Sex hormones associated with subclinical kidney damage and atherosclerosis in South African men: the SABPA study.

Nico T MALAN,[a] Mark HAMER,[a,b] Gavin W LAMBERT,[c] Aletta E SCHUTTE,[a] Hugo W HUISMAN,[a] Johannes M VAN ROOYEN,[a] Catharina M MELS,[a] Wayne SMITH,[a] Carla MT FOURIE,[a] Rudolph SCHUTTE,[a] Ruan KRUGER,[a] Leoné MALAN.[a]

Authors' Affiliation:

[a]Hypertension in Africa Research Team (HART), School for Physiology, Nutrition and Consumer Sciences, North West University, Potchefstroom, South Africa, [b]Department of Epidemiology and Public Health, University College of London, UK, [c]Human Neurotransmitters Laboratory, Baker IDI Heart & Diabetes Institute and Faculty of Medicine, Nursing & Health Sciences, Monash University, Melbourne, Australia.

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Correspondence: Prof Nico Malan, DSc, Hypertension of Africa Research Team (HART), School for Physiology, Nutrition and Consumer Sciences, North-West University, Potchefstroom Campus, Private Bag X6001, Hoffman Street, Potchefstroom, 2520, South Africa. Tel:+27 18 299 2438; fax+27 18 2991053; e-mail; nico.malan@nwu.ac.za

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INTRODUCTION

South Africa is facing an epidemic of hypertension and vascular disease but there still is inadequate information on the physiological factors that are contributing to this process [1]. It is known that blood pressure increases and that testosterone (T) levels decrease with age [1,2] in men and this might be exacerbated by environmental stress [3]. It has been reported that low T levels are associated with increased blood pressure in men [2]. In addition, estradiol (E2) levels also tend to decrease with age in men albeit not to the same extent as testosterone levels [2]. Low T levels are associated with an increase in visceral fat [4-6] resulting in an increase in aromatase enzyme activity [6]. This will tend to stabilise E2 levels and reduce T levels by metabolising T to E2 resulting in an increased E2:T ratio, which, as has been shown in previous studies, is associated negatively with a variety of health aspects in men [7,8].

Conversely, it has been reported that low levels of T [9] as well as increased levels of E2 in men are associated with cardiovascular complications such as atherosclerosis [7,11]. However, little is known about the association of E2 with cardiovascular and/or renal physiology in men.

Cardiovascular diseases like hypertension are associated with target organ damage including increased carotid intima media thickness (CIMT) and an increase in kidney damage as reflected by biomarkers such as albumin-to-creatinine ratio (ACR) [12,13]. Ageing is associated with an increased burden on the kidneys, as advancing age may cause functional and structural damage in the kidney, which could lead to increased ACR [14].

Microalbuminuria (albumin-to-creatinine ratio above 3.5-30 $mg.mmol^{-1}$) is an established risk factor for cardiovascular morbidity and mortality as well as for end-stage renal disease [15]. This is especially true for individuals with hypertension and diabetes mellitus [12]. Indeed, even low-grade albuminuria, well below 3.5 $mg.mmol^{-1}$, is associated with increased blood pressure [16,17], endothelial dysfunction [17,18] and the development of cardiovascular disease [19].

The role of sex hormones is not clear in this process. Low concentrations of T in men have been shown to be associated with metabolic syndrome symptoms and increased CIMT [5] and the possibility was raised that the age-related decrease in testosterone is associated with the severity of renal disease [20]. The role of E2 in renal disease is also not clear. Maric *et al.* found that estradiol supplementation attenuated renal disease in rats and that the rate of diabetic renal disease progression in women is slower than in men [21,22]. However, Meng *et al.* found that high doses of estrogen exerted a toxic effect on renal and cardiac injury in surgically menopausal mice [23].

The aim of this study was, therefore, to investigate the association of E2 with subclinical kidney damage and atherosclerosis in a cohort of African and Caucasian men. Since testosterone and E2 are closely related (as described above), we hypothesised that the effects of E2 would be most pronounced in participants with low T levels.

METHODS

Design and participants

Participants were recruited as part of the Sympathetic Activity and Ambulatory Blood Pressure in Africans (SABPA) comparative cohort population study conducted between February and May of 2008 and 2009. The study sample comprised urban African and Caucasian teachers working in the Kenneth Kaunda Education district in the North West Province of South Africa. The reason for this selection was to obtain a homogenous sample

from a similar working environment and socio-economic status although cultural differences could not be excluded.

We invited all eligible participants between the ages of 25 and 65 years to participate. Exclusion criteria were an ear temperature above 37.5 °C, chronic users of α- and β-blockers, psychotropic substance dependence or abuse, blood donors and individuals vaccinated in the past 3 months. A total of 201 men were initially included in the study. However, for the current sub-study all clinically diagnosed diabetics (N=8), antidepressant users (N=2) and HIV (N=16) infected participants were excluded. A total of 79 Africans and 98 Caucasians were thus included in the study (Figure 1). Participants were fully informed about the objectives and procedures of the study prior to their recruitment. All participants provided written, informed consent and the study was approved by The Ethics Review Board of the North-West University (Potchefstroom Campus: 0003607S6).

Participants were transported at 1630 h to the Metabolic Unit Research Facility of the North-West University and were familiarised with the protocol. After receiving a standardised dinner, participants were encouraged to go to bed at around 2200 h and were woken at 0545 h the following morning to undergo a battery of clinical assessments. Registered nurses collected fasting venous blood samples and obtained information about prescribed medications in addition to obtaining the participants' medical history.

Assessment of health behaviours

In order to assess physical activity, participants wore Actical® accelerometers (Montréal, Québec) around their hip during a normal working day. The Actical is an omnidirectional accelerometer (i.e., is sensitive to movements in all planes), and it has been validated during treadmill walking, running, and lifestyle activities performed in a laboratory (r = 0.94) [24]. The device was initialised using 15-s epochs and converted to 1-min epochs for data analysis. Smoking status was assessed using serum cotinine, which is a reliable and valid circulating

biochemical marker of nicotine exposure [25]. Participants with cotinine values above 14.99 g/L were categorised as smokers [26]. Serum gamma-glutamyl transferase (γ-GT) was measured as a marker of alcohol abuse [27].

Anthropometric measurements

Participants' body mass was determined to the nearest 0.1 kg using a calibrated digital scale and height to the nearest 0.1 cm using standardised procedures [28]. All anthropometric measurements were performed in triplicate by registered level II anthropometrists according to standardised procedures. Body surface area was calculated based on the Mosteller formula [29]. Waist-height ratio was calculated as waist circumference in cm divided by height in cm [30].

Cardiovascular measurements

On the morning prior to the clinical assessment an ambulatory blood pressure (ABPM) and 2-lead electrocardiograph were attached to participants on the non-dominant arm at their workplace. This apparatus was validated and approved by the British Hypertension Society (Meditech CE120 CardioTens®; Meditech, Budapest, Hungary). The ABPM apparatus was programmed to measure blood pressure at 30-min intervals during the day (0800–2200 h) and every hour during night time (2200–0600 h). The successful inflation rate over this period was 75.2% (± 9.8) in Africans and 84.7% (± 9.1) in Caucasians. Participants were asked to continue with normal daily activities and record any abnormalities such as headache, nausea and stress on their ambulatory diary cards. The data was analysed using the CardioVisions 1.19 Personal Edition software (Meditech). Hypertensive status was classified from 24 h ABPM as systolic and/or diastolic blood pressure >125/80 mm Hg [13].

Biochemical measurements

Blood sampling was completed before 09:00 avoiding circadian rhythm changes and the protocol was standardized for all individuals. Serum albumin levels were determined by means of the Cobas Integra 400plus apparatus (Roche, Basel, Switzerland) and the Unicel DXC800 apparatus (Beckman and Coulter, Germany). Serum samples were analysed for total testosterone (T), total E2 and sex hormone-binding globulin (SHBG) using an electro chemiluminescence immunoassay on the Elecsys 2010 apparatus (Roche, Basel, Switzerland). Both the intra- and interassay coefficients of variation (CV) for all the assays were less than 10%. Non SHBG-bound Testosterone (non SHBG-bound T) and -E2 (non SHBG-bound E) were calculated using the Sodergard equations [31].

Total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, high sensitivity C-reactive protein (CRP), cotinine and γ-glutamyl transferase (GGT) were analysed in serum (Unicel DXC 800, Beckman and Coulter, Germany; Modular ROCHE Automized, Switzerland and Konelab^{TM} 20I Sequential Multiple Analyzer Computer, ThermoScientific, Vantaa, Finland). Sodium fluoride plasma samples were used to determine fasting glucose levels using a timed-end-point method (Unicel DXC 800, Beckman and Coulter, Germany and Konelab^{TM} 20I Sequential Multiple Analyzer Computer, ThermoScientific, Vantaa, Finland). HbA1c levels were determined with aturbidometric inhibition immunoassay. (Integra 400, Roche, Switzerland).

Subclinical kidney damage

A fasting 8 hour overnight urine sample was collected from each participant, stored for less than 2 hours at 4°C after collection and then frozen at -80°C. ACR determined the presence of micro- or macro-albuminuria and was used as an indicator of renal impairment. Albumin concentration in the urine was determined in micrograms per litre by means of the Turbidimetric method after immuno-precipitation enhanced by polyethylene glycol at 450 nm

(Unicel DXC 800, Beckman and Coulter, Germany). Creatinine in the urine was determined with the QuantChrom creatinine assay kit from BioAssay Systems. Microalbuminuria is expressed as the urinary albumin-to-creatinine ratio (exceeding 2.9 mg/mmol) [15].

Subclinical atherosclerosis

High resolution ultrasound was applied to determine structural vascular changes as reflected by intima-media thickness in the carotid artery (CIMT) [32]. Standardized images [33] of the left and the right carotid artery were obtained, from at least two optimum angles using a Sonosite Micromaxx ultrasound system (SonoSite Inc., Bothell, WA, USA) and 6 - 13 MHz linear array transducer. The images were digitized and imported into the Artery Measurement Systems automated software (AMS) II v1.139 (Gothenburg, Sweden). The left far wall of the CIMT (L-CIMTf) of a 10 mm segment, 1 cm distal of the carotid bulb, was chosen for analysis [33].

Statistical analyses

Data was analysed using the computer software package Statistica® version 10.0 (Statsoft Inc., Tulsa, USA, 2010). Skewness of data was tested and gamma glutamyl transferase (γ-GT) values were logarithmically transformed. T-tests for independent groups determined differences between ethnic groups. Chi-square (χ^2) statistics calculated and compared proportions. Two-way ANCOVAs tested significant interactions on main effects (ethnicity X T) for each variable. Hereafter, the study population was divided into low and high T groups by means of median split. ANCOVAs compared significant differences by comparing low as well as high T ethnic male groups from least square means analyses, while adjusting for confounders. In line with the European Society of Hypertension Guidelines [13] we considered *a priori* covariates including age, body surface area (instead of body mass index),

physical activity, cotinine and log γ-GT. Multiple regression analyses were computed between ACR, L-CIMTf and cardio-metabolic markers within each ethnic and T group, independent of *a priori* covariates. Forward stepwise regression analyses were performed for various models (separately for ethnic groups and T values), with both ACR and L-CIMTf as dependent variables. Independent covariates considered in all the models were the five *a priori* covariates age, BSA, physical activity, log γ-GT and cotinine. E2, glucose, cholesterol, CRP, E2:T and systolic blood pressure (SBP) were included in those models, justified by significant correlations with ACR and L-CIMTf ($r \geq 0.30$, $p \leq 0.05$). Significance was noted as $p \leq 0.05$.

RESULTS

As reported previously, the African men compared to the Caucasian men, displayed lower physical activity and BSA [34] but higher waist-to-height-ratio (Table 1). The Africans also had elevated γ-GT levels compared to the Caucasians. Furthermore the Africans demonstrated elevated CRP, total E2, non SHBG-bound E2, E2:T, HbA1c, ACR and lower cholesterol levels compared to Caucasian men. All the T parameters as well as SHBG were lower in the Africans compared to the Caucasians. Higher mean ambulatory blood pressure (ABPM) and -heart rate were evident in African men compared to their counterparts. Hypertension was prevalent in 76.5% of Africans and 68.6% of Caucasians.

A single 2 x 2 ANCOVA showed interaction on main effects (ethnicity x T) for 24 hour SBP [$F(1, 111), 6.82$; $p = 0.01$]. Subsequently the subjects were divided by means of a median split into high and low T groups and the values were adjusted for confounders (age, BSA, physical activity, smoking (cotinine) and alcohol abuse (log γ-GT)) according to the ESH guideline (Table 2) [13]. When comparing the low T ethnic groups in Table 2, the low T African group demonstrated a more vulnerable cardio-metabolic profile with higher prevalence of hypertension, waist-to-height ratio, CRP, HbA1c, ACR, BP, heart rate and L-

CIMTf values than their Caucasian counterparts. In the high T group, except for serum cortisol, no differences in any of the variables occurred between the two ethnic groups.

In the low T African men (Table 2)

E2 and blood glucose explained 33% and 38% respectively of the variance in ACR while E2:T and SBP explained 22% and 36% respectively of the variance in L-CIMTf. In the high T African men E2 and SBP explained 40% and 38% respectively of the variance in ACR.

In the low T Caucasian men (Table 3) E2:T and SBP explained 35 % and 33% respectively of the variance in ACR. No associations existed between ACR and any sex hormone or blood pressure variables in high T Caucasian men.

DISCUSSION

Our main findings indicate that E2 levels were associated with the renal dysfunction marker, ACR, in both low and high T Africans and with ACR in low T Caucasians. E2 levels were also associated with subclinical atherosclerosis in low T Africans.

With urbanisation in Sub-Saharan Africa the prevalence of hypertension has increased significantly in recent years while people living in true rural conditions seem to be relatively protected against the advances of civilisation [1]. The African Union regards hypertension as the greatest health problem in Africa after AIDS [35], and 50% - 60% of a mixed South African population has been found to suffer from hypertension in 2005 [1]. The impact of hypertension is magnified given that high blood pressure increases the risk of the development of target organ damage including cardiac, cerebral, retinal and renal events [36].

Low T levels in men are associated with the development of hypertension, atherosclerosis and other cardio-metabolic diseases [4,6,8,37]. There are no generally adopted clinically important cut-off points for low T and it is also not clear whether older men can expect to have the same T concentrations as younger men [38]. Both the African and Caucasian groups were, therefore, divided into low T and high T groups by means of a median split. The mean

total T value of the low T African group (10.3 nmol/L) was below the 5th percentile for non-diabetic men between 40-44 years of age while the total T value of the low T Caucasians (12.2 nmol/L) was well below the 10th percentile [39]. There are also no generally accepted lower limits of normal total T values and values below 10.4 nmol/L are regarded as low T values. It has been proposed that testosterone replacement therapy becomes a possible treatment for patients with T levels below this value [40]. The African group's T levels are below this level while the Caucasians' levels are just above the upper limit of borderline hypogonadic status [39].

Although the age and socio-economic status of the Africans and the Caucasians were similar, the cardio-metabolic health profile of the Africans was clearly worse than that of the Caucasians. The prevalence of hypertension in both groups was very high (76.5% in Africans and 68.6% in Caucasians) and did not differ significantly. However, a larger difference in HT prevalence rates emerged when the low T groups were compared, indicating that the Africans had a staggering 90% prevalence. The values for both low T groups with regard to SBP, DBP [30], WheightR [30], as well as HbA1c [41] were above the cut-off points for cardiovascular, renal and metabolic diseases respectively. Combined with the very high CRP values of the African group, this pattern is indicative of a high risk for the development of serious cardiometabolic and structural vascular diseases [41]. Aminbakhsh *et al.* demonstrated that the risk of first myocardial infarction (MI) and stroke increases with a CIMT above 0.82 mm and 0.75 mm respectively [42]. The 0.74 mm L-CIMTf value in the low T African men suggests that this group has borderline risk for subclinical atherosclerosis. It is well known that blood pressure increases with age in urban societies [1]. Testosterone (T) also decreases with age in men while E2 levels decrease but generally not to the same extent as T [2]. This most likely contributed to the elevated E2:T ratio in the Africans compared to the Caucasians. It is clear that even though the E2 values of the high T groups were higher than those of the

low T groups, the E2:T ratio of the low T African group is still higher than any of the other groups.

According to Mattix, *et al*. the urine creatinine concentration of Afro-Americans is higher than their Caucasian counterparts as result of their larger muscle mass [43]. This might result in an under estimation of the ACR in African-Americans. In the current study the creatinine concentration of the African group was lower than their Caucasian counterparts with the result that the ACR of this group might be overestimated. This discrepancy with the results of Mattix may be the result of the anthropometric characteristics of the Setswana speaking ethnic group in South Africa which tend to be smaller than that of the Caucasians. However the urine albumin concentration of both low and high T African groups was so much higher than the Caucasians (factor 5.37) that it more than compensated for their lower creatinine value (factor 1.72). From a partial correlation analysis it was determined that ACR correlated best with measures of E2 as well as with blood pressure parameters (results not shown).

The association between high blood pressure and albuminuria is well documented [12]. Albuminuria has been associated with impaired nitric oxide (NO)-dependent and –independent vascular reactivity. Together with its association with greater levels of adhesion molecules it might provide a possible mechanism for the increased cardiovascular risk associated with albuminuria [44]. However, the role of E2 in the development of kidney and endothelial damage is controversial. Baylis reported that estrogens protect women against kidney damage and endothelial damage by maintaining a higher level of renal NO than in men [14]. Meng *et al.* on the other hand, found that high dose estrogen supplementation was toxic and contributed to the development of cardiac and renal injury [23]. Furthermore, E2 supplementation in foetal rats caused renal damage which was synergistically increased by alcohol supplementation but this was not found when T supplementation occurred [45]. Increased concentrations of E2 in men, on the other hand, have been shown to be associated

with the pathogenesis of diabetic nephropathy associated with diabetes mellitus as well as lower extremity artery disease in elderly men [21].

If E2 is indeed instrumental in the development of renal damage, especially in men, the high E2 levels in the African groups in our study, might contribute to increased ACR and L-CIMTf. In the African group the γ-GT which is used as an indicator of alcohol abuse [27] was much higher than in the Caucasian group and that could have contributed to endothelial damage, increases in ACR as well as vasoconstrictive effects in the macrovasculature and subsequent elevated blood pressure. However, if E2 is protective against subclinical kidney damage and atherosclerosis by maintaining higher NO levels [14], a more protective and compensatory role for E2 in low T groups should be considered. Contrary to our results, Maric *et al.* suggested that male sex hormones contribute to the pathogenesis of kidney disease in diabetic men [21]. We have found a positive association between high blood pressure, ACR and L-CIMTf in the low T African group. It is known that testosterone is negatively associated with blood pressure [2] supporting the notion that low testosterone may facilitate the development of subclinical kidney damage and atherosclerosis associated with increased blood pressure and estradiol. Furthermore, in the low T Caucasian men ACR was positively associated with blood pressure and the E2:T ratio indicating a negative association with the relative T concentration while suggesting a possible role for E2 in the increased ACR values. It is unclear why total E2 is the stronger predictor of ACR in Africans while E2:T ratio is stronger in Caucasians.

Several limitations should be noted. The sex hormones were sampled only once, thus circadian patterns could not be considered. The cross sectional design of the current study prevents us from being able to infer causality. Larger sample sizes are needed and should include interaction with psychological distress markers to verify the influence of stress on sex hormone profiles and sympathetic drive. In addition, further data on autonomic and

endothelial function are needed to delineate possible physiological mechanisms at play. A great deal of controversy exists concerning the therapeutic treatment of the effects of low testosterone levels. Testosterone replacement therapy (TRT) in combination with aromatase inhibition was found to attenuate renal injury in diabetic rats [46]. Some reports indicate that TRT had no positive or negative effects [10] while others warn about the possible negative side-effects of long-term TRT [47]. It is clear that, despite ethical concerns, prospective clinical trials with a large number of participants are needed before safe treatment could commence. Direct immunoassays used in this study could have resulted in higher values, especially of E2 in both the ethnic groups. Nevertheless, the assays used in the present study had intra- and inter-assay CVs below 10%.

In conclusion, this study shows that a relative increase in E2 levels in both African groups as well as low T in Caucasians is associated with an increase in subclinical kidney damage and atherosclerosis. Systolic blood pressure was also associated with an increase in ACR in the higher T Africans and the low T Caucasians. It seems, therefore, that both E2 as well as an increase in SBP may play a role in the development of subclinical functional and structural endothelial impairment in men. It is clear that progression and risk for established kidney damage and atherosclerosis in Africans should be estimated in prospective studies.

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contributions: N.T.M. had full access to the data; takes responsibility for the integrity of the data and accuracy of the data analyses. All authors contributed to the concept and design of study, drafting and critical revision of the manuscript.

Conflicts of interest

There are no conflicts of interest.

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Table 1: Comparing characteristics of African and Caucasian men

	African men (N=79)	Caucasian men (N=98)	P-value
Unadjusted values			
Age (years)	42.4 ± 8.26	44.8 ± 11.1	0.12
Body surface area (m^2)	1.95 ± 0.23	2.18 ± 0.21	<0.001
Physical activity (kcal)	2733.4 ± 831.3	3474.3 ± 718.8	<0.001
Cotinine (μg/L)	24.7 ± 48.1	31.5 ± 97.6	0.57
γ-GT, U/L	76.7 ± 72.3	34.2 ± 29.0	<0.001
Adjusted values (adjusted for age, BSA, physical activity, cotinine and γ-GT)			
Lifestyle and metabolic variables			
Waist:height ratio	0.59 (0.58, 0.60)	0.53 (0.52, 0.54)	<0.0001
Stature (cm)	172.2 (170.9,173.6)	180.0 (178.8,181.1)	<0.0001
Creatinine urine (mmol/L)	11.1 (9.16,13.0)	17.5 (15.8,19.2)	<0.0001
Albuminuria (mg/L)	13.1 (10.4,15.8)	4.37 (1.98,6.75)	<0.0001
Albumin:Creatinine ratio (mg/mmol)	1.47 (1.11,1.82)	0.37 (0.06,0.69)	<0.0001
C-reactive protein (mg/l)	5.51 (4.17, 6.85)	1.88 (0.70, 3.05)	<0.001
Glucose, fasting (mmol/L)	5.96 (5.63, 6.29)	5.87 (5.57, 6.16)	0.70
HbA1c (%)	6.34 (6.11, 6.57)	5.55 (5.35, 5.74)	<0.0001
Biochemical measurements			
Total Cholesterol (mmol/L)	4.79 (4.49, 5.10)	5.64 (5.37, 5.90)	0.0002
HDL Cholesterol (mmol/L)	1.04 (0.96,1.11)	1.03 (0.96,1.10)	0.93
Chol: HDL Chol ratio	5.13 (4.63,5.63)	5.88 (5.44,6.32)	0.04
Total Testosterone (nmol/L)	14.2 (12.9, 15.6)	19.0 (17.7, 20.2)	<0.0001
Non-SHBG bound T (nmol/L)	8.84 (8.08, 9.59)	10.5 (9.85, 11.2)	0.003
Total Estradiol (pmol/L)	95.0 (86.3, 103,7)	74.0 (66.4, 81.6)	0.001
Non-SHBG bound Estradiol (pmol/L)	72.7 (66.1, 79.3)	52.7 (46.9, 58.5)	<0.0001
E2:T ratio	0.009 (0.007, 0.01)	0.004 (0.003, 0.005)	<0.0001

DHEAS (µg/dL)	157.1 (134.6, 179.6)	200.4 (180.6, 220.1)	0.01
SHBG (nmol/L)	33.5 (29.7, 37.4)	41.0 (37.7, 44.4)	0.009
Cardiovascular measurements (24h ambulatory)			
L-CIMTf (mm)	0.70	0.67	0.27
SBP (mm Hg)	140 (137, 143)	126 (123, 129)	<0.0001
DBP (mm Hg)	89 (87, 91)	79 (77, 81)	0.003
Heart rate (HR) (beats per minute)	78 (76, 81)	72 (70, 75)	0.003
HT % (SBP>125 and/or DBP > mm Hg)	76.5	68.6	0.24
Use of Hypertension medicatio	16.1	9.09	0.16
Use of Statins	1.23	6.06	0.1

Mean (95% Confidence interval) and P values ≤ 0.05 regarded as statistically significant.

Where:γ-GT, Gamma glutamyl transferase; HbA1C, glycosylated hemoglobin; SHBG, sex hormone-binding globulin; DHEAS, dehydroepiandrosterone sulphate; E2:T, estradiol-testosterone ratio; L-CIMTf, left carotid intima media thickness of the far wall; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; HT, hypertension. Values adjusted according to ESH guidelines for age, BSA (body surface area instead of BMI); physical activity; smoking (cotinine) and γGT (for alcohol abuse).

Table 2: Adjusted cardiovascular and endocrine values in low and high Testosterone African vs Caucasian men.

	Low T African men (N = 40)	Low T Caucasian men (N = 44)	High T African men (N =39)	High T Caucasian men (N = 54)
Lifestyle and metabolic variables				
Waist:height ratio	0.64 (0.62,0.66)	0.57 (0.55,0.59)**	0.53 (0.51,0.55)	0.50 (0.49,0.52)
Stature (cm)	172.0 (170.2,173.8)	180.5 (178.8,182.2)**	173.6 (171.6,175.6)	178.9 (177.3,180.5)**
C-reactive protein (mg/l)	7.9 (5.17,10.6)	2.52 (-0.05,5.09)**	2.59 (1.92,3.26)	1.69 (1.15,2.37)
Creatinine urine (mmol/L)	10.6 (7.74,13.4)	18.2 (15.6,20.8)**	11.0 (8.18,13.9)	17.5 (15.1,19.8)**
Albuminuria (mg/L)	15.3 (11.2,19.4)	2.85 (-0.93,6.63)**	11.2 (7.3,15.0)	5.48 (2.33,8.63)*
Albumin:Creatinine ratio (mg/mmol)	1.69 (1.27,2.1)	0.09 (-0.3,0.49)**	1.36 (0.75,1.96)	0.52 (0.03,1.01)
Glucose, fasting (mmol/L)	6.45 (5.82,7.08)	6.0 (5.41,6.59)	5.37 (5.04,5.69)	5.83 (5.56,6.17)
HbA1c (%)	6.75 (6.31,7.19)	5.68 (5.3,6.29)**	5.84 (5.67,6.02)	5.50 (5.36,5.64)*
Total Cholesterol (mmol/L)	4.89 (4.38,5.4)	5.81 (5.33,6.29)*	4.64 (4.24,5.03)	5.53 (5.22,5.86)**
HDL-cholesterol (mmol/L)	0.95 (0.84,1.06)	0.98 (0.87,1.06)	1.12 (1.0,1.25)	1.07 (0.97,1.17)
Total cholesterol:HDL ratio	5.79 (4.9,6.68)	6.19 (5.35,7.03)	4.45 (3.87,5.03)	5.63 (5.15,6.1)**
Endocrine variables				
Total Testosterone (nmol/L)	10.26 (9.0,11.5)	12.2 (11.05,13.4)*	20.5 (18.9,22.0)	22.9 (21.6,22.2)*

Non-SHBG bound T (nmol/L)	6.48 (5.67,7.3)	8.0 (7.13,8.67)*	12.2 (11.2,13.2)	12.0 (11.2,13.2)
Total Estradiol (pmol/L)	92.7 (81.3,104.0)	57.3 (46.7,68.0)**	104.8 (91.4,118.2)	82.3 (71.4,93.2)*
Non SHBG-bound Estradiol (pmol/L)	72.2 (62.9,81.5)	44.3 (35.6,53.0)**	77.7 (67.6,87.9)	56.3 (48.0,64.6)**
E2:T ratio	0.012 (0.009,0.014)	0.005 (0.002,0.007)**	0.005 (0.004,0.006)	0.004 (0.003,0.004)**
DHEAS (µg/dL)	147.4 (112.6,182.1)	187.6 (154.9,220.2)	169.2 (137.1,201.2)	209.3 (183.1,235.5)
SHBG (nmol/L)	30.5 (26.7,34.2)	27.5 (24.0,31.0)	41.0 (35.0,47.0)	48.9 (44.0,53.8)
Cardiovascular measures				
L-CIMTf (mm)	0.74 (0.69,0.78)	0.65 (0.60,0.69)*	0.67 (0.62,0.72)	0.69 (0.64,0.73)
24hr SBP, mmHg	148 (143.1,153.1)	127 (122.4,131.8)**	130 (126.5,134.2)	126 (122.7,129.1)
24hr DBP, mmHg	93 (89.3,96.1)	80 (76.5,82.9)**	85 (81.6,87.5)	78 (75.5,80.4)**
24h Heart rate, beats/minute	81 (76.7,84.8)	76 (71.8,79.4)*	75 (70.8,78.2)	70 (67.2,73.2)
HT % (SBP>125 and/or DBP >80 mm Hg)	90.9	69.6**	59.5	67.9*

Values depicted as mean (95% confidence interval) and proportions as N (%). Covariates included age, body surface area (BSA), physical activity, log γ-GT and cotinine. Where: **P ≤ 0.01, *P ≤ 0.05. Where HbA1c, glycosylated hemoglobin; SHBG, sex hormone-binding

globulin; E2:T, estradiol-to-testosterone ratio; DHEAS, dehydroxyepiandrosterone sulfate; SBP, systolic blood pressure; DBP, diastolic blood pressure; HT, hypertension; L-CIMTf, left carotid intima media thicness – far wall.

Table 3: Forward stepwise regression analyses predicting relationships between estradiol, ACR and L-CIMTf in African men.

	Albumin:Creatinine	
	Low Testosterone Africans ß (±95% CI)	**Low Testosterone Caucasians** ß (±95% CI)
Adjusted R^2	**0.36**	**0.38**
Glucose (mmol/L)	0.38 (0.12,0.63), p=0.006	-
Total Estradiol (pmol/L)	0.33 (0.08,0.58), p=0.01	-
Estradiol:Testosterone ratio	-	0.35 (0.09,0.61) p=0.01
ABPM SBP (mmHg)	-	0.33 (0.11,0.65), p=0.02
	High Testosterone Africans	**High Testosterone Caucasians**
Adjusted R^2	**0.32**	No entry?
Total Estradiol (pmol/L)	0.40 (0.12,0.68), p=0.01	-
ABPM SBP (mmHg)	0.38 (0.11,0.65) p<0.01	-

	L-CIMTf	
	LowTestosteroneAfricans	**LowTestosteroneCaucasians**
	ß (±95% CI)	ß (±95% CI)
Adjusted R^2	**0.48**	No entry
Estradiol:Testosterone ratio	0.22 (0.0, 0.44), p=0.06	-
ABPM SBP (mmHg)	0.36 (0.14, 0.59),p=0.01	-
	High TestosteroneAfricans	**High TestosteroneCaucasians**
Adjusted R^2	No entry	No entry
	-	-

ß denotes standardized regression coefficient. Covariates for models included age, body surface area, physical activity, cotinine, log γ-GT. Structural vascular disease models were additionally adjusted for log CRP, cholesterol and mean 24h blood pressure.

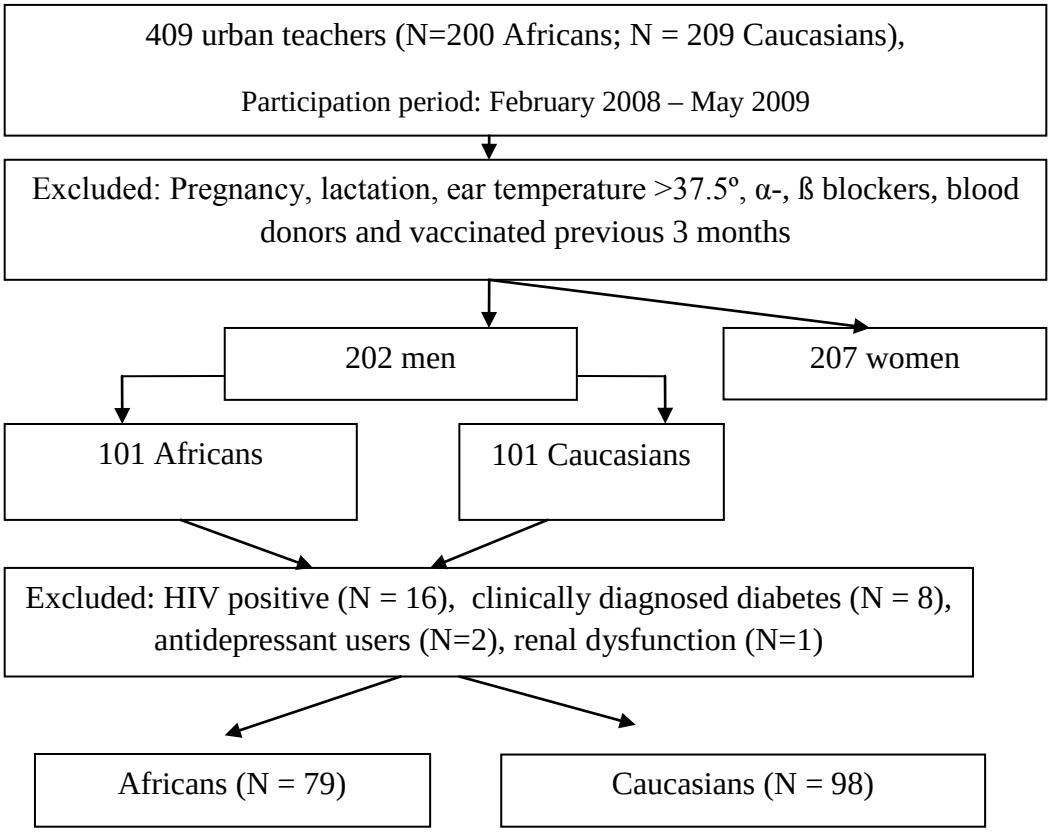

Figure 1: Selection sequence of study participants.