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# Leptin and the vasculature in young adults: The African-PREDICT study

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## Abstract

**Background and aim:** Information regarding the effect of leptin on the vasculature in young healthy adults at risk for cardiovascular disease development is limited. We therefore examined the associations between measures of subclinical atherosclerosis (carotid intima-media thickness, carotid cross-sectional wall area), large artery stiffness (pulse wave velocity) and a measure of endothelial dysfunction (von Willebrand factor [vWF]) with leptin in young healthy men and women.

**Methods:** In a cross-sectional study in South Africa involving 820 normotensive individuals (337 men and 483 women) aged 20-30 years, we measured carotid intima-media thickness, carotid cross-sectional wall area, pulse wave velocity, vWF from citrated plasma and leptin from serum.

**Results:** Despite sevenfold higher leptin in women than men ($P < 0.001$), only in young healthy men, we observed negative, independent associations between measures of carotid wall thickness (carotid intima-media thickness: $R^2 = 0.05$; $\beta = -0.20$; $P = 0.036$; carotid cross-sectional wall area: $R^2 = 0.05$; $\beta = -0.20$; $P = 0.035$) with leptin in multivariable-adjusted regression analyses. When reviewing these associations across body mass index categories, we found an association to be evident only in overweight men (carotid intima-media thickness: $R^2 = 0.15$; $\beta = -0.41$; $P = 0.007$; carotid cross-sectional wall area: $R^2 = 0.21$; $\beta = -0.47$; $P = 0.002$). No association was observed in the women or between pulse wave velocity and vWF with leptin.

**Conclusion:** In young healthy men, we found a beneficial inverse association between measures of carotid wall thickness and circulating leptin, thereby supporting a potential vascular protective role of leptin.

**KEYWORDS**
atherosclerosis, carotid intima-media thickness, endothelium and sex, healthy, overweight

# 1 | INTRODUCTION

Obesity is a growing public health concern worldwide[1] and is also now considered an important factor in the development of atherosclerotic cardiovascular disease (CVD), diabetes and other related metabolic disorders.[2] But the mechanisms by which adiposity contributes to alterations in both the anatomy and physiology of blood vessels remain only partially understood.[2] Accumulating evidence suggests a link between adipose tissue and the vasculature, implicating the product of the obesity (*ob*) gene, namely leptin.[3]

Leptin is a pleiotropic[4] and vasoactive hormone,[5] with a wide range of functions beyond the regulation of energy intake and expenditure.[6] The presence of leptin's receptor within the vasculature[7] also laid credence for leptin's involvement in the regulation of vascular function[8] which itself has yielded conflicting reports.[9] For example, leptin was shown to predict atherosclerosis, stroke, myocardial infarction and also coronary events in either overweight or obese individuals.[10-12] In contrast, other studies have reported a vascular protective role[8] of leptin against atherosclerosis in either overweight or obese humans or animals.[13,14] Recently, a meta-analysis involving 4257 participants with CVD and 26 710 controls showed that elevated leptin levels may not be associated with the risk of developing coronary heart disease and stroke in both men and women.[15]

Suggested concepts which may explain the disparity between the beneficial and detrimental effects of leptin include the widespread cardiovascular and dose-dependent effects of leptin, as well as the concept of selective leptin resistance.[16] It is currently unknown whether leptin plays a role during the early phases of atherosclerosis development in young adults in the absence of overt CVD. Previous studies investigating the involvement of leptin in endothelial dysfunction and atherosclerosis development focused generally on older (mean age, 45 years) overweight or obese individuals, those with the metabolic syndrome (hypertension and diabetes), diseased (CVDs; myocardial infarction, coronary heart disease and stroke) or in Western populations.[9,10,17-19] However, the role of leptin on vascular health especially in young adults (20-30 years of age), and potential disparities in men and women at risk of obesity and CVD development, is unknown. To address this, we investigated young healthy men and women and determined whether measures of subclinical atherosclerosis, large artery stiffness and a marker of endothelial dysfunction (von Willebrand factor [vWF])[20,21] are associated with leptin.

# 2 | MATERIALS AND METHODS

## 2.1 | Study population

Participants were recruited as part of the larger African-PREDICT study (African Prospective study on the Early Detection and Identification of Cardiovascular disease and Hypertension). The African-PREDICT study is aimed at following up the healthy young adults over a period of 10 years, in order to identify and track potential markers of early cardiovascular risk.

The current cross-sectional sub-study includes the first consecutive 820 participants (men N = 337; women N = 483; white N = 389 and black N = 431) enrolled in the African-PREDICT study, with measurements performed on campus of the North-West University in South Africa. The study adhered to all applicable requirements of the Helsinki Declaration, and the African-PREDICT study and this present sub-study have been approved by the Health Research Ethics Committee of the North-West University. All subjects participated voluntarily in the study and also provided written informed consent. Inclusion criteria for eligible participants were the following: black and white men and women aged 20-30 years; normal clinic BP (BP <140/90 mm Hg after three consecutive readings) and blood glucose; no known CVD; not using any antihypertensive medication; no chronic disease (or treatment thereof); HIV-free and not pregnant or breast feeding.

## 2.2 | Questionnaire data

Demographic and lifestyle questionnaires as well as the global physical activity questionnaire (GPAQ) were used to determine medical history, lifestyle, socioeconomic status, traditional risk factors and physical activity.

## 2.3 | Body composition and physical activity

Weight (kg; SECA electronic scales, SECA, Birmingham, UK), height (cm; SECA stadiometer, SECA) and waist circumference were measured with a nonflexible tape measure (Holtain, Crymych, UK). Body mass index was calculated using the standard formula of weight (kg)/height ($m^2$). Bioelectrical impedance was used to assess lean body mass and body fat percentage (Bodystat 1500MDD dual-frequency analyser; Bodystat, Ltd, Ballakaap, British Isles). For the assessment of active energy expenditure (AEE; estimation of physical activity), each participant wore a combined heart rate (HR) and accelerometer, namely an ActiHeart device (CamNtech, Cambridge, UK) for a maximum of 7 consecutive days and data were collected at 60-s epochs. The AEE was further indexed by dividing AEE by the weight of the participants, to compensate for increased energy expenditure accompanied with increase in body mass.

## 2.4 | Biochemical measurements

In the early morning, a research nurse took a fasting blood sample, and samples were prepared on site and stored at −80°C. We performed analyses of serum high-sensitivity C-reactive protein, total cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides, gamma-glutamyltransferase, and glucose in sodium fluoride plasma (Cobas Integra 400plus; Roche, Basel, Switzerland). Serum cotinine was determined with a chemiluminescence method on the Immulite (Siemens, Erlangen, Germany). Serum leptin levels were determined in duplicate using an enzyme-linked immunosorbent assay kit (Quantikine, R&D Systems, MN, USA), and adiponectin levels were determined with the Human Adiponectin ELISA kit (BioCat GmbH, Heidelberg, Germany). We determined vWF in citrate plasma using a sandwich ELISA. Polyclonal rabbit anti-vWF antibody and rabbit anti-vWF-horseradish peroxidase antibody (DAKO, Glostrup, Denmark) were used to perform the assay.

## 2.5 | Cardiovascular measurements

Participants were fitted with a 24-hour ambulatory blood pressure and ECG apparatus (CardioXplore®, CE0120; Meditech, Budapest, Hungary), using an appropriate sized cuff on the participant's nondominant arm. If <70% of the recordings were successful, the measurement was repeated the next day.

Carotid intima-media thickness (CIMT) was measured on the left and right common carotid artery (General Electric Vivid E9; GE Vingmed Ultrasound A/S, Horten, Norway), by a single medical technologist. Images from at least two optimal angles of the left and right common carotid artery were obtained. A single reader conducted measurements using a semi-automated program, namely the Artery Measurement Systems software (AMS) II v1.139 (Chalmers University of Technology, Gothenburg, Sweden). The cross-sectional wall area (CSWA) was calculated to confirm structural and not functional changes in luminal diameter: $\mathrm{CSWA} = \pi(d/2 + \mathrm{CIMT})^2 - \pi(d/2)^2$, where $d$ denotes luminal diameter.

Carotid-femoral pulse wave velocity (PWV) was measured using the Sphygmocor® XCEL device (AtCor Medical Pty. Ltd, Sydney, NSW, Australia) according to the manufacturer's instructions. PWV was measured along the descending thoraco-abdominal-aorta using the foot-to-foot velocity method, while the participant was in a supine position. Prior to this test, participants were not allowed to eat at least 8 hours before the procedure. During this measurement, PWV was captured at the right carotid and femoral arterial pulse points. The femoral artery wave form was captured via an appropriate sized cuff placed around the thigh, and the carotid arterial waveform was captured simultaneously via applanation tonometry. The distances between the pulsated sites were measured using an infantometer, and 80% of these distances were used as the pulse wave travelled distance.

## 2.6 | Statistical analyses

Variables that were not normally distributed were log transformed and represented as geometric mean with 5th and 95th percentiles. Normally distributed variables were presented as mean ± standard deviation and categorical variables represented as percentages. We tested the interaction effects of either ethnicity or BMI for the associations between vWF, PWV, CIMT and CSWA with leptin. Independent $t$ tests were done to compare means of men and women, and chi-square tests ($\chi^2$) to compare frequencies. Linear regression analyses were conducted to determine the associations of vWF, PWV, CIMT and CSWA with leptin. These analyses were performed in the total group, in groups according to leptin tertiles, and in groups based on BMI categories. Regression models all included the following covariates: leptin, age, ethnicity, socioeconomic score, body fat percentage, 24-hour mean arterial blood pressure, LDL-cholesterol, C-reactive protein, glucose, gamma-glutamyltransferase and moderate-vigorous physical activity.

# 3 | RESULTS

## 3.1 | Characteristics of the study population

Aligned with our aim, we grouped our participants into men and women, also due to reported differences in leptin concentrations with respect to sex[22,23] (Table 1). When comparing men and women, we found that men had lower vWF ($P = 0.018$), but higher PWV ($P < 0.001$), CIMT ($P = 0.021$) and CSWA ($P < 0.001$) than women. Women had higher BMI ($P = 0.004$), body fat percentage and leptin (all $P < 0.001$) than men.

## 3.2 | Pearson analysis

We performed Pearson correlation analyses between vWF, PWV, CIMT and CSWA with leptin in men and women and found a negative association between CIMT and CSWA with leptin only in men (all $P < 0.001$; Figure 1). There were no significant associations between vWF and PWV in men, as well as between vWF, PWV, CIMT and CSWA with leptin women.

## 3.3 | Multivariable-adjusted regression analyses

We conducted a forward stepwise multiple variable adjustment analysis (Table 2) where we determined the associations between vWF, PWV, CIMT and CSWA with leptin in

**TABLE 1** Basic characteristics of young men and women

| Number of participants | Men (N = 337) | Women (N = 483) | *P* |
|---|---|---|---|
| Ethnicity, black, N (%) | 168 (49.9) | 263 (54.45) | 0.19 |
| Age (y) | 24.9 ± 3.0 | 24.5 ± 3.1 | 0.67 |
| Socioeconomic status | | | |
| Low, N (%) | 127 (37.7) | 187 (38.7) | 0.051 |
| Middle, N (%) | 81 (24.0) | 129 (26.7) | |
| High, N (%) | 129 (38.3) | 167 (34.6) | |
| Body composition | | | |
| Body mass index (kg/m$^2$) | 24.2 [17.8; 33.6] | 25.3 [18.3; 37.8] | 0.004 |
| Waist circumference (cm) | 82.0 [64.8; 108] | 77.4 [63.0; 103] | <0.001 |
| Body fat percentage (%) | 17.5 ± 6.73 | 32.0 ± 8.63 | <0.001 |
| Lean body mass (kg) | 61.5 ± 11.7 | 45.7 ± 6.61 | <0.001 |
| Biochemical variables | | | |
| Leptin (ng/mL) | 4.27 [0.39; 32.5] | 29.0 [6.58; 96.7] | <0.001 |
| Von Willebrand factor (%) | 80.7 [41.0; 173] | 84.9 [39.0; 203] | 0.018 |
| Adiponectin (μg/mL) | 3.07 [0.66; 10.1] | 4.56 [1.13; 14.5] | 0.008 |
| Total cholesterol (mmol/L) | 4.19 [2.81; 6.12] | 4.09 [2.80; 5.97] | 0.062 |
| LDL-C (mmol/L) | 2.73 [1.54; 4.57] | 2.56 [1.45; 4.25] | 0.11 |
| HDL-C (mmol/L) | 1.16 [0.75; 1.76] | 1.33 [0.81; 2.10] | 0.50 |
| Triglycerides (mmol/L) | 0.95 [0.45; 2.21] | 0.76 [0.39; 1.65] | 0.002 |
| Glucose (mmol/L) | 4.75 ± 0.80 | 4.60 ± 0.67 | <0.001 |
| C-reactive protein (mg/L) | 0.76 [0.10; 6.72] | 1.45 [0.14; 12.7] | 0.14 |
| Cardiovascular measurements | | | |
| 24-h systolic BP (mm Hg) | 122 ± 8.01 | 113 ± 8.53 | 0.21 |
| 24-h diastolic BP (mm Hg) | 70.1 ± 5.92 | 68.1 ± 5.63 | 0.31 |
| 24-h mean arterial BP (mm Hg) | 90.7 ± 5.94 | 86.1 ± 6.34 | 0.19 |
| Pulse wave velocity (m/s)[a] | 6.65 ± 0.84 | 6.04 ± 0.83 | <0.001 |
| Carotid intima-media thickness (mm)[a] | 0.44 ± 0.07 | 0.43 ± 0.07 | 0.021 |
| Carotid cross-sectional wall area (mm$^2$)[a] | 8.42 ± 1.63 | 7.69 ± 1.61 | <0.001 |
| Lifestyle | | | |
| Active energy expenditure (kCal) | 328 [136; 672] | 390 [147; 924] | 0.035 |
| AEE/body weight (kCal/kg) | 36.5 [14.8; 74.7] | 43.4 [16; 103] | 0.035 |
| MVPA (min/d) | 53.8 [6.43; 406] | 50.5 [7.14; 360] | 0.51 |
| Self-reported tobacco use, N/total (%) | 203/452 (44.91) | 113/313 (36.10) | 0.015 |
| Cotinine (ng/mL) | 7.60 [1.00; 411] | 2.16 [1.00; 228] | <0.001 |
| Gamma-glutamyltransferase (U/L) | 27.0 [12.6; 82.8] | 18.7 [7.90; 54.8] | 0.22 |
| Self-reported alcohol use, N/total (%) | 108/179 (60.3) | 211/593 (35.6) | <0.001 |

Values are expressed as arithmetic mean ± standard deviation or geometric mean (5th to 95th percentile intervals) for logarithmically transformed variables, or number of participants and percentages (%).

AEE, active energy expenditure; BMI, body mass index; BP, blood pressure; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; MVPA, moderate-vigorous physical activity and $P \leq 0.05$ were considered significant.

[a]The values of carotid intima-media thickness, cross-sectional wall area as well as pulse wave velocity were adjusted for 24-h mean arterial pressure (ANCOVA).

men and women. From the result, only men showed an independent inverse association between CIMT (Std $\beta = -0.27$; $P < 0.001$; $R^2 = 0.07$) and CSWA (Std $\beta = -0.24$; $P = 0.002$; $R^2 = 0.06$) with leptin.

## 3.4 | Sensitivity analysis

We conducted a forward stepwise multiple regression analysis between vWF, PWV, CIMT and CSWA with leptin in

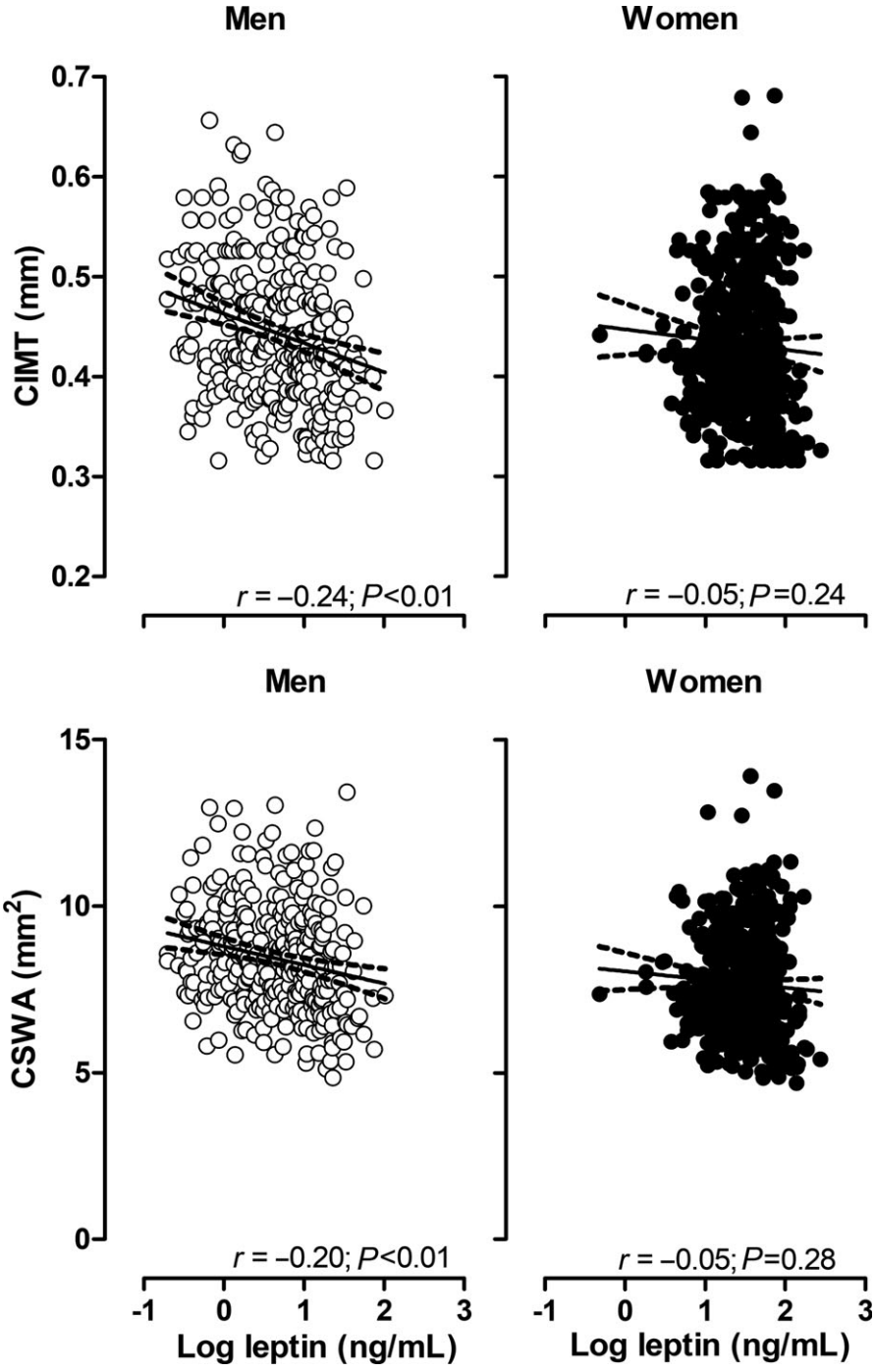


**FIGURE 1** Leptin plotted against carotid intima-media thickness and cross-sectional wall area in both men and women. Solid and dashed lines represent the regression line and the 95% CI boundaries

the men and women after stratifying according to leptin tertiles and BMI categories. Based on leptin tertiles (Table 3), men showed an independent inverse association between PWV with leptin in the first tertile (Std $\beta = -0.21$; $P = 0.047$; $R^2 = 0.19$) and women between vWF with leptin in the second tertile (Std $\beta = -0.21$; $P = 0.018$; $R^2 = 0.12$). In the BMI categories (Table 4), only in overweight men did we observed a negative association between CIMT (Std (Std $\beta = -0.45$; $P < 0.001$; $R^2 = 0.16$) and CSWA (Std $\beta = -0.43$; $P < 0.001$; $R^2 = 0.23$) with leptin. No association was found in the lean men and in any of the BMI categories of women.

There was no interaction of ethnicity (Table S1) between our variables of interest with leptin, but to further strengthen our findings and based on a previous study that has showed differences in leptin levels based on ethnicity,[10] we also divided the subjects based on sex and ethnicity (Table S2). Again, both the black and white men showed a negative association between CIMT (black men, Std $\beta = -0.29$; $P = 0.034$; $R^2 = 0.08$; white men, Std $\beta = -0.24$; $P = 0.042$; $R^2 = 0.07$) and CSWA (black men, Std $\beta = -0.22$; $P = 0.038$; $R^2 = 0.10$; white men, Std $\beta = -0.25$; $P = 0.022$; $R^2 = 0.08$) with leptin. There was no association between CIMT and CSWA with leptin in either the black or white women.

**TABLE 2** Forward stepwise multiple regression analysis between von Willebrand factor, pulse wave velocity, carotid intima-media thickness and cross-sectional wall area with leptin in men and women

| | Men (N = 337) | | | | Women (N = 483) | | | |
|---|---|---|---|---|---|---|---|---|
| **Variable** | **Adj $R^2$** | **Adj *P*** | **$\beta$ (95% CI)** | ***P*** | **Adj $R^2$** | **Adj *P*** | **$\beta$ (95% CI)** | ***P*** |
| vWF (%) | **0.01*** | **0.04** | - | | 0.01 | 0.10 | - | - |
| PWV (m/s) | **0.12*** | **<0.001** | - | | **0.10*** | **<0.001** | - | - |
| CIMT (mm) | **0.07*** | **<0.001** | **−0.27 [−0.41; −0.12]** | **<0.001** | **0.02*** | **0.016** | - | - |
| CSWA ($mm^2$) | **0.06*** | **0.003** | **−0.24 [−0.40; −0.09]** | **0.002** | **0.02*** | **0.016** | - | - |

Standardized $\beta$ (Std $\beta$) represents the change in the dependent variable for every 1 SD change in the independent variable. $\beta$, partial regression coefficients; 95% CI, 95% confidence interval; Adjusted $R^2$, coefficient of determination of each total regression model; Models for the regression were all included at once and included: leptin, age, ethnicity, socioeconomic score, body fat percentage, 24-h mean arterial blood pressure, LDL-cholesterol, C-reactive protein, glucose, gamma-glutamyl-transferase and moderate-vigorous physical activity. Bold values indicate $P \leq 0.05$.

Adj $R^2$, adjusted $R^2$ and Adj $P$ represents, $P$ values for each regression model; CIMT, carotid intima-media thickness; CSWA, cross-sectional wall area; PWV, pulse wave velocity; vWF, von Willebrand factor.

*$R^2$ values at $P \leq 0.05$.

**TABLE 3** Forward stepwise multiple regression analysis of von Willebrand factor, pulse wave velocity, carotid intima-media thickness and cross-sectional wall area with leptin after stratifying into leptin tertiles in men and women

| | Men Tertile 1 (N = 114) | | | | Women Tertile 1 (N = 161) | | | |
|---|---|---|---|---|---|---|---|---|
| | **Adj $R^2$** | **Adj *P*** | **$\beta$ (95% CI)** | ***P*** | **Adj $R^2$** | **Adj *P*** | **$\beta$ (95% CI)** | ***P*** |
| vWF (%) | 0.04 | 0.09 | - | - | **0.07*** | **0.03** | - | - |
| PWV (m/s) | **0.19*** | **0.002** | **−0.21 [−0.42; −0.01]** | **0.047** | **0.13*** | **<0.001** | - | - |
| CIMT (mm) | 0.03 | 0.12 | - | - | **0.06*** | **0.05** | - | - |
| CSWA ($mm^2$) | **0.07*** | **0.037** | - | - | **0.10*** | **0.01** | - | - |
| | **Tertile 2 (N = 111)** | | | | **Tertile 2 (N = 161)** | | | |
| vWF (%) | 0.01 | 0.26 | - | - | **0.12*** | **0.002** | **−0.21 [−0.38; −0.04]** | **0.018** |
| PWV (m/s) | **0.07*** | **0.04** | - | - | **0.07*** | **0.02** | - | - |
| CIMT (mm) | 0.03 | 0.15 | - | - | 0.01 | 0.17 | - | - |
| CSWA ($mm^2$) | 0.04 | 0.14 | - | - | 0.01 | 0.18 | 0.09 [−0.01; 0.28] | 0.29 |
| | **Tertile 3 (N = 112)** | | | | **Tertile 3 (N = 161)** | | | |
| vWF (%) | 0.02 | 0.19 | - | - | 0.01 | 0.13 | - | - |
| PWV (m/s) | **0.10*** | **0.02** | - | - | **0.08*** | **0.04** | - | - |
| CIMT (mm) | **0.08*** | **0.040** | −0.15 [−0.39; 0.007] | 0.18 | 0.04 | 0.06 | −0.20 [−0.39; −0.02] | 0.034 |
| CSWA ($mm^2$) | **0.13*** | **0.011** | −0.15 [−0.36; 0.07] | 0.19 | 0.05 | 0.07 | −0.17 [−0.36; 0.02] | 0.077 |

Standardized $\beta$ (Std $\beta$) represents the change in the dependent variable for every 1 SD change in the independent variable. $\beta$, partial regression coefficients; 95% CI, 95% confidence interval; Adjusted $R^2$, coefficient of determination of each total regression model; Models for the regression were all included at once and included: leptin, age, ethnicity, socioeconomic score, body fat percentage, 24-h mean arterial blood pressure, LDL-cholesterol, C-reactive protein, glucose, gamma-glutamyl-transferase and moderate-vigorous physical activity. Bold values indicate $P \leq 0.05$.

Adj $R^2$, adjusted $R^2$ and Adj $P$ represents, $P$ values for each regression model; CIMT, carotid intima-media thickness; CSWA, cross-sectional wall area; PWV, pulse wave velocity; vWF, von Willebrand factor.

*$R^2$ values at $P \leq 0.05$.

## 4 | DISCUSSION

Our key finding is that in young healthy men, leptin independently and negatively associated with measures of subclinical atherosclerosis, but not in women. This result was robust when performed separately in black or white men. Within BMI categories, we found this association to be evident specifically in overweight men. When reviewing leptin's relationship with other measures of arterial structure and function, leptin consistently showed beneficial inverse associations. Across leptin tertiles, we observed negative associations between arterial stiffness and leptin within the first leptin tertile for men and between vWF (as a measure of endothelial function) and leptin within the second tertile

**TABLE 4** Forward stepwise multiple regression analysis of von Willebrand factor, pulse wave velocity, carotid intima-media thickness and cross-sectional wall area with leptin in lean and overweight men and women

| | **Men Lean (N = 167)** | | | | **Women Lean (N = 225)** | | | |
|---|---|---|---|---|---|---|---|---|
| | **Adj $R^2$** | **Adj *P*** | **$\beta$ (95% CI)** | ***P*** | **Adj $R^2$** | **Adj *P*** | **$\beta$ (95% CI)** | ***P*** |
| vWF (%) | **0.03*** | **0.03** | - | - | **0.06*** | **0.01** | [−0.05; 0.27] | 0.17 |
| PWV (m/s) | **0.16*** | **<0.001** | - | - | **0.17*** | **<0.001** | - | - |
| CIMT (mm) | 0.01 | 0.16 | −0.18 [−0.36; 0.01] | 0.067 | 0.01 | 0.15 | - | - |
| CSWA ($mm^2$) | 0.03 | 0.13 | −0.13 [−0.32; 0.07] | 0.20 | 0.01 | 0.19 | - | - |
| | **Overweight (N = 96)** | | | | **Overweight (N = 124)** | | | |
| vWF (%) | 0.02 | 0.22 | - | - | 0.04 | 0.06 | - | - |
| PWV (m/s) | **0.15*** | **0.01** | - | - | **0.12*** | **<0.001** | - | - |
| CIMT (mm) | **0.16*** | **0.003** | **−0.45 [−0.67; −0.22]** | **<0.001** | 0.04 | 0.09 | −0.15 [−0.36; 0.06] | 0.17 |
| CSWA ($mm^2$) | **0.23*** | **<0.001** | **−0.43 [−0.66; −0.20]** | **<0.001** | 0.02 | 0.18 | - | - |
| | | | | | **Obese (N = 105)** | | | |
| vWF (%) | | | | | 0.01 | 0.24 | - | - |
| PWV (m/s) | | | | | **0.23*** | **0.003** | - | - |
| CIMT (mm) | | | | | 0.02 | 0.28 | - | - |
| CSWA ($mm^2$) | | | | | 0.01 | 0.29 | - | - |

Standardized $\beta$ (Std $\beta$) represents the change in the dependent variable for every 1 SD change in the independent variable. $\beta$, partial regression coefficients; 95% CI, 95% confidence interval; Adjusted $R^2$, coefficient of determination of each total regression model; Models for the regression were all included at once and included: leptin, age, ethnicity, socioeconomic score, body fat percentage, 24-h mean arterial blood pressure, LDL-cholesterol, C-reactive protein, glucose, gamma-glutamyl-transferase and moderate-vigorous physical activity.

Adj $R^2$, adjusted $R^2$ and Adj *P* represents, *P* values for each regression model; CIMT, carotid intima-media thickness; CSWA, cross-sectional wall area; PWV, pulse wave velocity; vWF, von Willebrand factor.

Bold values indicate $P \leq 0.05$.

We did not conduct regression analysis for underweight (N = 24) and obese men (N = 49) as well as in underweight women (N = 29) due to their small sample sizes.

*$R^2$ values at $P \leq 0.05$.

of women. Collectively, these findings support the notion of potential beneficial vascular protective effects of leptin, especially in men.

Physiologically, leptin is suggested to be an important factor in the maintenance of vascular homoeostasis and wall integrity.[9] However, many studies have shown leptin's detrimental effects on cardiovascular function, including atherosclerosis.[24,25] This was reported especially in older overweight and obese individuals with conditions such as the metabolic syndrome, hypertension and type 2 diabetes.[9,10,26,27] We observed a negative link between CIMT and CSWA with leptin in men. In contrast to the previously mentioned studies, the men in our study were young and healthy. Our finding may therefore suggest that the beneficial effects of leptin signalling previously reported in the vasculature[28] may be intact. Leptin is known to induce nitric oxide synthesis[29] and stimulate coronary artery vasodilation in humans.[30] Nitric oxide may be the potential mechanism underlying the observed negative associations between leptin and carotid wall thickness in our young healthy men, due to its important role in reducing platelet adhesion and vascular smooth muscle cell proliferation.[31] In fact, Rodríguez et al[32] demonstrated leptin's ability to inhibit vasoconstriction (angiotensin II) and in turn reduce smooth muscle proliferation in an experimental animal model.

Additionally, Momin et al[33] pointed out leptin's beneficial and direct effects on vascular smooth muscle tone regulation through hyperpolarization, independently of vascular endothelium-derived nitric oxide synthesis in humans with coronary artery disease. Other studies have also showed leptin's potential to recruit beneficial vascular endothelial progenitor cells into the vasculature[8,13,16]—known to maintain vascular homoeostasis and reduce plaque formation.[34] More so, not only is leptin suggested to be a beneficial factor in the regulation of myocardial metabolism as well as cardiac function,[28] the injection of leptin in ob/ob mice has showed a significant reduction in the wall thickness and size of cardiac myocytes.[28,35] This anti-hypertrophic potential of leptin on cardiac myocyte cells also suggests leptin as an important factor that may play a role in the reduction of neointima growth.[36]

Our finding in the overweight healthy young men is aligned with a previous study[17] showing that leptin is independently associated with flow mediated dilation in overweight patients with diabetes using insulin and not in the lean group. Also, Simiti et al[37] showed that elevated leptin concentrations were associated with a better prognosis in overweight patients with coronary heart disease. Furthermore, leptin has been shown to reverse the positive association that existed between BMI and mortality in older overweight men with coronary heart disease and heart failure.[38] Importantly, our finding of a beneficial association between leptin and carotid wall thickness was absent in lean men as well as in lean and obese women, but was significant in overweight men. Circulating leptin concentrations in this group (8.95 ng/mL) is similar to the normal physiological level at which leptin is noted to modulate normal physiological responses, namely 8.70 ng/mL[9] and 10 ng/mL.[16] Yet, this finding was absent in lean men (2.17 ng/mL) and was thought to be related to the U-shaped relationship between leptin and cardiovascular risk, where leptin associated with increased risk both at low and high doses.[17,39] A study by Wallace et al[40] proposed a risk threshold for future coronary events in otherwise hypercholesterolaemic men at risk of coronary artery disease, only from the 4th and 5th quintiles of leptin. In order to clarify the dose dependency of leptin in the young healthy adults, we investigated these associations within tertiles of leptin. Within our young healthy population, a U-shaped relationship between leptin and cardiovascular risk was not evident, as shown in previous studies[39,40] such as one conducted in 392 elderly patients (62 years old) with atherosclerosis.[39] This may become evident as our study participants age[27] or develop an associated metabolic disorder such as dyslipidaemia or atherosclerosis.[39,40] We rather observed leptin's negative association with large artery stiffness in the tertile with the lowest leptin concentrations, which was lost with increasing leptin tertiles. The observed negative associations between carotid wall thickness with leptin within the third tertile of leptin were not statistically significant, but reflected a similar trend as observed in the overweight men (BMI category; $P < 0.001$).

Importantly, our finding was more prominent in men than in women, who had higher body fat percentages and almost sevenfold higher leptin levels than men. Leptin may have different functionalities in men and women. Previous studies showed gender differences in the association between measures of autonomic function with leptin to be higher in men than women.[23,41] Another study indicated that women show more resistance to the physiological effects of leptin than men.[42] More studies are recommended to explain the sex-specific effects of leptin. Although a study has shown that in either obese or lean healthy women without any metabolic complications, leptin did not predict either endothelial function or CIMT.[43] The negative association between leptin and vWF—an established haemostatic risk factor for cardiovascular disease[21]—in young normotensive women (second leptin tertile), suggests a beneficial effect of leptin on endothelial function. This is in contrast to Guagnano et al[19] that showed a positive association between vWF and leptin in obese women compared to controls. Whether these contrasting findings could be explained by age differences or their states of adiposity requires more detailed investigation.

Our findings must be interpreted within the context of its limitations and strengths. As there is a lack of information on leptin's role in young healthy populations, our findings shed light on a potential beneficial role of leptin. Secondly, the study is limited to a small sample size for the underweight men and women as well as in obese men prohibiting detailed analyses in these groups. Furthermore, the study is limited to a cross-sectional design and we can only speculate on the possible mechanisms underlying the potential beneficial vascular effect of leptin in young healthy adults. Despite this, the study highlights novel findings and was furthermore well designed and performed under highly controlled conditions in a Hypertension Research Clinic.

## 5 | CONCLUSION

In conclusion, in young healthy men, measures of subclinical atherosclerosis were independently and negatively associated with circulating leptin, supporting the notion of vascular protective effects of leptin. The presence of these beneficial associations in specifically the healthy young overweight men shows that leptin is not associated with adverse vascular function in this group.

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### CONFLICT OF INTEREST

The authors declared no conflict of interest.

### AUTHOR'S CONTRIBUTIONS

AOB performed the literature search, data cleaning, statistical analyses, interpretation of data and writing of the

draft manuscript. AES is the principal investigator of the African-PREDICT study, and AES, WS and LL were responsible for the research planning and design, acquisition of data, interpretation of data and revising article critically for intellectual content. All authors approved the final version.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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