectin have not previously been analysed.

Materials and methods

Study design and participants’ characteristics

The Black urban African women in this cross-sectional study were participants in the Study of Women Entering and in Endocrine Transition. They were the biological mothers and caregivers of the children in the Birth-to-Twenty plus (BT20 plus) cohort (16). Owing to infrastructure and timeline constraints, not all of the 2200 participants, who were still in contact with BT20, could be recruited into the study. A group of 902 women (the maximum number of women contactable within the above-mentioned limits of the study) were randomly chosen from the available 2200 BT20 caregivers, contacted by a team member and invited to participate in the study. Exclusion criteria were <40 years and >60 years and pregnancy and ethnicity other than Black African. Within this group of 902 women, 200 women did not participate. The reasons were as follows: 35 were now older than 60 years and 79 refused to participate. In addition, 37 women were deceased, 3 were terminally ill and 46 had become untraceable, or now living outside the study area. Ultimately, 702 women agreed to participate in the study. However, of these participants, only 648 had data from dual-energy X-ray absorptiometry scans due to subjects being too obese to obtain an accurate scan or refusal to have scans taken. All participants signed informed consent forms. The Human Research Ethics Committee (Medical) of the University of the Witwatersrand approved the protocol (ethics certificate number M090620).

Simple measures of body anthropometry

Participants wearing light clothing and without shoes were weighed and their height measured, using, respectively, a calibrated electronic scale and a fixed-wall stadiometer, (Holtain, Crymych, UK). A soft measuring tape was used to measure waist and hip circumferences to the nearest 0.5 cm; the former at the smallest girth above the umbilicus and the latter at the greatest circumference of the hips. The intra-observer coefficient of variances (CVs) for height, weight and hip circumference were less than 1% and less than 2% for waist circumference. Inter-observer CV for height, weight, hip circumference and waist circumference was less than 1%. BMI was calculated. Blood pressure was recorded on the left arm while the participant was seated using a digital reader (Omron M6; Omron, Kyoto, Japan) and appropriate cuffs. Three readings were taken with a 2-min interval between each reading. The first reading was discarded, and the remaining two values were averaged.

Dual-energy X-ray absorptiometry measurements

Whole-body composition was measured using a Hologic QDR 4500A DXA machine and analysed using Apex software version 4.0.2 (Hologic Inc., Bedford, USA). The machine was calibrated daily using a phantom spine, and coefficients of variation during the course of the study were <2% for total fat mass. All standard DXA measurements were analysed using Hologic APEX 3.1 software (Hologic). The APEX software defines gluteofemoral fat (gynoid fat) as lying in a region from the head of the femur to the mid-thigh, while leg fat was measured in a region demarcated by an oblique line passing through the femoral neck and joining a central vertical line between the legs (see Supplementary Fig. 1, see section on supplementary materials given at the end of this article). Whole-body (excluding head) fat mass was recorded. Abdominal visceral adipose tissue and s.c. adipose tissue were measured according to previously described methodology (17).
Blood analyte assays and diagnosis of the metabolic syndrome and menopause staging

Fasting blood samples were obtained in the morning before 11:00 h. Serum and plasma samples were collected and aliquoted into cryovials and immediately stored at −80°C until assays were performed. Levels of lipids, blood glucose, insulin and adiponectin were measured.

An immunoassay was performed for insulin as per manufacturer's instructions (ADVIA Centaur XP Systems, Siemens Healthcare Diagnostics). The homeostasis model assessment (HOMA) method was used to calculate insulin resistance. Total cholesterol, high-density lipoprotein cholesterol (HDL), triglycerides and glucose were measured using the Advia 1800 Chemistry Systems analyser (Siemens Healthcare Diagnostics). Low-density lipoprotein cholesterol (LDL) levels were calculated using the Friedewald formula (18). Total adiponectin was measured with an ELISA Quantikine kit (R&D systems, Boston Biochem). All biochemical analyses were performed in the laboratory of Department of Chemical Pathology, National Health Laboratory Services, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa. The metabolic syndrome was diagnosed using the harmonised guidelines (19).

The age range of the women in this study was 40–60 years, and therefore, menopause status was assessed in all subjects. Each study participant was asked about their menstrual bleeding patterns and were categorised as pre- or postmenopausal using the Stages of Reproductive Aging Workshop + 10 (STRAW+10) guidelines (20), which had previously been validated in this study population (16). The women were also questioned on their use of hormone therapy, contraceptive agents and previous hysterectomy.

Data analysis

Univariate correlations of adiponectin with body fat measures and CMD risk factors were assessed using univariate regression analysis. Multivariable linear regression models were developed for the following dependent (outcome) variables: adiponectin, glucose, insulin resistance (HOMA), total cholesterol, LDL, HDL, triglycerides and systolic and diastolic blood pressure. The purpose of these models was to isolate the principal body fat depots that had the strongest association with each of these variables. Therefore, all the models included the following independent (effector) variables: gluteofemoral (or total leg), visceral, total arm and abdominal s.c. fat. All models also included age and height. These variables were chosen because they covered the main body fat depots and collinearity between them was low. Collinearity was assessed using the variance inflation factor (VIF) which was found to be >5.00 for total body fat, BMI and s.c. fat, and therefore, only the latter variable was used in the regression models. Collinearity was also observed between total leg (VIF = 10.0) and gluteofemoral fat (VIF = 11.6) due to the high correlation (Pearson r = 0.94) between these variables. This is due to the wide anatomical overlap of these body regions on the DXA scans (see Supplementary Fig. 1). Therefore, due to the strong anatomical and analytical relationship between these fat depots, we decided that for each of the dependent variables, two separate multivariable regression models should be used which included either gluteofemoral or total leg fat alongside the other independent variables listed above. Neither hip nor waist circumference was used in the regression models as these are proxy measures of gluteofemoral and visceral fat, respectively, with measures of both these fat depots being provided by the DXA scans. Menopausal status may affect body fat distribution, blood pressure and serum levels of adiponectin, glucose, insulin or lipids. Therefore, all the multivariable linear regression models were performed with and without adjustment for menopause status.

The level of mediation by adiponectin of the association of body fat depots with each of the selected CMD risk factors was assessed by adding adiponectin to each of the multivariable linear regression models described above. The effect of adiponectin on the standardised β coefficient for each body fat depot was then observed. Structural equation modelling was used to confirm the extent of the indirect effects of adiponectin in the multivariable linear regression models. In order to assess whether the models were acceptable, we focused on the following fit indices: root mean squared error of approximation (RMSEA) <0.08, comparative fit index (CFI) and Tucker-Lewis index (TLI) >0.90. The fit statistics for the structural equation models for the various cardiometabolic variables suggested a good fit (all TLI > 0.90 and CFI > 0.90 and RMSEA < 0.07).

The sample size for this study was calculated based on the multivariable regression models described above. Assuming a minimum effect size of 0.05, statistical power of 0.90, the maximum number of independent variables as 7 and an alpha level of 0.05 (21), the calculated minimal sample size was 373.
Results

Participants’ characteristics

Table 1 provides data on anthropometric and cardiometabolic variables for the study population. The sample number is lower for the blood analysis data due to insufficient blood volume to run all the tests. The data show that this population had a mean age of just below 50.0 years and had a high mean BMI of 32.6 ± 6.29 kg/m² (Table 1). The prevalence of obesity (BMI ≥ 30.0) was 66.8% and metabolic syndrome was 49.6%. Menopause status could be assessed in 559 of the 648 participants, with 89 women not able to be staged due to current use of contraceptives (n = 58) or a previous hysterectomy (n = 31). Thus, of these 559 women, 48.1% were postmenopausal and 51.9% were premenopausal. No women reported the use of hormone therapy.

Univariate regression models for adiponectin

Univariate regression analyses for adiponectin showed that all fat depots, with the exception of total leg fat (P=0.26) and gluteofemoral fat (P=0.07), correlated strongly (P<0.0001) and negatively with serum adiponectin levels (Supplementary Table 1). Height did not correlate with adiponectin levels (P=0.38). Age, cholesterol levels and diastolic and systolic blood pressures did not correlate with adiponectin while insulin resistance and triglycerides both correlated strongly (P<0.0001) and negatively with adiponectin as did fasting plasma glucose levels (P=0.001), while HDL levels correlated (P<0.0001) positively (Supplementary Table 1). Menopause status was also associated with adiponectin levels which were higher in post- than in premenopausal women (9.31 ± 7.03 vs 7.86 ± 5.00 µg/mL; P=0.0003).

Multivariable linear regression model for adiponectin

Table 2 shows multivariable linear regression models for adiponectin. In model 1, visceral and s.c. abdominal fat correlated negatively with adiponectin, while gluteofemoral fat correlated positively (all three P-values <0.0001). Gluteofemoral fat was shown to be collinear with total leg fat, and therefore, the former variable was replaced by total leg fat in regression model 2 (see Table 2). The R² for both models was 0.26; the β-coefficient for total leg fat (0.43; P<0.0001) was slightly lower than that for gluteofemoral fat at 0.48 (P<0.0001). In both models, arm fat showed a negative, non-significant association with adiponectin, height also did not correlate with adiponectin, whereas age correlated positively and significantly. Both models explained 26.0% of the variance in serum adiponectin levels at P<0.0001, and the VIFs for each model demonstrated that multicollinearity was low.

Relationship of body fat depots with cardiometabolic variables and role of adiponectin

Multivariable linear regression models were used to analyse the association of the different body fat depots with cardiometabolic variables and whether these associations were mediated by adiponectin (see Table 3 and Supplementary Table 2). The mediation effect was assessed using structural equation modelling (SEM), and these results are given in Table 4.

In the glucose model, gluteofemoral fat has by far the strongest association (in a negative direction) with glucose (P<0.0001), but there is minimal mediation from adiponectin as demonstrated by very small changes in any of the β-coefficients once adiponectin is added to the model (see Table 3). This is confirmed by SEM where all the β-coefficients for the indirect effects (i.e. via adiponectin) are small (see Table 4). This trend is repeated in the models for cholesterol, LDL and diastolic and systolic blood pressure, where no mediating effect of adiponectin was observed. Gluteofemoral fat has only weak associations
Table 2  Multivariable linear regression models including either gluteofemoral or total leg fat showing the relationship of serum adiponectin level (logged) with body fat depots.

<table>
<thead>
<tr>
<th>Model number</th>
<th>Dependent variable</th>
<th>Independent variables with ( \beta )-coefficients(^a) and ( R^2 ) and ( P )-level for full model(^b)</th>
<th>VIF</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Adiponectin</td>
<td>Gluteofemoral fat: 0.48; &lt;0.0001 Visceral fat: −0.32; &lt;0.0001 Subcutaneous fat(^c): −0.49; &lt;0.0001 Arm fat: −0.05; 0.38 Height: −0.06; 0.11 Age: 0.10; 0.003 Total leg fat: 0.43; &lt;0.0001 Visceral fat: −0.31; &lt;0.0001 Subcutaneous fat(^c): −0.39; &lt;0.0001 Arm fat: −0.06; 0.10 Height: −0.04; 0.23 Age: 0.10; 0.006</td>
<td>3.28 1.62 4.57 3.11 1.09 1.05 3.28 1.65 3.89 3.29 1.07 1.05</td>
</tr>
<tr>
<td>2</td>
<td>Adiponectin</td>
<td>Gluteofemoral fat: 0.48; &lt;0.0001 Visceral fat: −0.32; &lt;0.0001 Subcutaneous fat(^c): −0.49; &lt;0.0001 Arm fat: −0.05; 0.38 Height: −0.06; 0.11 Age: 0.10; 0.003 Total leg fat: 0.43; &lt;0.0001 Visceral fat: −0.31; &lt;0.0001 Subcutaneous fat(^c): −0.39; &lt;0.0001 Arm fat: −0.06; 0.10 Height: −0.04; 0.23 Age: 0.10; 0.006</td>
<td>3.28 1.62 4.57 3.11 1.09 1.05 3.28 1.65 3.89 3.29 1.07 1.05</td>
</tr>
</tbody>
</table>

\(^a\)Data given as standardised \( \beta \)-coefficients; \(^b\)abdominal s.c. fat; \(^c\)adjusted \( R^2 \).

VIF, variance inflation factor.

with the dependent variables in all of these models. The models for LDL and systolic blood pressure are not shown due to their similarities to the models for cholesterol and diastolic blood pressure, respectively.

In the regression model for HOMA, gluteofemoral and visceral fat had the strongest associations \( (P<0.0001\) for both) with this variable, with the former fat depot having a negative effect and the latter a positive effect. Subcutaneous \( (P=0.001)\) and arm fat \( (P=0.006)\) also correlated positively with HOMA. Including adiponectin in this model attenuated all \( \beta \)-coefficients and SEM showed that adiponectin mediated 20.7%, 19.2% and 25.9% of the associations of gluteofemoral, visceral and s.c. fat, respectively, with HOMA (see Table 4).

The regression model for HDL shows that visceral \( (P=0.001)\) and arm fat \( (P=0.01)\) were the only fat depots that correlated substantially (and negatively) with HDL. Addition of adiponectin to this model attenuated the \( \beta \)-coefficient for visceral fat and SEM showed that adiponectin mediated 47.1% of the visceral fat association (see Table 4). In the model for triglycerides, gluteofemoral and visceral fat both had strong \( (P<0.0001\) for both) associations with this lipid, the former in a negative and the latter in a positive direction. Addition of adiponectin to this model demonstrated that this adipokine mediated 16.1% and 12.5% of the associations of gluteofemoral and visceral fat, respectively (see Table 4).

Adiponectin had strong and negative associations with HOMA \( (P<0.0001)\) and triglycerides \( (P=0.0003)\) and a positive association with HDL \( (P<0.0001)\).

When all the regression models and SEM described above were repeated with gluteofemoral fat being replaced with total leg fat, the associations and trends observed in the gluteofemoral models (Tables 3 and 4) were very similar to those in the models with total leg fat (Supplementary Tables 2 and 3). However, in models where these fat depots had significant associations with the dependent variable, that is glucose, insulin resistance and triglycerides, the \( \beta \)-coefficients for gluteofemoral fat were higher than those for total leg fat, with or without the inclusion of adiponectin.

All the multivariable regression models were adjusted for menopausal status and this did not alter mediation by adiponectin. All models shown are those without menopause status.

**Logistic regression model for metabolic syndrome**

Multivariable logistic regression models were developed for metabolic syndrome using the same variables used in the linear regression models, and the results are shown in Table 5 and Supplementary Table 4 (gluteofemoral fat replaced with total leg fat). The odds ratios in the models that were not adjusted for adiponectin levels are very similar to those in the model in which adiponectin was included. This suggests that the mediation effect of adiponectin on the association of the body fat depots with metabolic syndrome is minimal. Visceral fat was shown to have the strongest positive association with metabolic syndrome \( (P<0.0001\) in both models) with gluteofemoral fat \( (P<0.0001)\) and total leg fat \( (P<0.0001)\) having weak negative associations \( (P=0.05\) and 0.07, respectively). Adiponectin was also shown to have a negative association with metabolic syndrome \( (P=0.002\) in both models).
Gluteofemoral fat: 0.11; 0.11

With adjustment for adiponectin

Gluteofemoral fat: –0.32; <0.0001
Visceral fat: 0.06; 0.28
Subcutaneous fat: 0.22; 0.01
Arm fat: 0.09; 0.20
Adiponectin: −0.05; 0.30
R^2 = 0.055, P < 0.0001

Gluteofemoral fat: –0.23; 0.0007
Visceral fat: 0.20; <0.0001
Subcutaneous fat: 0.20; 0.01
Arm fat: 0.16; 0.01
Adiponectin: –0.19; <0.0001
R^2 = 0.22, P < 0.0001

Discussion

No previous studies have explored the ability of adiponectin to mediate the association of gluteofemoral fat with CMD risk factors. The results from this study suggest that gluteofemoral fat has a positive association with adiponectin independent of visceral and s.c. fat mass. In addition, there was a negative correlation between gluteofemoral fat and fasting glucose and triglyceride levels but did not mediate the association with glucose levels. Due to the strong collinearity between gluteofemoral and total leg fat, the associations described for gluteofemoral fat were also observed for leg fat.

As expected, we found that adiponectin correlates negatively with different measures of total body adiposity and body fat distribution (12, 22). However, within these univariate analyses, adiponectin correlates weakly (P=0.07) and negatively with gluteofemoral fat, but this correlation becomes much stronger and positive in a multivariate linear regression model that includes other fat depots (Table 2). This is because gluteofemoral fat correlates with other fat depots which obscures the positive relationship between adiponectin and gluteofemoral fat in a univariate analysis. One other study has also shown that gluteofemoral fat does correlate positively with serum adiponectin levels but only in multivariable models that have been adjusted for other fat depots (11). A longitudinal study conducted...
Table 4  Structural equation modelling for the association between fat depots and CMD risk factors and the mediation effect of adiponectin.

<table>
<thead>
<tr>
<th>Fat parameters</th>
<th>Glucose</th>
<th>Insulin resistance*</th>
<th>Cholesterol</th>
<th>HDL</th>
<th>Triglycerides</th>
<th>DBP</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAT (direct)</td>
<td>−0.32b</td>
<td>−0.23c</td>
<td>−0.04</td>
<td>0.01</td>
<td>−0.26b</td>
<td>0.07</td>
</tr>
<tr>
<td>GAT (indirect)</td>
<td>−0.02</td>
<td>−0.06c</td>
<td>0.002</td>
<td>0.08b</td>
<td>−0.05c</td>
<td>0.01</td>
</tr>
<tr>
<td>GAT (total) (% mediated)</td>
<td>−0.30d (6.67)</td>
<td>−0.29e (20.7)</td>
<td>−0.04 (5.00)</td>
<td>0.09 (88.9)</td>
<td>−0.31d (16.1)</td>
<td>0.08 (12.5)</td>
</tr>
<tr>
<td>VAT (direct)</td>
<td>0.06</td>
<td>0.20d</td>
<td>0.12d</td>
<td>−0.10</td>
<td>0.27d</td>
<td>0.13d</td>
</tr>
<tr>
<td>VAT (indirect)</td>
<td>0.01</td>
<td>0.05b</td>
<td>−0.002</td>
<td>−0.08b</td>
<td>0.04d</td>
<td>−0.01</td>
</tr>
<tr>
<td>VAT (total) (% mediated)</td>
<td>0.07 (14.3)</td>
<td>0.26d (19.2)</td>
<td>0.12d (1.67)</td>
<td>−0.17d (47.1)</td>
<td>0.32d (12.5)</td>
<td>0.12d (8.33)</td>
</tr>
<tr>
<td>SAT (direct)</td>
<td>0.22d</td>
<td>0.20d</td>
<td>0.07</td>
<td>0.07</td>
<td>0.11</td>
<td>0.06</td>
</tr>
<tr>
<td>SAT (indirect)</td>
<td>0.02</td>
<td>0.07c</td>
<td>−0.003</td>
<td>−0.10b</td>
<td>0.06c</td>
<td>−0.01</td>
</tr>
<tr>
<td>SAT (total) (% mediated)</td>
<td>0.24d (8.33)</td>
<td>0.27d (25.9)</td>
<td>0.01 (30.0)</td>
<td>−0.03 (333.0)</td>
<td>0.18d (33.3)</td>
<td>0.05 (20.0)</td>
</tr>
<tr>
<td>Arms (direct)</td>
<td>0.09</td>
<td>0.16d</td>
<td>−0.08</td>
<td>−0.17d</td>
<td>−0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>Arms (indirect)</td>
<td>0.003</td>
<td>0.01</td>
<td>−0.0004</td>
<td>−0.02</td>
<td>0.01</td>
<td>−0.002</td>
</tr>
<tr>
<td>Arms (total) (% mediated)</td>
<td>0.09 (3.33)</td>
<td>0.17d (5.88)</td>
<td>−0.08 (0.50)</td>
<td>−0.19d (10.5)</td>
<td>−0.05 (20.0)</td>
<td>0.05 (4.00)</td>
</tr>
</tbody>
</table>

All models are adjusted for age and height; data are presented as standardized β coefficients for direct, indirect (through adiponectin) and total effects with % mediated in parentheses.

*Assessed using HOMA method; *P < 0.0001; **P < 0.01; ***P < 0.05;  HOMA, HDL, and DBP variables were log transformed to normality.

on women transitioning through the menopause has also shown that changes in gynoid fat mass correlate positively with changes in adiponectin levels (23). These and the current study are the only ones to have specifically measured gluteofemoral fat and show that it correlates positively with adiponectin levels. Three previous studies have also shown that leg fat measured by DXA correlated positively with blood adiponectin levels (12, 13, 14), and we observed the same association. This relationship was due to the marked anatomical overlap between gluteofemoral and total leg fat which led to a strong correlation and hence a high level of collinearity between these two fat depots. This strong collinearity led to both fat depots correlating positively with serum adiponectin levels. It is possible that the association of leg fat with adiponectin is driven by the gluteofemoral section of the leg fat measurement; however, we cannot rule out the possibility that leg fat that is inferior to the gluteofemoral depot may also contribute to the associations observed for leg fat.

Two longitudinal studies have demonstrated that during the transition through menopause, serum adiponectin levels rise (23, 24). We also found that adiponectin levels were higher in post- than in premenopausal women. The reason for the increase in adiponectin levels during the menopause transition is not known and requires further confirmation, since one previous study found no difference in adiponectin levels between pre- and postmenopausal women, although a positive association with FSH levels was observed (25).

The cellular mechanisms whereby an increase in particular body fat depots attenuates serum adiponectin concentrations, while higher gluteofemoral and leg fat mass causes adiponectin levels to rise, are not known. The physiological purpose of these opposing influences on adiponectin secretion is also not fully understood. Studies have shown that a number of factors do affect adipocyte secretion of adiponectin. Free fatty acids (26) and inflammatory cytokines (27) all suppress adipocyte production of adiponectin and may possibly

Table 5 Multivariable logistic regression model showing the relationship between body fat depots and the metabolic syndrome and the effect of adiponectin on these associations.

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Independent variables with odds ratio (95% CIs) and P-level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabolic syndrome</td>
<td>Without adjustment for adiponectin</td>
</tr>
<tr>
<td>Gluteofemoral fat: 0.98 (0.97, 1.00); 0.05</td>
<td></td>
</tr>
<tr>
<td>Visceral fat: 1.18 (1.11, 1.25); &lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Subcutaneous fat*: 1.02 (0.99, 1.04); 0.12</td>
<td></td>
</tr>
<tr>
<td>Arm fat: 1.27 (0.99, 1.63); 0.05</td>
<td></td>
</tr>
<tr>
<td>Height: 0.35 (0.02, 5.84); 0.47</td>
<td></td>
</tr>
<tr>
<td>Age: 1.03 (1.00, 1.07); 0.05</td>
<td></td>
</tr>
<tr>
<td>With adjustment for adiponectin</td>
<td></td>
</tr>
<tr>
<td>Gluteofemoral fat: 0.99 (0.97, 1.01); 0.26</td>
<td></td>
</tr>
<tr>
<td>Visceral fat: 1.15 (1.08, 1.23); &lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Subcutaneous fat*: 1.01 (0.98, 1.03); 0.44</td>
<td></td>
</tr>
<tr>
<td>Arm fat: 1.24 (0.96, 1.59); 0.09</td>
<td></td>
</tr>
<tr>
<td>Height: 0.26 (0.01, 4.56); 0.35</td>
<td></td>
</tr>
<tr>
<td>Age: 1.04 (1.01, 1.08); 0.01</td>
<td></td>
</tr>
<tr>
<td>Adiponectin: 0.94 (0.91, 0.98); 0.002</td>
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*Abdominal s.c. fat.
play a role in obesity-related hypoadiponectinaemia. Further investigation is required to understand whether these factors have attenuated effects on gluteofemoral adipocytes. In addition, type 2 diabetes is also associated with lower serum adiponectin levels and a study has shown that adiponectin protein levels and gene expression are higher in both gluteal and abdominal s.c. adipocytes from non-diabetic compared to diabetic subjects (Fisher et al., 2002). These data exemplify the importance of s.c. adiposity for adiponectin synthesis and demonstrate the association of diabetes with attenuated adipocyte production of adiponectin.

Multivariable regression analyses demonstrated that gluteofemoral fat correlated negatively with insulin resistance, glucose and triglyceride levels. These findings confirm previous studies demonstrating that gluteofemoral or leg fat has an inverse association with CMD risk factors. These include a study conducted on a large American population which showed that gluteofemoral fat correlates negatively with triglyceride levels and positively with HDL levels in women only (28). Similarly, a study performed on Chinese women demonstrated that gluteofemoral fat correlated negatively with blood pressure, glucose and triglyceride levels and positively with HDL levels (29). A large population cohort study has also shown that gluteofemoral fat correlates negatively with insulin resistance (30). In addition, studies using MRI to assess body fat distribution have demonstrated that non-obese subjects who are metabolically unhealthy have low levels of gluteofemoral and leg fat (31), and low percentage levels of these lower body fat depots are strongly associated with poor metabolic health and high carotid intima-media thickness, independent of visceral fat mass (32). It has also been observed that low levels of both adiponectin and lower body adiposity are associated with the presence of non-alcoholic fatty liver disease (NAFLD) (33), but it is not known whether the effects of leg and gluteofemoral fat on hepatic steatosis are mediated by adiponectin. A longitudinal study of 2683 lean, postmenopausal females has also demonstrated that low levels of leg fat are associated with an increased risk of incident cardiovascular events (34). A recent genome-wide association study has shown that a polygenic risk score for lower gluteofemoral and leg fat is associated with higher blood pressure and triglyceride levels and higher risk for diabetes and coronary disease (35). Other studies, using proxy measures of gluteofemoral fat, for example, hip circumference, have also shown that this fat depot has a negative association with various CMD risk factors (10).

Our findings of the partial mediation of gluteofemoral and leg fat associations with cardiometabolic variables by adiponectin are supported by data from another study, which was performed in men and showed that adiponectin partially explained the association of lower body fat (leg fat with some gluteal fat) with fasting serum lipid levels (36). There may be other mechanisms, in addition to adiponectin secretion, by which gluteofemoral fat may protect against CMD. One theory suggests that gluteofemoral fat may act as a preferred storage site for triglycerides and free fatty acids thus reducing deposition in the visceral fat depot or at ectopic sites such as skeletal muscle and liver (10). In addition, gluteofemoral fat has a different adipokine secretory profile when compared to other fat depots, which favours higher insulin sensitivity and lower systemic inflammation (10, 30). In terms of differential secretory profiles of body fat depots, fatty acid outputs from these sites have also been analysed. Thus, the fatty acid palmitoleate (16:1n-7), which is associated with improved insulin sensitivity in humans (37), has been shown to be secreted at higher levels from the gluteofemoral compared to the abdominal s.c. fat depot (38). This molecule may therefore be an additional factor that contributes to the negative association of lower body fat with CMD risk.

It is unlikely that the mediating effect of adiponectin on the association of gluteofemoral fat with CMD risk factors is specific to African females. A previous study performed in a male, Danish population observed mediation of the negative association of lower body fat with serum lipids by adiponectin (36), and a number of studies conducted in a variety of different ethnic groups have shown negative relationships of proxy (10) and direct (11) measures of gluteofemoral fat with CMD risk factors.

Although many mechanistic and cross-sectional epidemiological studies have demonstrated a possible role for adiponectin in the aetiology of various CMDs (12, 39), not all investigations support these findings. Some prospective studies have shown that low adiponectin levels are not related to a higher risk for stroke (40, 41) or coronary heart disease (CHD) (40, 42) but do predict incident diabetes (43), CVD (44), hypertension (3, 45) and non-alcoholic fatty liver disease (46). Also, studies using Mendelian randomization have failed to show causal relationships of low adiponectin levels with dyslipidaemia (47), coronary artery disease (48) and CHD (49) but have found associations with diabetes (50), sub-clinical atherosclerosis (51) and insulin resistance (52). In addition, there is debate over the association of adiponectin with insulin resistance with studies suggesting that this is a
bi-directional relationship (53). The data from the current investigation must therefore be interpreted in the context of the above investigations and highlight the need for functional studies to analyse the role of adiponectin in the aetiology of multiple CMDs.

The major limitation of this study is that it is cross-sectional, and therefore, we were only able to measure statistical associations between body fat depot sizes and CMD risk factors and to quantify the mediating effect of adiponectin on these associations; causality cannot be established. Therefore, it would be important to interrogate these findings via functional studies in rodents and longitudinal studies in humans. A further limitation of our study is that it was conducted on women only. However, we are currently collecting data on men. Lastly, the assay used for the measurement of adiponectin did not measure the high molecular weight (HMW) form of the protein but rather, total adiponectin. The HMW multimer is thought to be the major bioactive form and correlates more strongly with insulin sensitivity than does total adiponectin (54). It is therefore possible that the associations of adiponectin with the various CMD risk factors observed in this study are underestimates. A major strength of this study was that it used DXA to give specific measurements of gluteofemoral fat and other body fat depots. It must be noted that CT and MRI are considered ‘gold standard’ techniques for measuring body fat depots and that DXA has been shown to be comparable to both methods for assessing visceral fat levels (17, 55, 56) with an acceptable level of precision (57); however, the measurement of gluteofemoral fat by DXA has not been validated using other imaging technologies. This is the first study to analyse the effects of gluteofemoral fat on CMD risk factors in a sub-Saharan African population and is only one of three studies that have analysed the relationship between gluteofemoral fat and serum adiponectin levels. It is also the first study to assess whether adiponectin mediates the association of gluteofemoral fat with CMD risk factors.

In conclusion, gluteofemoral and total leg fat correlate positively with adiponectin levels and negatively with fasting glucose and triglyceride levels and insulin resistance. Furthermore, this study provides evidence that the association of gluteofemoral and leg fat with triglycerides and insulin resistance was partially mediated by adiponectin, suggesting that other factors must also be involved. Further investigations are required to determine what these factors may be, and the mechanisms whereby they attenuate CMD risk. Such studies are particularly important in understudied sub-Saharan African populations where the prevalence of both obesity and metabolic syndrome is high, particularly in midlife females (58).

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**Supplementary materials**

This is linked to the online version of the paper at [https://doi.org/10.1530/EC-22-0156](https://doi.org/10.1530/EC-22-0156).

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**Declaration of interest**

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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**Funding**

This work was supported by the Medical Research Council of South Africa (MRC); the National Health Laboratory Service Research Trust; the University of the Witwatersrand Iris Ellen Hodges Cardiovascular Research Trust and the National Research Foundation (NRF) of South Africa. BT20 plus is supported by the Wellcome Trust and Gates Foundation. S A N is supported by the DSI-NRF Centre of Excellence in Human Development at the University of the Witwatersrand, Johannesburg, South Africa.

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**Author contribution statement**

P J G: conceptual design, investigation, formal analysis, data curation, methodology, project administration, writing-original draft. N G J: investigation, methodology, project administration, writing-original draft. S A N: conceptual design, supervision, funding acquisition, writing-review and editing. M T: data curation, methodology, writing-review and editing. N J C: conceptual design, supervision, formal analysis, funding acquisition, validation, writing-original draft.

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**Acknowledgements**

The authors thank the staff of the MRC/Wits Developmental Pathways for Health Research Unit, Department of Paediatrics, Faculty of Health Sciences, for assistance with data collection and the National Health Laboratory Service routine laboratory at Charlotte Maxeke Johanneburg Academic Hospital for performing the assays.

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Received in final form 20 September 2022
Accepted 28 September 2022
Accepted Manuscript published online 28 September 2022