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ORIGINAL ARTICLE

# Serum calcium revisited: associations with 24-h ambulatory blood pressure and cardiovascular reactivity in Africans

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**Sub-Saharan Africans face an increasing burden of hypertension. Although controversial, recent experimental evidence strongly suggests that serum calcium contributes to elevated blood pressure through increased vascular resistance. We investigated the associations of 24-h blood pressure and cardiovascular reactivity with serum calcium in African men stratified by age. The study consisted of 50 younger (median age: 38 years) and 49 older (median age: 49 years) participants. We measured 24-h ambulatory blood pressure with a mean successful inflation rate of 72.6%. Total peripheral resistance and stroke volume reactivity were obtained using a Finometer device during application of the Stroop color and word conflict test. Total serum calcium was adjusted for serum albumin. Results showed that serum calcium levels were similar between the younger and older groups. However, in the younger group, 24-h systolic blood pressure, 24-h diastolic blood pressure and total peripheral resistance reactivity correlated positively, whereas stroke volume reactivity correlated negatively with serum calcium in single and multiple regression analyses (systolic blood pressure: $B$=34.99, $P$=0.017; diastolic blood pressure: $B$=34.93, $P<0.001$; total peripheral resistance reactivity: $B$=65.44, $P$=0.048; stroke volume reactivity: $B$=−45.40, $P$=0.017). No associations were evident in the older African men. In conclusion, 24-h ambulatory systolic and diastolic blood pressures are positively associated with serum calcium in African men younger than 43 years. The blood pressure–serum calcium relationship seems to be mediated through increased vascular resistance during stress.**
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**Keywords:** Africans; blood pressure; cardiovascular reactivity; serum calcium

## INTRODUCTION

The positive association between serum calcium in the normal physiological range and blood pressure is a much disputed topic.[1–4] The first report of this association appeared in 1976[1] and was subsequently confirmed,[2,5] but also contradicted[3,4] by others. To add to the confusion, this association seems to be present mostly in men.[2,6] In addition, serum calcium tends to increase with age only in women,[5] while blood pressure increases with age in both genders,[7] although the increase is less pronounced in women.[8] Kesteloot and Geboers[2] also noticed that the blood pressure–serum calcium relationship was stronger in younger compared with older men. More recently, elevated serum calcium was found to be a predictor,[9] as well as an independent prospective risk factor,[10] for myocardial infarction in middle-aged men. All of the above studies involved Caucasian populations. The Third National Health and Nutrition Examination Survey reported the first results on African-Americans.[11] However, this study could not confirm a blood pressure–serum calcium relationship in either men or women.[11] Similar results were found in African men from South Africa almost two decades ago,[12–14] an ethnic group that today has the highest prevalence of hypertension in South Africa.[15,16]

Cardiovascular reactivity studies show that African men have greater α-adrenergic vasoconstriction compared with African women and Caucasian men and women.[17–19] Serum calcium was not included in these studies. Animal studies do indeed suggest that serum calcium increases blood pressure because of an abnormally high calcium permeability of vascular smooth muscle cells when α-adrenergic receptors are activated, thereby increasing vasoconstriction.[20] Considering the above human and animal studies, one could hypothesize that in Africans, when placed under stress, an increased sensitivity of vascular smooth muscle cells to serum calcium could possibly contribute to increased blood pressure. It therefore seems relevant to reinvestigate the blood pressure–serum calcium relationship using 24-h ambulatory and cardiovascular reactivity measurements. In addition, it would be clinically informative to investigate whether

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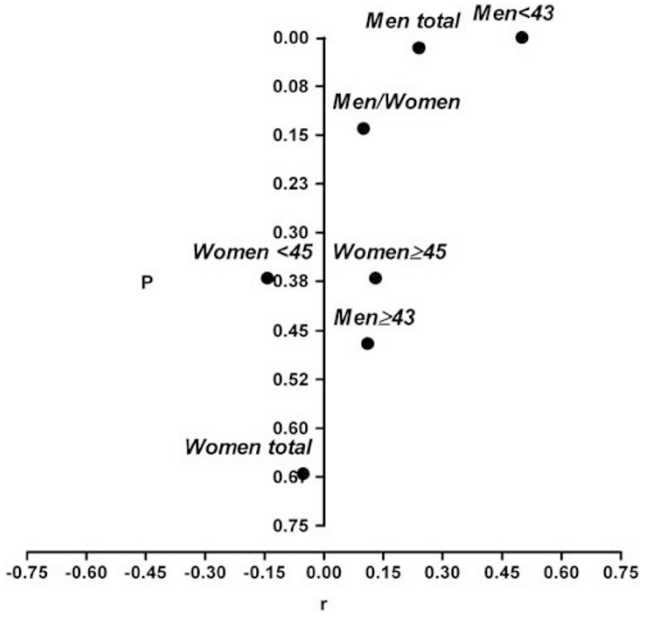


**Figure 1** *P*-values as a function of single regression coefficients (*r*) of the association between 24-h diastolic blood pressure and serum calcium in African men and women.

associations are more prominent in younger subjects, as noticed by Kesteloot and Geboers.[2] The aim of this study was to investigate the associations of 24-h ambulatory blood pressure and cardiovascular reactivity with serum calcium in African men stratified by age.

## METHODS

### Study population

This study is nested in the Sympathetic Activity and Ambulatory Blood Pressure in Africans study conducted between February and May 2008. We recruited 200 urban African male ($n$=101) and female ($n$=99) educators working in the Dr Kenneth Kaunda district in the North-West Province, South Africa. The reason for this selection was to obtain a homogenous sample from a similar socio-economic class. We invited all eligible participants between the ages of 25 and 65 years to participate. Exclusion criteria were an oral temperature above normal, psychotropic substance dependence or abuse, regular blood donors and individuals vaccinated in the past 3 months. Among the 200 participants, we excluded all women due to the absence of associations between blood pressure and serum calcium as reported previously.[2,6] To confirm this, the single regression coefficients and *P*-values of the 24-h diastolic and serum calcium relationship in men and women are presented in Figure 1. In addition, to serve as motivation for the median split–age stratification, the regression coefficients and *P*-values are also presented. Among the 101 men, we excluded two subjects from all analyses due to missing necessary basic variables.

Participants were fully informed about the objectives and procedures of the study before their recruitment. Assistance was available for any participant who requested conveyance of information in their home language. All participants signed an informed consent form. The study complied with all applicable international regulations, in particular the Helsinki declaration of 1975 (as revised in 1983) for investigation of human participants. The Ethics Review Board of North-West University (Potchefstroom Campus) approved the study.

### Cardiovascular measurements

We conducted 24-h ambulatory blood pressure measurements during working days. At approximately 0800 hours, a 24-h ambulatory blood pressure measurement apparatus (Meditech CE120 CardioTens; Meditech, Budapest, Hungary) was attached to the participants' non-dominant arm at their workplace. The 24-h ambulatory blood pressure measurement apparatus was programmed to measure blood pressure at 30-min intervals during the day (0800–2200 hours) and every hour during nighttime hours (2200–0600 hours). Participants were asked to continue with normal daily activities and record any abnormalities, such as headache, nausea and stress, on their ambulatory diary cards.

Participants were admitted at 1630 hours to the Metabolic Unit Research Facility of North-West University. This facility consists of 10 bedrooms, 2 bathrooms, a living room and a kitchen. Participants received a standardized dinner (containing 166.7 mg calcium, 1396.4 mg potassium and 1536.6 mg sodium) and had their last beverages (tea or coffee) and two biscuits (containing 6.0 mg sodium, 49.1 mg potassium and 84.5 mg sodium) at 2030 hours. Thereafter, they relaxed by reading, watching television or social interaction and refrained from consuming alcohol, caffeine, smoking and doing exercise. They were requested to go to bed at around 2200 hours. At 0600 hours, the 24-h ambulatory blood pressure measurement apparatus was removed and subsequent measurements commenced. We downloaded the 24-h blood pressure and electrocardiogram data into a database using the CardioVisions 1.7.2 Personal Edition (Meditech, Budapest, Hungary). The successful inflation rate over the 24-h period was 72.6%. Participants were regarded as hypertensive if their mean 24-h systolic and diastolic blood pressures exceeded 130/80 mm Hg.[21]

The validated[22,23] Finometer device (FMS, Finapres Measurement Systems, Amsterdam, The Netherlands) was connected, and after a 10-min resting period, a 5-min continuous measurement of resting cardiovascular parameters was carried out. During the recording, after 2-min, a return-to-flow systolic calibration was performed to provide an individual subject-level adjustment of the finger arterial pressure with the brachial artery pressure.[23] The highest precision in cardiovascular measurements is obtainable only after this calibration,[23] and blood pressure levels met the requirements of the Association for the Advancement of Medical Instrumentation.[24] Thereafter, the participant was exposed to the Stroop color and word conflict test for 1-min. Reportedly, the Stroop color and word conflict test shows 2-h[25] and 1-month[26] reproducibility on cardiovascular reactivity. The Finometer was used to compute the stroke volume and total peripheral resistance online and store the data in result files.[27] We obtained the average of the last 2-min of the resting recordings and the average of the last 15 s of the stressor recordings. The cardiovascular reactivity was calculated for each participant as the percentage change from the resting value.

### Anthropometric measurements

The height (stature) and weight of participants wearing their underwear were measured using calibrated instruments (Precision Health Scale, A & D Company, Tokyo, Japan; Invicta Stadiometer, IP 1465, Invicta, London, UK). Measurements were taken in triplicate using standard methods.[28]

### Biochemical measurements

After completion of the anthropometric measurements, a registered nurse obtained a blood sample with a sterile winged infusion set from the antebrachial vein branches. Serum was stored at −80°C.

Fasting serum samples for total calcium, sodium, potassium, albumin, total cholesterol and high-sensitivity C-reactive protein were analyzed using the sequential multiple analyzer computer (Konelab 20i, Thermo Scientific, Vantaa, Finland). Serum glucose was determined using the Olympus AU 2700 analyzer (Olympus UK, London, UK). The method of analysis incorporated an automated hexokinase method with ultraviolet detection at 340 nm. Assay linearity was carried out across a concentration range of 11–811 mg per 100 ml with a coefficient of variation of 3%.

All biochemical measurements were performed by an independent laboratory that was blinded to the subjects' cardiovascular profile.

### Statistical analyses

For database management and statistical analyses, we used SAS software version 9.1 (SAS Institute, Cary, NC, USA). The group was stratified by age, by means of median split (43 years). The distributions of serum albumin, glucose, sodium, potassium, high-sensitivity C-reactive protein and physical activity were normalized by logarithmic transformation. The central tendency and spread of these variables were represented by the geometric mean and the 5th

and 95th percentile intervals. We compared the means and proportions by a standard *t*-test and the $\chi^2$-test, respectively. Albumin-corrected serum calcium was calculated as follows: total serum calcium ($mmol\,l^{-1}$)+0.02(41.5−serum albumin ($g\,l^{-1}$)) where 41.5 represents the average albumin level of the total group in gram per liter. Hereafter, the albumin-corrected serum calcium is referred to as serum calcium. Mean values of serum calcium were plotted by tertiles of the cardiovascular variables to ensure that linear correlation techniques were appropriate. We investigated associations between the cardiovascular variables and serum calcium using single and multiple linear regressions and identified covariates using a forward stepwise procedure in which the *P*-values required to enter and to stay in the model were set at 0.15. Covariates considered for entry into the model were age, body mass index, serum sodium, serum potassium, total cholesterol, serum glucose, high-sensitivity C-reactive protein, 24-h pulse rate and smoking status. Of these variables, only age, total cholesterol and smoking status failed to enter any of the models as significant covariates. However, because of their physiological significance, these variables were forced into the models.

We also repeated the multiple regression analyses after excluding HIV-positive subjects and subjects using anti-hypertensive medication. All *P*-values refer to two-sided hypotheses.

## RESULTS

### Characteristics of participants

Table 1 lists the characteristics of the African men stratified by age. The younger men ($n$=50) had lower serum cholesterol ($P$=0.051), glucose ($P$=0.003) and C-reactive protein ($P$=0.002) levels compared with the older men ($n$=49). The younger men also smoked less ($P$=0.012) and had lower 24-h systolic ($P$=0.005) and diastolic ($P$=0.005) blood pressures. One (2.0%) younger subject and six (12.2%) older subjects used calcium channel blockers ($P$=0.047). No difference existed for serum calcium between the younger and older groups. However, when the younger and older groups were stratified according to hypertensive status, serum calcium tended to be higher in the younger hypertensives ($n$=29; aged 37.2 ± 3.9 years) compared with the younger normotensives ($n$=21; aged 36.0 ± 5.1 years), that is, 2.46 ± 0.15 *vs.* 2.39 ± 0.11 $mmol\,l^{-1}$; $P$=0.057. There was no difference between the older hypertensives ($n$=39; aged 49.3 ± 5.6 years) and normotensives ($n$=10; aged 50.7 ± 4.2 years), that is, 2.43 ± 0.15 *vs.* 2.40 ± 0.10 $mmol\,l^{-1}$; $P$=0.46.

### Unadjusted analysis

In single regression analyses (Figure 2), 24-h systolic blood pressure ($r$=0.34, $P$=0.016), 24-h diastolic blood pressure ($r$=0.50, $P<0.001$) and total peripheral resistance reactivity ($r$=0.37, $P$=0.011; Figure 3) correlated positively with serum calcium in the younger men, whereas no associations were evident in the older men (Figures 2 and 3). In addition, stroke volume reactivity correlated negatively with serum calcium ($r$=−0.30, $P$=0.041) in the younger men, whereas this association was also absent in the older men (Figure 3).

**Table 1 Characteristics of study population**

| | *African men <43 years* (n=*50*) | *African men ⩾43 years* (n=*49*) | P |
|---|---|---|---|
| Age, years | 36.7 ± 4.4 | 49.6 ± 5.3 | <0.001 |
| Body mass index, $kg\,m^{-2}$ | 26.9 ± 5.8 | 28.1 ± 5.8 | 0.33 |
| *Biochemical measurements* | | | |
| Albumin-corrected serum calcium, $mmol\,l^{-1}$ | 2.43 ± 0.13 | 2.42 ± 0.14 | 0.59 |
| Total serum calcium, $mmol\,l^{-1}$ | 2.45 ± 0.20 | 2.40 ± 0.18 | 0.24 |
| Serum albumin, $g\,l^{-1}$ | 43.3 (35.9–57.5) | 41.9 (36.3–55.4) | 0.23 |
| Total cholesterol, $mmol\,l^{-1}$ | 4.50 ± 1.02 | 4.98 ± 1.26 | 0.051 |
| Serum glucose, $mmol\,l^{-1}$ | 5.30 (4.32–6.87) | 6.21 (4.56–12.93) | 0.003 |
| High-sensitivity C-reactive protein, $mg\,l^{-1}$ | 1.92 (0.17–10.80) | 3.97 (0.95–16.1) | 0.002 |
| Serum $Na^+$, $mmol\,l^{-1}$ | 146 (120–195) | 142 (121–185) | 0.37 |
| Serum $K^+$, $mmol\,l^{-1}$ | 4.56 (3.51–6.03) | 4.35 (3.53–5.68) | 0.21 |
| *Cardiovascular measurements* | | | |
| 24-h Systolic blood pressure, mm Hg | 133 ± 14 | 142 ± 17 | 0.005 |
| 24-h Diastolic blood pressure, mm Hg | 85 ± 9 | 91 ± 11 | 0.005 |
| 24-h Pulse rate, b.p.m | 78 ± 12 | 80 ± 11 | 0.28 |
| Stroke volume (resting), ml | 98.3 ± 18.0 | 102.1 ± 31.6 | 0.48 |
| Stroke volume reactivity, % | −7.64 ± 12.99 | −9.38 ± 14.20 | 0.54 |
| Total peripheral resistance (resting), $mm\,Hg\,ml^{-1}\,s^{-1}$ | 1.06 ± 0.29 | 1.10 ± 0.35 | 0.49 |
| Total peripheral resistance reactivity, % | 0.67 ± 21.67 | 6.86 ± 22.95 | 0.18 |
| Non-dippers, *n* (%) | 18 (36.0) | 23 (46.9) | 0.31 |
| *Lifestyle* | | | |
| Physical activity, $kcal\,day^{-1}$ | 2667 (1614–4355) | 2524 (1767–3845) | 0.34 |
| *Smoking* | | | 0.012 |
| Never, *n* (%) | 31 (62.0) | 16 (32.7) | |
| Former, *n* (%) | 7 (14.0) | 15 (30.6) | |
| Ever, *n* (%) | 12 (24.0) | 18 (36.7) | |
| Current drinking, *n* (%) | 19 (38.0) | 21 (42.9) | 0.62 |
| HIV-positive, *n* (%) | 8 (16.0) | 5 (10.2) | 0.39 |

Abbreviation: b.p.m, beats per minute.
Values are arithmetic mean ± s.d., geometric mean (5–95th percentile interval), or number of subjects (%).

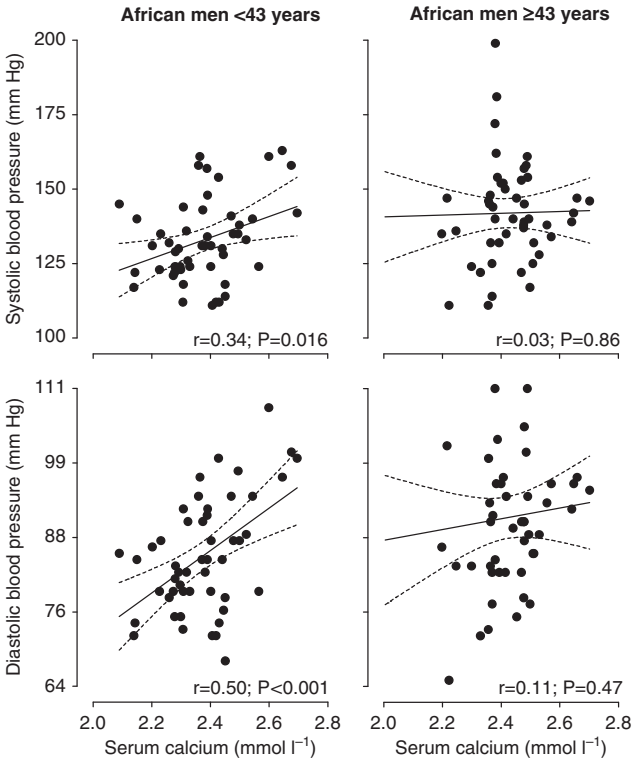


**Figure 2** 24-h blood pressure as a function of serum calcium in 50 African men (<43 years) and 49 African men (⩾43 years) in single regression analysis.

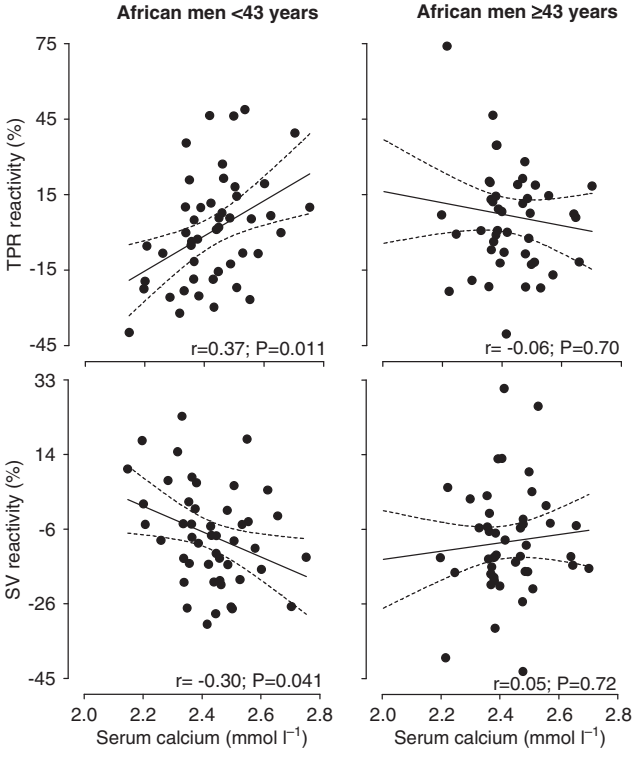


**Figure 3** Total peripheral resistance and stroke volume reactivity as a function of serum calcium in 50 African men (<43 years) and 49 African men (⩾43 years) in single regression analysis.

In exploratory analyses (Table 2), we investigated the characteristics of the younger men across tertiles of serum calcium. There were no associations of age or body mass index with increasing serum calcium. In addition, there were also no significant associations with serum cholesterol, glucose or high-sensitivity C-reactive protein. However, 24-h systolic blood pressure tended to increase ($P$=0.076 for the difference between the lowest and highest tertile), whereas 24-h diastolic blood pressure increased significantly with increasing serum calcium ($P$=0.015 for the trend). In addition, stroke volume tended to decrease ($P$=0.036 for the difference between the lowest and highest tertile), although total peripheral resistance tended to increase with increasing serum calcium during application of the stressor ($P$=0.050 for the difference between the lowest and highest tertile).

### Adjusted analysis

The independent associations between the cardiovascular variables and serum calcium in the younger and older men are shown in Table 3. With total adjustment, the above associations were confirmed. In the younger men, 24-h systolic blood pressure, 24-h diastolic blood pressure and total peripheral resistance reactivity correlated positively, whereas the negative association between stroke volume reactivity and serum calcium remained significant. Again, the associations between 24-h systolic blood pressure, 24-h diastolic blood pressure, total peripheral resistance reactivity and stroke volume reactivity were absent in the older men.

### Sensitivity analysis

We excluded 2 men from the younger group and 13 men from the older group for using anti-hypertensive medication. In addition, because it is known that a HIV-positive status is associated with cardiovascular effects,[29] eight younger and five older HIV-positive men were also excluded. By doing so, our associations not only remained consistent but were also strengthened (Table 4).

## DISCUSSION

This study sought evidence for an age-specific association between 24-h ambulatory blood pressure and serum calcium in African men. The main finding of the study was that, although the mean serum calcium levels were similar between the groups, both systolic and diastolic blood pressures were significantly associated with serum calcium only in the younger men. In addition, in the younger men, total peripheral resistance reactivity was positively associated and stroke volume reactivity was negatively associated with serum calcium, suggesting that blood pressure increases with increasing calcium in the younger men because of a possible increased sensitivity to serum calcium and a resulting increased vascular resistance.

These findings confirm previous reports,[1,5,30] but in particular, as noted by Kesteloot and Geboers,[2] they also confirm the more prominent association between blood pressure and serum calcium in younger men. Not surprisingly, associations from large population-based studies have generally been quite weak ($r$<0.20),[1,4,6,31] which is possibly the result of failing to stratify by age and gender. Indeed, our preliminary results, as indicated in Figure 1, also showed this masking effect of age and gender. More recently, a prospective study by Lind *et al.*[10] found that serum calcium was an independent risk factor for myocardial infarction in a cohort of Swedish middle-aged men. In another Swedish study, Jorde *et al.*[9] also found a similar association in a group of 12 865 men but not in women.

All the above evidence is based on Caucasian studies. Studies investigating the blood pressure–serum calcium relationship in Africans are not only limited but also fail to confirm this relationship.

**Table 2 Characteristics of young African men (<43 years) across tertiles of serum calcium**

| | *Tertiles of the distribution of serum calcium* | | | P-*values* | |
|---|---|---|---|---|---|
| *Characteristics* | *Low* | *Medium* | *High* | *For trend* | *For low vs. high* |
| Limits of tertiles, mmol $l^{-1}$ | <2.37 | 2.37–2.49 | ⩾2.49 | | |
| Number | 18 | 16 | 16 | | |
| Age, years | 35.4 ± 5.7 | 37.6 ± 4.5 | 37.1 ± 2.5 | 0.34 | 0.28 |
| BMI, kg $m^{-2}$ | 26.4 ± 5.2 | 26.8 ± 6.4 | 27.6 ± 6.0 | 0.86 | 0.59 |
| 24-h Systolic blood pressure, mm Hg | 126.9 ± 8.5 | 135.7 ± 15.6 | 135.8 ± 15.4 | 0.11 | 0.072 |
| 24-h Diastolic blood pressure, mm Hg | 79.6 ± 4.8 | 85.9 ± 8.0 | 88.3 ± 11.3 | 0.015 | 0.005 |
| 24-h Pulse rate, b.p.m | 79.8 ± 13.6 | 76.8 ± 9.3 | 77.3 ± 12.9 | 0.74 | 0.56 |
| Stroke volume (resting), ml | 98.5 ± 19.4 | 94.1 ± 19.4 | 103.1 ± 14.4 | 0.37 | 0.48 |
| Stroke volume reactivity, % | −1.9 ± 13.4 | −8.5 ± 11.2 | −12.0 ± 13.4 | 0.104 | 0.036 |
| Total peripheral resistance (resting), mm Hg $ml^{-1}$ $s^{-1}$ | 0.99 ± 0.33 | 1.14 ± 0.30 | 1.02 ± 0.19 | 0.26 | 0.76 |
| Total peripheral resistance reactivity, % | −9.3 ± 18.7 | 3.5 ± 21.8 | 6.52 ± 22.3 | 0.11 | 0.050 |
| Total cholesterol, mmol $l^{-1}$ | 4.59 ± 1.04 | 4.25 ± 1.00 | 4.68 ± 1.02 | 0.44 | 0.81 |
| Serum glucose, mmol $l^{-1}$ | 5.29 (3.53–10.43) | 5.05 (3.53–5.29) | 5.61 (4.31–9.96) | 0.26 | 0.37 |
| High-sensitivity C-reactive protein, mg $l^{-1}$ | 2.00 (0.15–54.46) | 2.00 (0.16–10.80) | 1.76 (0.21–6.72) | 0.95 | 0.78 |
| Serum $Na^+$, mmol $l^{-1}$ | 140.1 (117.6–223.2) | 144.8 (117.8–219.9) | 154.8 (125.2–194.8) | 0.26 | 0.11 |
| Serum $K^+$, mmol $l^{-1}$ | 4.29 (3.51–5.76) | 4.42 (3.10–6.02) | 5.02 (3.58–6.59) | 0.056 | 0.025 |

Abbreviations: BMI, body mass index; b.p.m, beats per minute.
Values are arithmetic mean ± s.d. or geometric mean (5th–95th percentile interval).

**Table 3 Independent associations between blood pressure and serum calcium**

| | *24-h SBP, mm Hg* | | *24-h DBP, mm Hg* | | *SV-reactivity, %* | | *TPR-reactivity, %* | |
|---|---|---|---|---|---|---|---|---|
| | B *(± s.e.)* | P | B *(± s.e.)* | P | B *(± s.e.)* | P | B *(± s.e.)* | P |
| *African men (<43 years; N=50)* | | | | | | | | |
| $R^2$ | 0.475 | | 0.534 | | 0.299 | | 0.226 | |
| Serum calcium, mmol $l^{-1}$ | 34.99 ± 14.07 | 0.017 | 34.93 ± 8.55 | <0.001 | −45.40 ± 18.21 | 0.017 | 65.44 ± 31.91 | 0.048 |
| Age, years | −0.094 ± 0.41 | 0.82 | 0.061 ± 0.25 | 0.81 | −0.068 ± 0.45 | 0.88 | 0.35 ± 0.80 | 0.67 |
| Body mass index, kg $m^{-2}$ | 0.87 ± 0.38 | 0.027 | 0.33 ± 0.23 | 0.16 | 0.62 ± 0.45 | 0.17 | −0.32 ± 0.78 | 0.68 |
| Total cholesterol, mmol $l^{-1}$ | −1.16 ± 1.77 | 0.52 | −1.22 ± 1.07 | 0.26 | 2.46 ± 2.04 | 0.24 | −3.89 ± 3.57 | 0.28 |
| hsCRP, log mg $l^{-1}$ | 1.45 ± 4.25 | 0.73 | 1.95 ± 2.58 | 0.45 | −12.02 ± 5.09 | 0.024 | 9.35 ± 8.92 | 0.30 |
| Blood glucose, log mmol $l^{-1}$ | 23.88 ± 22.08 | 0.29 | 18.95 ± 13.41 | 0.17 | 5.65 ± 28.76 | 0.85 | −29.57 ± 50.40 | 0.56 |
| Serum $K^+$, log mmol $l^{-1}$ | −59.58 ± 41.51 | 0.16 | −34.45 ± 25.22 | 0.18 | −38.68 ± 47.05 | 0.42 | 30.66 ± 82.45 | 0.71 |
| Serum $Na^+$, log mmol $l^{-1}$ | 21.10 ± 46.73 | 0.65 | 8.95 ± 28.38 | 0.75 | 67.68 ± 58.08 | 0.25 | −5.33 ± 101.77 | 0.96 |
| 24-h Pulse rate, b.p.m | 0.32 ± 0.16 | 0.057 | 0.16 ± 0.10 | 0.12 | 0.18 ± 0.19 | 0.36 | −0.040 ± 0.34 | 0.91 |
| Smoking (0,1) | −3.19 ± 4.25 | 0.46 | −1.75 ± 2.58 | 0.50 | −1.72 ± 4.99 | 0.73 | −2.27 ± 8.75 | 0.46 |
| *African men (⩾43 years; N=49)* | | | | | | | | |
| $R^2$ | 0.315 | | 0.322 | | 0.294 | | 0.205 | |
| Serum calcium, mmol $l^{-1}$ | −2.15 ± 19.06 | 0.91 | 5.32 ± 12.81 | 0.68 | 23.12 ± 16.65 | 0.17 | −21.31 ± 28.16 | 0.45 |

Abbreviations: *B*, partial regression coefficient; b.p.m, beats per minute; DBP, diastolic blood pressure; hsCRP, high-sensitivity C-reactive protein; SBP, systolic blood pressure; SV, stroke volume; TPR, total peripheral resistance.

Cooper and Shamsi[32] were unsuccessful in obtaining this association in a group of 50 hypertensive subjects, consisting of African-American men and women. Similarly, results from the Third National Health and Nutrition Examination Survey failed to confirm the blood pressure–serum calcium relationship in both African-American men and women.[11] Previous studies on African men from South Africa even revealed a negative association between total serum calcium and blood pressure[12,13] and found serum calcium to be lower in hypertensives (median age: 35.1 years) compared with normotensives (median age: 33.4 years).[14] This is in contradiction with our results in men of comparable age, in that our younger group revealed strong positive associations between serum calcium and blood pressure and, when stratified by hypertensive status, serum calcium tended to be higher in the younger hypertensives compared with the younger normotensives.

A study in spontaneously hypertensive rats revealed that the microcirculation is primarily affected, with increased noradrenalin-induced permeability of arteriolar smooth muscle cell to extracellular calcium.[20] Therefore, when noradrenaline binds to α1-adrenoceptors, the arterioles are more sensitive to calcium, possibly owing to a higher

**Table 4 Sensitivity analyses**

| | *Serum calcium, mmol l$^{-1}$* | | | | | |
|---|---|---|---|---|---|---|
| | *African men (<43 years; N=40)*[a] | | | *African men (⩾43 years; N=31)*[b] | | |
| | $R^2$ | B (± s.e.) | P | $R^2$ | B (± s.e.) | P |
| 24-h SBP, mm Hg | 0.484 | 38.66 ± 15.18 | 0.016 | 0.547 | 7.89 ± 28.33 | 0.78 |
| 24-h DBP, mm Hg | 0.572 | 35.06 ± 8.98 | <0.001 | 0.534 | 8.85 ± 19.27 | 0.65 |
| SV-reactivity, % | 0.433 | –61.60 ± 19.18 | 0.003 | 0.450 | 29.51 ± 23.84 | 0.23 |
| TPR-reactivity, % | 0.258 | 82.30 ± 37.29 | 0.036 | 0.365 | –23.73 ± 36.83 | 0.53 |

Abbreviations: *B*, partial regression coefficient; DBP, diastolic blood pressure; SBP, systolic blood pressure; SV, stroke volume; TPR, total peripheral resistance.
Adjusted for age, body mass index, total cholesterol, high-sensitivity C-reactive protein, blood glucose, serum potassium, serum sodium and 24-h heart rate.
[a]Eight HIV-positive and two subjects using anti-hypertensive medication excluded.
[b]Five HIV-positive and 13 subjects using anti-hypertensive medication excluded.

α1-adrenoceptor density, causing increased calcium inflow, vasoconstriction and increased blood pressure.[20] Interestingly, other animal studies also indicate that the α1-receptor density seems to decrease with increasing age, attenuating the responsiveness to α1-adrenergic agonists.[33–36] This is supported by a human study, in which infusion of the α1-adrenergic receptor agonist, phenylephrine, in young (mean age: 25.1 years) and old (mean age: 72.2 years) subjects at rest caused a less pronounced vasoconstriction response in the leg arteries of the older subjects.[37] Therefore, a higher α1-adrenergic receptor density would render peripheral arteries more sensitive to serum calcium. Not surprisingly, calcium infusion in humans also raised systolic and diastolic blood pressure and total peripheral resistance in both hypertensive and normotensive subjects with no change in cardiac output, and infusion of the calcium channel blocker, verapamil, attenuated this increase in both total peripheral resistance and blood pressure.[38,39] In addition, African men show increased vascular α1-adrenergic vasoconstriction (and therefore, a higher calcium sensitivity) when exposed to stress compared with Caucasian men and women,[17–19] and they also seem to respond better to calcium channel blockers compared with Caucasians.[15,40,41] This experimental evidence supports our results on associations in the younger men, indicating an increased vascular resistance and decreased stroke volume with increasing serum calcium when subjected to stress. The decrease in stroke volume emphasizes the arteriolar vasoconstrictive effect because an increase in vascular resistance decreases stroke volume.[42,43] As the younger and older subjects had similar serum calcium levels, one may speculate that the peripheral vessels of our younger subjects are more sensitive to serum calcium during stress, possibly because of a higher α1-adrenergic receptor density, which increases vascular resistance and in turn increases blood pressure. Therefore, clinically one may also speculate that calcium channel blockers may be more effective in treating hypertension in younger African men because of this higher α1-adrenergic receptor density and consequent increased sensitivity of vascular smooth muscle cells to calcium.

Our current study must be interpreted within the context of its limitations and strengths. Even though our results were consistent after multiple adjustments, we cannot exclude residual confounding effects. Although all subjects received a standardized meal the night before the study, dietary intake of calcium was not assessed. Normal or high normal serum calcium levels do not exclude low calcium intake because of compensatory increases in circulating levels of calcium-regulating hormones, such as parathyroid hormone and calcitriol. These hormones were not measured and could have influenced the results. We applied a cross-sectional target population design to investigate the associations between blood pressure and serum calcium and, therefore, cannot infer causality. Apart from this, we conducted a well-designed study under controlled conditions and made use of ambulatory blood pressure and independent cardiovascular reactivity measurements. To our knowledge, this is the first study to investigate associations between 24-h ambulatory blood pressure, cardiovascular reactivity and serum calcium in African men from South Africa. If our results are applicable to other African populations, it could have far-reaching implications in combating the increasing burden of cardiovascular morbidity and mortality observed in this population group.

To conclude, 24-h ambulatory systolic and diastolic blood pressures are positively associated with serum calcium in African men younger than 43 years. The blood pressure–serum calcium relationship seems to be mediated by increased vascular resistance during stress.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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