The relationship between obesity, leptin, adiponectin and the components of metabolic syndrome in urban African women, Free State, South Africa

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Introduction

Obesity is a global epidemic. In 2008, 1.4 billion adults were overweight. This number had escalated in 2014 to an alarming 1.9 billion adults worldwide, including over 600 million obese men (11%) and women (15%). The high prevalence of overweight and obesity is observed in developing countries as well as countries undergoing epidemiological transition, such as South Africa. Various studies have reported that the overall prevalence of overweight and obesity is the highest among South African women. A comparison of the 2013 data from the South African National Health and Nutrition Examination Survey (SANHANES-1) with 2007 data released by the South African Department of Health revealed that obesity increased dramatically from 27% to 39.2% amongst females. Urban women had a significantly higher body mass index (BMI) than rural women. This disturbing increase in obesity is of great concern due to the association of obesity with type II diabetes, dyslipidaemia and cardiovascular disease.

Metabolic syndrome refers to the cluster of risk factors that increase the risk of developing type II diabetes mellitus and cardiovascular diseases. These components include central obesity (increased waist circumference), elevated triglycerides, reduced high density lipoprotein cholesterol (HDL-C) levels, elevated blood pressure or treatment of previously diagnosed hypertension, and elevated fasting glucose. It is widely accepted that a number of peptide hormones are released from adipocytes. The adipocyte-derived biologically active substances (adipokines), leptin and adiponectin, are implicated in the pathogenesis of metabolic syndrome. These proteins secreted by adipose tissue are dysregulated when the adipocyte mass is markedly altered. With increasing adiposity, adipokines with pro-inflammatory properties, for example leptin, are overproduced, while anti-inflammatory adipokines, for example adiponectin, are decreased. Various South African studies have demonstrated ethnic differences in leptin levels. Normal and overweight African women had significantly higher serum leptin levels than their Caucasian counterparts, when matched for age and obesity.

Various studies have also focused on the critical role of adiponectin and leptin in the development of metabolic syndrome. Wolfson et al. observed a strong positive association between adiponectin levels and HDL-C, but a negative association with triglycerides and glucose. They could not demonstrate an association between adiponectin and BMI. Schutte et al. reported significant inverse correlations in African women between adiponectin and age, BMI and fasting blood glucose, and positive correlations with HDL-C. Martins et al. found that BMI as an index of overall adiposity was significantly associated with serum leptin and reported a strong correlation between leptin and insulin resistance. The leptin:adiponectin

Objectives: Obesity prevalence is increasing worldwide. In South Africa, older and urbanised African women have significantly higher rates of obesity. Limited information is available on the relationship between anthropometric parameters, adipokines and metabolic health status of African women. This study investigated the relationship between obesity, adipokines and the components of metabolic syndrome in urban African women.

Methods: This study included 135 urban African women that were 26–63 years of age, identified with metabolic syndrome in the urban leg of the Assuring Health for All in the Free State (AHA-FS) study. To establish anthropometric status, the following measures were taken: body weight, height and waist circumference. Blood was drawn to determine leptin, adiponectin levels and metabolic status.

Results: Adiponectin levels in obese women were significantly decreased compared to normal weight women. Leptin levels and leptin:adiponectin ratios (L:A) were increased in the obese group compared to the overweight and normal weight groups. Leptin and L:A showed strong positive correlations with body mass index and waist circumference. Adiponectin levels decreased as the number of components of metabolic syndrome increased. The L:A ratio was significantly lower in women with elevated triglycerides and significantly higher in women with elevated blood glucose levels. Adiponectin levels were significantly lower in women with elevated blood glucose.

Conclusion: This study confirms the inverse relationship between adiponectin and leptin with increased body adiposity. Results indicate that waist circumference, fasting blood glucose and triglyceride levels are the metabolic syndrome components most closely associated with altered adiponectin and leptin levels and L:A in urban African women with metabolic syndrome.

Keywords: adipokines, adiponectin, components of metabolic syndrome, leptin, L:A ratio, obesity
ratio (L:A ratio) has also been indicated as a potentially clinically useful marker for metabolic syndrome. A study conducted in Japanese women investigated the association between L:A and components of metabolic syndrome. The results indicated a significant and gradual increase in L:A corresponding to the increase in the number of components of metabolic syndrome. A positive association between HDL-C, age and L:A ratio, but an inverse association between BMI, triglycerides and L:A ratio, was found.

Despite increased understanding of the relationship between anthropometric, metabolic and inflammatory parameters, limited information is available on these parameters and the metabolic health status of urban African women. The increasing prevalence of metabolic syndrome in developing countries such as South Africa requires strategic health policies and intervention programmes. The primary goal of this study was to investigate the relationship between anthropometric and metabolic parameters, leptin and adiponectin in urban African women with metabolic syndrome. Consequently, the following objectives were pursued in this study population: (i) determine fasting adiponectin and leptin levels; (ii) investigate the association between BMI status and leptin and adiponectin levels; (iii) investigate the relationship between leptin and adiponectin levels and the components of metabolic syndrome; (iv) determine which components of metabolic syndrome are more closely related to altered adiponectin and leptin levels; and (v) determine whether ageing is associated with the an increasing number of components of metabolic syndrome.

Methods

Materials and setting

This study formed part of the baseline phase of the Assuring Health for All in the Free State (AHA-FS) epidemiological study. The urban leg of the study commenced in 2009 in Mangaung in the Free State. The number of plots in the Mangaung University Community Partnership Programme (MUCPP) service area was counted on a municipal map. An estimate was made of additional squatter households in open areas. A stratified proportional cluster sample was selected, stratified by area and formal plot/squatter households in open areas. Using randomly selected X and Y coordinates, 100 starting points were selected. From each starting point five adjacent households were approached.

Study population and sample

A total of 387 households were included in the urban leg of this study. The total study sample consisted of 565 participants (adults and children). However, this article will focus only on anthropometric and metabolic parameters as well as adipokine levels of African female participants (26–63 years) identified with metabolic syndrome.

Procedures

Adult participants were interviewed to complete household sociodemographic and individual health questionnaires. The questionnaires also included information regarding risk factors for metabolic syndrome, for example a history of hypertension and diabetes.

To establish anthropometric status, body weight, height and waist circumference were measured. Anthropometric evaluation was done after an overnight fast, in an examination gown and without shoes. Weight was determined using a Seca® (Germany) digital electronic foot scale. Anthropometric indices calculated included BMI [weight in kilograms divided by height in meters squared (kg/m²)]. Underweight was defined as BMI < 18.5 kg/m², normal weight as BMI 18.5–24.9 kg/m², overweight (pre-obese) as BMI 25–29.9 kg/m² and obese as BMI ≥ 30 kg/m². The cut-off point for central obesity was a waist circumference of ≥ 80 cm for women. The World Health Organisation’s STEPwise Approach to Surveillance (WHO STEPS) protocol for measuring waist circumference was followed.

Participants underwent medical examinations and blood sampling to determine leptin, adiponectin levels and metabolic profiles. Blood pressure was measured in the supine position with a DS-175, auto-inflate digital electronic blood pressure monitor (Nissei, Japan). Urgent medical conditions were referred on the day of medical examination. All data forms and blood sample results were reviewed for referral. Communities were visited by medical practitioners again and participants could obtain results of biochemical tests and referral letters. If participants did not attend the individual feedback appointments, referral letters were delivered to participants by community workers. Patients were referred to local clinics, local health care providers and health care centers.

All serum and plasma samples were prepared in the laboratory according to standard methods and stored at -80°C until analyses were performed. Leptin levels were determined using a quantitative sandwich enzyme immunoassay technique (Quantikine®, Human Leptin Immunoassay, R&D Systems, Inc., Cat. No. SLP00, USA). Adiponectin levels were determined using a quantitative sandwich enzyme immunoassay technique (Quantikine® ELISA Human Total Adiponectin/Acrp30 Immunoassay, R&D Systems, Inc., Cat. No. SRP300, USA). Adiponectin and leptin analyses were performed by the Centre of Excellence in Nutrition, Faculty of Health Sciences, University of North West, Potchefstroom, South Africa.

Blood specimens for the measurement of fasting venous plasma glucose (FVPG) were drawn into fluoride tubes. Analyses were performed by an accredited private laboratory in Bloemfontein, South Africa. Samples were centrifuged within four hours and FVPG was measured immediately after centrifuge using the glucose oxidase method, on a Beckman LX20® auto-analyser (Beckman Coulter, USA). Low density lipoprotein (LDL) (normal levels for women: > 40 mg/dL [1.04 mmol/L]) were measured using enzymatic assay kits on a Beckman LX20® auto-analyser (Beckman Coulter, USA). Low density lipoprotein (LDL) cholesterol levels (normal value < 100 mg/dL [2.59 mmol/L]) were calculated indirectly with the Friedewald equation, namely:

$$\text{LDL cholesterol} = \frac{\text{total cholesterol} - \text{HDL cholesterol} - \text{triglyceride}}{5}$$

The International Diabetes Federation (IDF) and American Heart Association/National Heart, Lung and Blood Institute (AHA/NHLBI) criteria for the clinical diagnosis of the metabolic syndrome were applied in this study. Female participants were diagnosed with metabolic syndrome if they presented with at least three of the following five components, namely (i) waist circumference ≥ 80 cm; (ii) triglycerides ≥ 150 mg/dL; (iii) HDL-C < 50 mg/dL; (iv) blood pressure ≥ 130/85 mm Hg or history of treatment of previously diagnosed hypertension; and (v) fasting blood glucose ≥ 110 mg/dL (≥ 5.6 mmol/L).
Statistical analysis
Data were analysed by the Department of Biostatistics, Faculty of Health Sciences, University of the Free State (UFS). Due to skew data distributions, medians and percentiles for the numerical variables were calculated. Significance of associations between categorical variables and numerical variables were determined using 95% confidence intervals (CI) for differences in percentages or medians. Correlations between continuous variables were determined using Spearman’s correlation coefficients.

Validity and reliability
To ensure validity, all methods (questionnaire, blood sampling and medical examination) aimed at achieving the objectives of the study as listed above. Participants were interviewed by trained final-year and postgraduate students from the Department of Nutrition and Dietetics at the UFS, supervised by lecturers, to complete standardised questionnaires related to household sociodemographic information and individual health information. Where necessary, Sesotho, Setswana and isiXhosa interpreters assisted the researchers. In order to address reliability of questionnaires, 10% of all interviews were repeated. Where the percentage of given responses to questions differed with more than 20%, the question was considered unreliable. No questions were found to be unreliable. All analyses on blood samples were done in accredited laboratories and the reliability of the blood sampling procedures was ensured by using standard laboratory techniques. Appropriate standardised measuring techniques were used to obtain reliable anthropometric measurements.

Ethical considerations
The study protocol was approved by the Ethics Committee of the Faculty of Health Sciences, UFS (ETOVS number 21/7), the Department of Health and local municipalities. All participants gave informed written consent.

Results
Adult participants included 22.1% male and 77.9% female participants, with a mean age of 42.5 years. Only 11 male participants had three or more components of metabolic syndrome, compared to 144 female participants. Due to the small number of suitable male participants, this article will only report on the results of urban African women identified with metabolic syndrome. Nine of the 144 female participants were excluded due to their blood samples being inadequate to perform complete analyses. The mean age of the study group was 46.9 years.

Anthropometric and inflammatory parameters
Table 1 shows the association between BMI status and inflammatory parameters of urban African women identified with metabolic syndrome. Adiponectin levels in obese women (BMI ≥ 30 kg/m²) were significantly decreased compared to that of normal weight women (BMI 18.5–24.9 kg/m²) (95% CI −9.86; −2.94). Leptin levels in the obese group were significantly higher compared with the overweight (95% CI 14.91; 31.86) and normal weight group (95% CI 25.22; 45.63). A strong positive association was observed between L:A and BMI, with the obese group having significantly higher ratios than the normal weight group (95% CI 4.13; 8.52) and overweight group (95% CI 2.18; 6.20). The adiponectin levels of the overweight group were significantly lower than that of the normal weight group (95% CI −8.98; −0.91), whereas the leptin levels (95% CI 5.93; 18.37) and the L:A ratio (95% CI 0.81; 2.70) were significantly higher.

Table 1: Anthropometric and inflammatory parameters of urban African women identified with metabolic syndrome

| Variable per body mass index (BMI) category | Median [Inter Quartile Range (IQR)] |
|--------------------------------------------|-----------------------------------|
| Normal weight (BMI 18.5–24.9 kg/m²) (n = 17)* |                                    |
| Age                                        | 50.0 [47.0–52.0]                   |
| Adiponectin (μg/mL)                        | 14.4 [9.3–19.1]                    |
| Leptin (ng/mL)                             | 9.1 [5.7–14.1]                     |
| L:A ratio                                  | 0.7 [0.5–1.3]                      |
| Overweight (BMI ≥ 30 kg/m²) (n = 83)       |                                    |
| Age                                        | 48.0 [41.0–56.0]                   |
| Adiponectin (μg/mL)                        | 6.4 [4.1–10.2]                     |
| Leptin (ng/mL)                             | 44.1 [31.8–75.7]                   |
| L:A ratio                                  | 7.9 [3.6–11.9]                     |

*Only one woman had a BMI in the underweight category and was grouped with the normal weight participants.
*L:A ratio = leptin/adiponectin ratio.

Components of metabolic syndrome, leptin and adiponectin
The association between the individual components of metabolic syndrome, leptin and adiponectin in urban African women are reflected in Table 2. Leptin levels were significantly higher (95% CI 9.27; 42.23) in women with increased waist circumference and there was a trend for adiponectin levels to be lower in these women (95% CI −6.63; 3.62). L:A was also significantly higher in women with increased waist circumference (95% CI 0.71; 8.01) and women with elevated fasting blood glucose (95% CI 0.36; 4.85), whereas L:A was significantly lower in women with elevated triglycerides (95% CI 3.27; 0.18). In women with elevated fasting blood glucose levels, adiponectin was significantly lower (95% CI −4.20; −0.88).

Six participants presented with all five components of metabolic syndrome. This group had a higher median age and a higher BMI (not significantly) than the participants identified with three or four components (Table 3). A trend towards an inverse association between adiponectin levels and the number of components was observed [three versus four components (95% CI −0.65; 2.72), three versus five components (95% CI −1.1; 6.54) and four versus five components (95% CI −2.28; 5.83)]. L:A ratio tended to be higher in the group identified with five components of metabolic syndrome [three versus five components (95% CI −14.22; 1.12) and four versus five components (95% CI −13.67; 1.73)].

As summarised in Table 4, leptin showed a strong positive correlation with BMI (r = 0.77) and waist circumference (r = 0.67). L:A ratio showed a strong positive correlations with both BMI (r = 0.67) and waist circumference (r = 0.55) and a weak positive correlation with fasting blood glucose (r = 0.27). Adiponectin showed a weak negative correlations with BMI (r = −0.27) and fasting blood glucose (r = −0.28). No significant correlations were found between triglycerides, HDL-C and the inflammatory markers.
Various studies have confirmed the involvement of leptin and adiponectin, both derived from adipose tissue, in the pathogenesis of obesity and metabolic syndrome.\(^2\)\(^2\) The results of the present study confirm previous observations illustrating an inverse relationship between adiponectin and leptin with altered adipocyte mass.\(^8\)\(^,\)\(^11\)\(^,\)\(^23\) We also observed an increase in the calculated L:A ratio with increasing adiposity. Leptin and L:A were identified as markers that have strong positive correlations with both BMI and waist circumference.

It is well known that adiponectin has anti-inflammatory properties, cardio-protective characteristics (anti-atherogenic) and improves insulin sensitivity (anti-diabetic), while leptin has pro-inflammatory properties. Prior studies have demonstrated that adiponectin levels decrease across the glycemic spectrum, while leptin levels increase. Schutte et al.\(^11\) found an inverse association between adiponectin and fasting blood glucose. We confirmed this inverse association between adiponectin and elevated fasting blood glucose levels, but could not illustrate a significant inverse association with elevated triglyceride levels as reported by Wolfson et al.\(^16\) Interestingly, we observed that L:A ratio had an inverse association with triglycerides and a direct association with fasting blood glucose levels.

Aging is an important biological factor for metabolic syndrome as it is associated with increased adiposity, mostly visceral obesity and altered adiponectin and leptin levels.\(^2\)\(^2\) The present study (age range 26–63 years) observed a trend between increasing age, BMI and the number of components identified for metabolic syndrome.

Previous studies have found that leptin, adiponectin, and the L:A ratio are informative biomarkers to evaluate the risk for metabolic syndrome.\(^12\)\(^,\)\(^23\)\(^–\)\(^26\) Yu et al.\(^27\) demonstrated that adiponectin levels decreased while leptin levels increased with an increased number of metabolic syndrome components in women. We observed that circulating adiponectin gradually decreased as the number of components for metabolic syndrome increased, although we could not illustrate an association between leptin

### Table 2: Association between the components of metabolic syndrome, leptin and adiponectin levels in urban African women

| Components of metabolic syndrome (cut-off points) | Median (Inter Quartile Range [IQR]) |
|--------------------------------------------------|-------------------------------------|
| Increased waist circumference*                    |                                     |
| ≥ 80 cm (n = 129)                                 |                                    |
| < 80 cm (n = 6)                                   |                                    |
| Elevated triglycerides                            |                                     |
| ≥ 150 mg/dl (1.7 mmol/L) (n = 45)                 |                                    |
| < 150 mg/dl (1.7 mmol/L) (n = 90)                 |                                    |
| Reduced HDL-C                                    |                                     |
| < 50 mg/dl (1.3 mmol/L) (n = 120)                 |                                    |
| ≥ 50 mg/dl (1.3 mmol/L) (n = 15)                  |                                    |
| Elevated fasting glucose                         |                                     |
| ≥ 100 mg/dl (≥ 5.6 mmol/L) (n = 35)               |                                    |
| < 100 mg/dl (≥ 5.6 mmol/L) (n = 100)              |                                    |
| Treatment of previously diagnosed hypertension   |                                     |
| No (n = 7)                                       |                                    |
| Yes (n = 128)                                    |                                    |

*Cut-off points for sub-Saharan Africans.

### Table 3: Adiponectin and leptin values, and L:A ratio in urban African women identified with metabolic syndrome, according to number of metabolic syndrome components present

| Variable                                  | Number of components | Median (Inter Quartile Range) |
|-------------------------------------------|----------------------|------------------------------|
| Age (years)                               | Three (n = 89)       | 47.0 [40.0–53.0]             |
| BMI (kg/m²)                               | Four (n = 40)        | 50.0 [42.5–57.0]             |
| Adiponectin (μg/mL)                       | Five (n = 6)         | 53.5 [53.0–65.0]             |
| Leptin (ng/mL)                            |                      |                              |
| L:A ratio                                 |                      |                              |

### Table 4: Spearman correlation coefficients between adiponectin, leptin and L:A ratio and metabolic syndrome-related risk factors

| Variable                                  | Adiponectin (μg/mL) | Leptin (ng/mL) | L:A ratio |
|-------------------------------------------|---------------------|----------------|-----------|
| Age (years)                               | 0.16                | −0.01          | −0.01     |
| BMI (kg/m²)                               | −0.27               | 0.77           | 0.67      |
| Waist circumference                       | −0.16               | 0.67           | 0.55      |
| Triglycerides                             | 0.07                | −0.15          | −0.14     |
| HDL-C                                     | 0.14                | 0.10           | 0.01      |
| Fasting glucose                           | −0.28               | 0.16           | 0.27      |

### Discussion

The prevalence of obesity is increasing worldwide and according to WHO reports, overweight and obesity are the fifth leading risk for global mortality.\(^3\) South African Demographic and Health Survey (SADHS) data indicate that older and urbanised African women in South Africa have significantly higher rates of obesity.\(^3\)\(^5\)

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| Reduced HDL-C                                    |                                     |
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| Treatment of previously diagnosed hypertension   |                                     |
| No (n = 7)                                       |                                    |
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| L:A ratio                                 |                      |                              |

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|-------------------------------------------|---------------------|----------------|-----------|
| Age (years)                               | 0.16                | −0.01          | −0.01     |
| BMI (kg/m²)                               | −0.27               | 0.77           | 0.67      |
| Waist circumference                       | −0.16               | 0.67           | 0.55      |
| Triglycerides                             | 0.07                | −0.15          | −0.14     |
| HDL-C                                     | 0.14                | 0.10           | 0.01      |
| Fasting glucose                           | −0.28               | 0.16           | 0.27      |

#### Discussion

The prevalence of obesity is increasing worldwide and according to WHO reports, overweight and obesity are the fifth leading risk for global mortality.\(^3\) South African Demographic and Health Survey (SADHS) data indicate that older and urbanised African women in South Africa have significantly higher rates of obesity.\(^3\)\(^5\)
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concentrations and the increasing number of components. This may depend on the nature of the components. For example, waist circumference may not have the same impact as a history of previously diagnosed hypertension. Kotani and Sakane demonstrated a significant and gradual increase in the L:A ratio corresponding to the number of components of metabolic syndrome. We also observed a significant increase in the L:A ratio in the group of women who presented with five components of metabolic syndrome. However, the small number of women in this group, precludes firm conclusions.

The present study also aimed to investigate which components of metabolic syndrome were more closely related to altered adiponectin and leptin levels in urban African women with metabolic syndrome. We found that fasting leptin and adiponectin levels and L:A ratio were associated with various components of metabolic syndrome.

This is one of the first studies investigating the relationship between obesity, pro-inflammatory and anti-inflammatory markers as well as the components of metabolic syndrome in urban African women with metabolic syndrome. Apart from the fact that our main study highlighted the need for serious recognition of the increasing burden of obesity and metabolic syndrome in urban African communities in South Africa, our findings also indicate that leptin, adiponectin and L:A ratio are significantly related to indices of adiposity. Elevated leptin, decreased adiponectin and an increased L:A ratio should be considered as warning signs, particularly in the obese individual, as these are associated with components of metabolic syndrome.

Limitations
The authors acknowledge the following limitations of the study. Only 11 male participants in the AHA-FS urban leg were identified with three or more risk factors for metabolic syndrome. Therefore, this article only reports on the results of urban African women identified with metabolic syndrome. We also acknowledge that due to the much younger mean age of the subject group identified with no components of metabolic syndrome, no suitable age-matched control group was available in the dataset.

Conclusion and Recommendations
This study emphasises the associations between obesity, inflammatory biomarkers (adiponectin, leptin and L:A ratio) and components of metabolic syndrome. Based on the findings of this study, we recommend follow-up research to consider the following:

- to evaluate the predictive value of these biomarkers to detect multiple risk factors in urban African women; and
- to indicate which combinations of the metabolic syndrome criteria best predict cardiovascular risk in these communities.

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