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Large artery stiffness is associated with marinobufagenin in young adults: the African-PREDICT study

Michél Strauss^a, Wayne Smith^{a,b}, Wen Wei^c, Alexei Y. Bagrov^c, Olga V. Fedorova^c, and Aletta E. Schutte^{a,b}

^aHypertension in Africa Research Team (HART), North-West University, Potchefstroom, South Africa

^bMRC Research Unit: Hypertension and Cardiovascular Disease, North-West University, Potchefstroom, South Africa

^cNational Institute on Aging, NIH, Baltimore, Maryland, USA

Abstract

Objectives: The cardiotonic steroid, marinobufagenin (MBG), has been shown to play a physiological natriuretic role in response to salt intake. However, recent studies in clinical and animal models demonstrated possible links between elevated levels of endogenous MBG and increased arterial stiffness. Large artery stiffness is a known predictor of future cardiovascular disease. We, therefore, investigated whether large artery stiffness relates to 24-h urinary MBG excretion in young apparently healthy black and white adults.

Methods: This study included data of 711 participants (black 51%, men 42%, mean age 24.8±3.02 years). We measured the carotid-femoral pulse wave velocity (cfPWV), 24-h urinary MBG and sodium excretion.

Results: In single, partial and multivariable adjusted (Adj.) regression analyses, we found a persistent positive association between cfPWV and MBG excretion in women [Adj. $R^2 = 0.23$; standardized (std.) $\beta = 0.15$; $P = 0.002$], but not men (Adj. $R^2 = 0.17$; std. $\beta = 0.06$; $P = 0.31$). Multiple regression models were adjusted for ethnicity, age, waist-to-height ratio, mean arterial pressure, high-density lipoprotein cholesterol, C-reactive protein, γ -glutamyl transferase and glucose.

Conclusion: In conclusion, already at a young age heightened endogenous MBG levels may contribute to large artery stiffness in women via pressure-independent mechanisms, increasing their risk for future cardiovascular disease.

Keywords

healthy; marinobufagenin; pulse wave velocity; salt; sodium; young adult

Correspondence to Aletta E. Schutte, PhD, Hypertension in Africa Research Team (HART), North-West University, Private Bag X6001, Potchefstroom 2520, South Africa. Tel: +27 18 299 2444; fax: +27 18 285 2432; Alta.Schutte@nwu.ac.za.

Conflicts of interest

There are no conflicts of interest.

INTRODUCTION

The pathophysiological role of sodium in the cause of cardiovascular disease remains obscure. The mammalian bufadienolide marinobufagenin (MBG), a steroidal Na^+/K^+ -ATPase inhibitor with natriuretic properties has been identified as an endogenous biomarker released from the adrenal cortex in response to high- sodium intake [1]. Several studies have implicated MBG in blood pressure regulation via its inhibitory function on Na^+/K^+ -ATPase [2–4]. However, increasing evidence from experimental animal models [5,6] and in-vitro [7] studies have suggested alternate pathways through which MBG may adversely contribute to cardiovascular disease development beyond blood pressure.

Evidently, MBG was shown to exhibit profibrotic properties by promoting collagen deposition in rat aortic explants [7] – possibly contributing to large artery stiffness [7,8]. Indeed, MBG has been associated with arterial stiffness, an established predictor of cardiovascular risk and mortality [9–11], in a small prehypertensive population ($n = 11$, mean age 60 ± 2 years) [7].

To increase our understanding, however, on the functioning of MBG, it is important to explore whether large artery stiffness relates to MBG, particularly in a young apparently healthy population previously reported to consume excessive amounts of salt [12]. Should our study already indicate an association between MBG and arterial stiffness in young healthy adults, it would underline specific population-targeted approaches to reduce sodium intake and avoid or delay arterial stiffening at young ages.

Due to known reports on the sex-specific effects of MBG [2,4,13], we investigated these relationships in men and women, aged 20–30 years. Further taking into consideration the steroidal nature of MBG [1], we tested for cross- immunoreactivity of the anti-MBG monoclonal antibody used in this study with hormonal contraceptives.

This is the first study to the best of our knowledge to explore the relationship between large artery stiffness and 24-h urinary MBG excretion in a young healthy black and white population.

METHODS

Study design and participant recruitment

The African Prospective study on the Early Detection and Identification of Cardiovascular disease and Hypertension (African-PREDICT) assesses and monitors the lifestyle, biochemical and cardiovascular profiles of young apparently healthy black and white individuals from communities in close proximity to the Potchefstroom area, in the North West province of South Africa. Participant recruitment for this study took place on a voluntary basis, and community members were screened before inclusion into the study. Eligibility for participation was determined by the following inclusion criteria: normotensive based on office blood pressure ($<140/90$ mmHg); HIV uninfected; microalbuminuria less than 30 mg/ml; not pregnant or lactating and no previously diagnosed chronic illness (self-

reported). In addition, none of the participants included into the study made use of medication for hypertension or other chronic diseases.

For the purpose of this study, we analysed the crosssectional data of the first 711 (black 51%, men 42%) consecutively enrolled participants from February 2013 to October 2016, with complete 24-h urinary data.

The African-PREDICT study was approved by the Health Research Ethics Committee of the North-West University. The study is registered at [ClinicalTrials.gov](https://clinicaltrials.gov) (Nr. NCT03292094). All procedures conformed to the relevant principles outlined by institutional guidelines and the Declaration of Helsinki. All procedures were thoroughly explained, and each participant provided written informed consent prior to initial screening and participation in the advanced measurements of this study.

Questionnaire and anthropometric data

Detailed information on demographics and lifestyle habits were obtained through general health questionnaires completed by each participant. Questionnaire data included age, sex, ethnicity, socioeconomic status, family history, self-reported smoking, alcohol, and hormonal contraceptive use.

Anthropometric measurements were performed in triplicate, according to the guidelines set by the International Society for the Advancement of Kinanthropometry [14]. The body weight (kg; SECA 813 Electronic Scales), height (m; SECA 213 Portable Stadiometer; SECA, Hamburg, Germany) and waist circumference (cm; Lufkin Steel Anthropometric Tape; W606PM; Lufkin, Apex, USA) were measured, and the BMI [weight (kg)/height (m²)] calculated.

Cardiovascular measurements

Arterial stiffness—We used the Sphygmocor XCEL device (AtCor Medical Pty Ltd., Sydney, Australia) to measure carotid-femoral pulse wave velocity (cfPWV) noninvasively while participants rested in a supine position. Both the femoral and carotid artery waveforms were captured simultaneously by means of a femoral cuff placed on the upper right thigh, and carotid artery applanation tonometry. In order to determine the cfPWV travel distance, 80% of the distance measured between the arterial points (carotid to cuff measured using an infantometer, and femoral to cuff via a tape measure) was calculated [15]. cfPWV was automatically calculated as distance/pulse transit time. In addition, we determined supine brachial SBP and DBP. Mean arterial pressure (MAP) was subsequently calculated using supine brachial blood pressures [bDBP + 1/3(bSBP – bDBP)]. cfPWV as well as blood pressure measurements were performed in duplicate, and repeated if PWV differed by more than 3 m/s.

Ambulatory blood pressure monitoring

Each participant was fitted with a validated CardioXplore apparatus (Meditech, Budapest, Hungary, British Hypertension Society) on their nondominant arm so to obtain 24-h ambulatory blood pressure measurements. Blood pressure measurements were recorded over

30-min intervals during the day (0600–2200 h), and hourly at night (2200–0600 h). The 24-h blood pressure data was considered successful with at least 70% of the total 24-h blood pressure readings being valid; or at least 20 valid daytime and seven nighttime measurements [16].

Biological sampling and biochemical analyses

Participants refrained from eating or drinking at least 8 h before measurements took place. Early morning biological sampling included blood and spot urine collection, by a trained research nurse and 24-h urine was collected from participants starting on the morning of participation. Participants were requested to discard the first passed urine after which all subsequent urinary voids were collected. The 24-h urine samples were considered complete if the total urinary volume was at least 300 ml [17].

The 24-h urinary potassium, sodium, creatinine, and albumin were measured using the Cobas Integra 400plus (Roche, Basel Switzerland). The 24-h urinary MBG was analysed using a solid-phase Dissociation-Enhanced Lanthanide Fluorescent Immunoassay, based on a 4G4 anti-MBG mouse monoclonal antibody, described in detail by Fedorova *et al.* [18]. For our competitive reverse-phase immunoassays, we use a highly specific anti-MBG monoclonal antibody (mAb; clone 4G4) with very low immunoreactivity to several substances, including aldosterone. We also tested for immunoreactivity with contraceptive hormones [18]. The typical contraceptives reported by women in the present study include Nur-Isterate (progesterone), Yasmin (drospirenone and ethinyl estradiol), Triphasil (ethinyl estradiol and levonorgestrel), Minerva (progesterone with estrogens), and Ginette (progesterone with estrogens). MBG, progesterone, drospirenone, ethinyl estradiol, and levonorgestrel (MilliporeSigma, St Louis, Missouri, USA) were diluted in the assay buffer and tested in our 4G4 immunoassay to compete with immobilized antigen (MBG-thyroglobulin) for a limited number of binding sites on 4G4 anti-MBG mAb. Furthermore, we used the C18 column for sample extraction. This column extracts a panel of steroids and other hydrophobic substances from the sample because of the nature of its silica-based sorbent [19].

We analysed serum low-density lipoprotein cholesterol, high-density lipoprotein cholesterol (HDL-C), triglycerides, C-reactive protein (CRP), glucose and gamma glutamyl-transferase (GGT) using the Cobas Integra 400plus (Roche, Basel, Switzerland). The chemiluminescence method on the Immulite (Siemens, Erlangen, Germany) was used to measure serum cotinine. We measured serum aldosterone using the RIA Aldosterone Kit (Beckman Coulter, Immunotech, Radiova, Czech Republic).

Statistical analyses

All statistical analyses were performed with Statistica version 13.2 (Dell Inc., Tulsa, Oklahoma, USA) and figures drawn with GraphPad Prism version 5.0 (GraphPad Software Inc., La Jolla, California, USA). Data following a normal distribution was presented as the arithmetic mean $\pm$ standard deviation. Variables following a non-Gaussian distribution were logarithmically transformed, and the central tendency and spread presented as the geometric mean; 5th and 95th percentile intervals. Independent *t* tests were done to compare the

anthropometric, cardiovascular, 24-h urinary, biochemical, and lifestyle profiles of black and white participants, and chi-square tests for categorical data (socioeconomic status, smoking, alcohol, and hormonal contraceptive use). We performed analyses of covariance to determine significant differences in cfPWV across increasing quartiles of MBG excretion, while adjusting for age, waist-to-height ratio (WHtR), and MAP. Pearson regression analyses, partial regression analyses and multiple regression analyses were done in each group, with cfPWV as dependent variable, to explore the relationship with MBG excretion. Although several covariates were considered for inclusion as possible independent variables, we ultimately included: ethnicity, age, WHtR, MAP, high-density lipoprotein cholesterol (HDL-C), C-reactive protein (CRP), γ -glutamyl transferase (GGT), and glucose based on the strongest bivariate associations, with MBG excretion and cfPWV.

RESULTS

Cross-immunoreactivity of 4G4 anti-marinobufagenin monoclonal antibodies with contraceptive hormones

We found low cross-immunoreactivity of 4G4 anti-MBG mAb with contraceptive hormones (less than 0.001–0.01%; Table 1, Fig. 1). This insured that MBG was reliably measured in the nonextracted urine samples in the presence of other steroids and hormones.

Participant characteristics

Due to known reports on salt-sensitivity in black populations [20,21], we tested for an interaction of ethnicity on the relationships between MBG excretion and cfPWV, but found no interaction (Table 2).

The general characteristics of this study population (mean age 24.8 ± 3.02 years) are outlined in Table 3. Clear sex differences were evident in the cardiovascular profiles, with men demonstrating higher 24-h SBP, 24-h DBP, MAP, and cfPWV compared with women (all $P < 0.001$).

Even though 24-h urinary volume output was similar for men and women, men had higher MBG excretion ($P < 0.001$) and Na^+ excretion ($P = 0.003$). In entirety, 78.3% of this study population was on a high-salt habitual diet, consuming more than 5g of salt per day with a collective mean intake of 7.69g/day and a Na^+/K^+ ratio greater than 3.

Regression analyses

Single and partial regression analyses are presented in Table 4, with multiple regression analyses depicted in Table 5. We found that cfPWV associated positively across quartiles of MBG excretion in women ($P = 0.001$), adjusting for age, WHtR and MAP (Fig. 2).

Multivariable adjusted regression analyses underlined this positive association of cfPWV with MBG excretion women (Adj. $R^2 = 0.23$; std. $\beta = 0.15$; $P = 0.002$).

Sensitivity analyses

Taking into account, our sex-specific results as well as the steroidal nature of endogenous MBG, we performed a sensitivity analyses to determine the possible confounding role of

hormonal contraceptive use on the relationship between cfPWV and MBG in women (Table 5). This association between cfPWV and MBG remained significant (Adj. $R^2 = 0.21$; std. $\beta = 0.14$; $P = 0.005$). Noteworthy, however, was the borderline significant association between cfPWV and hormonal contraceptive use (Adj. $R^2 = 0.21$; std. $\beta = 0.090$; $P = 0.067$). In addition, we performed separate analyses in women who either use or do not use hormonal contraceptives. When plotting cfPWV across quartiles of MBG excretion, we found a significant trend in women not using hormonal contraceptives ($P = 0.005$), and no relationship in those who do use contraceptives ($P = 0.27$; see Figure, Supplemental Digital Content 1, <http://link-s.lww.com/HJH/A981>, demonstrating cfPWV across quartiles of MBG excretion in women who made use of hormonal contraceptives and women who did not). In multiple regression analyses, our results were confirmed in women not using hormonal contraceptives ($N = 217$; Adj. $R^2 = 0.19$; std. $\beta = 0.18$; $P = 0.005$; see Table, Supplemental Digital Content 2, <http://links.lww.com/HJH/A981>, which illustrates multiple regression analyses in women who made use of hormonal contraceptives and women who did not). In women who used contraceptives, the association was lost ($N = 140$; Adj. $R^2 = 0.22$; std. $\beta = 0.06$; $P = 0.50$).

We additionally performed a sensitivity analyses for aldosterone, which is known to influence sodium excretion, to determine whether the relationship between MBG excretion and cfPWV in women is robust (see Table, Supplemental Digital Content 3, <http://links.lww.com/HJH/A981>, demonstrating the sensitivity analyses for aldosterone). We found that the relationship of MBG excretion with cfPWV was not confounded by aldosterone (Adj. $R^2 = 0.23$; std. $\beta = 0.16$; $P = 0.001$), and that aldosterone did not associate with cfPWV (Adj. $R^2 = 0.23$; std. $\beta = -0.013$; $P = 0.81$).

Several studies report the known relationship between NaCl intake and MBG excretion, also confirmed in our population ($r = 0.49$; $P < 0.001$; Fig. 3). We, therefore, performed additional sensitivity analyses to determine if the associations of cfPWV with MBG excretion in women were confounded by NaCl intake (Table 5). In doing so, we found that the association of cfPWV with MBG excretion was independent of NaCl intake (Adj. $R^2 = 0.23$; std. $\beta = 0.14$; $P = 0.012$), confirming the robustness of our results.

DISCUSSION

We investigated whether large arterial stiffness is related to MBG excretion in a young healthy population. This study showed for the first time a positive and independent association between large artery stiffness and MBG excretion in women. In addition, our findings suggest that MBG may contribute to sodium and pressure-independent vascular changes at an early age, in the absence of detected cardiovascular disease.

Intriguingly, it has been previously proposed that large artery stiffness may play a precursory role in the development of hypertension as opposed to being a mere complication thereof [22]. Arterial stiffness characterizes the reduced capability of the vessel wall to expand in response to hemodynamic changes [23]. The relationship between arterial stiffness and MBG in this young population may be attributed to MBG-mediated alterations in the composition of the scaffolding proteins, specifically collagen [7], essential in determining

the mechanical properties of the arterial wall. Indeed, MBG has been shown to stimulate collagen-I synthesis via the Na^+/K^+ -ATPase-PKC δ -Fli-1 signalling pathway [6,7], and has been associated with arterial stiffness in an aged prehypertensive population ($n = 11$) [8]. Importantly, the relevance of our results are underlined in a recently published study demonstrating that arterial stiffness is a strong independent predictor of elevated blood pressure and hypertension in young normotensive adults (aged 38 ± 5 years) [24].

Our findings additionally suggest that women may be more sensitive to the vascular effects of MBG despite having lower urinary MBG excretion compared with men. Although evidence from human studies explaining our sex-specific result is limited, Goel *et al.* [25] demonstrated increased PKC β 2 expression in female rat aorta, previously shown to sensitize Na^+/K^+ -ATPase to MBG. Also, our study supports the role of sex hormones on the relationship between arterial stiffness and MBG, indicated by the sensitivity analyses performed for hormonal contraceptive use in women. The clear finding of an association only in women not using hormonal contraceptives, support the notion that sex hormones may interact with MBG as a steroidal hormone and its association with arterial stiffness, and should be accounted for in future analyses. In spite of the fact that our results add to a restricted body of literature with regards to MBG and sex, we did not specifically explore the relationship between MBG and sex hormones. Therefore, we cannot discuss causality. A more in-depth investigation into the relationship between MBG and sex hormones is imperative.

A strength of this study includes the measurement of arterial stiffness as cfPWV, accepted as the golden standard [26]. Due to the cross-sectional nature of our analyses, the findings of this study should be interpreted within the appropriate context. Also, although urinary MBG was used to reflect plasma MBG levels, the measurement of plasma MBG for future studies would shed further light on the relationship with arterial stiffness.

In conclusion, in a young healthy population, natriuretic MBG may contribute to large artery stiffness early in life via a pressure independent manner, only in women, despite men exhibiting significantly higher estimated 24-h sodium excretion, MBG, and higher arterial stiffness. This article highlights the possible harmful implications of elevated MBG, shown to increase with a high-salt diet, on large artery stiffness, thereby potentially increasing the risk for future CVD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviation:

MBG marinobufagenin

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FIGURE 1.

Displacement of binding 4G4 anti-marinobufagenin monoclonal antibody to marinobufagenin-thyroglobulin conjugate by marinobufagenin, progesterone, drospirenone, ethinyl estradiol, and levonorgestrel in dissociation-enhanced fluoroimmunoassay competitive reverse phase immunoassay. DELFIA, dissociation-enhanced fluoroimmunoassay

FIGURE 2.

Arterial stiffness according to increasing quartiles of marinobufagenin excretion within men (■) and women (●). Black men <mi>; white men <mi>; black women (□), and white women (○). Adjusted for age, waist-to-height ratio, and mean arterial pressure.

FIGURE 3.

Pearson correlation between 24-h urinary marinobufagenin excretion and estimated NaCl intake.

TABLE 1.

Cross-immunoreactivity of 4G4 monoclonal antimarinobufagenin antibody with the components of the contraceptive treatments

Cross-reactants	Cross-reactivity (%)
MBG	100
Progesterone	0.0007 (<0.001)
Drospirenone	0.0014
Ethinyl estradiol	0.0007 (<0.001)
Levonorgestrel	0.0034

MBG, marinobufagenin.

TABLE 2.

Interaction of ethnicity on the relationship between marinobufagenin excretion and arterial stiffness in the total group, men and women

	Marinobufagenin excretion (nmol/day)		
	Total group (N = 711)	Men (N = 296)	Women (N = 415)
cfPWV (m/s) ^a	P = 0.60	P = 0.90	P = 0.35

cfPWV, carotid–femoral pulse wave velocity.

^aAdjusted for mean arterial pressure.

TABLE 3.

Basic characteristics of young men and women

	Men (N = 296)	Women (N = 415)	P
Ethnicity, black, N(%)	145 (49.0)	217 (52.3)	0.39
Age (years)	24.9 ± 2.95	24.8 ± 3.08	0.70
Socio economic status, N(%)			0.27
Low	113 (38.2)	158 (38.0)	
Middle	65 (22.0)	111 (26.8)	
High	118 (39.8)	146 (35.2)	
Anthropometric measurements			
Height (m)	1.75 ± 7.65	1.63 ± 0.07	<0.001
Weight (kg)	76.2 ± 18.6	69.3 ± 16.6	<0.001
BMI (kg/m ²)	24.8 ± 5.32	26.0 ± 6.15	0.008
WC (cm)	83.4 ± 13.1	78.7 ± 13.0	<0.001
Waist-to-height ratio	0.48 ± 0.07	0.48 ± 0.08	0.42
Cardiovascular profile			
24-h SBP (mmHg)	121 ± 8.26	113 ± 8.44	<0.001
24-h DBP (mmHg)	69.8 ± 5.99	68.1 ± 5.36	<0.001
MAP (mmHg)	92.2 ± 7.61	88.7 ± 7.91	<0.001
CfPWV (m/s) ^a	6.71 ± 0.81	5.95 ± 0.79	<0.001
24-h urinary profile			
Volume (l/24-h)	1.42 ± 0.75	1.38 ± 0.83	0.51
MBG conc. (nmol/l)	3.29 (1.26; 7.23)	2.26 (0.70; 5.79)	<0.001
MBG exc. (nmol/day)	4.13 (1.46; 10.2)	2.69 (0.92; 7.92)	<0.001
MBG/Na ⁺ ratio	0.03 (0.01; 0.08)	0.02 (0.01; 0.06)	<0.001
Na ⁺ exc. (mmol/day)	141 (41.1; 360)	123 (45.8; 326)	0.003
NaCl intake (g/day)	8.32 (2.42; 21.2)	7.27 (2.70; 19.2)	0.003
K ⁺ exc. (mmol/day)	41.6 (12.6; 104)	39.3 (15.9; 101)	0.35
Na:K ratio	3.41 (1.36; 7.14)	3.22 (1.40; 6.78)	0.15
Albumin (mg/l)	3.55 (2.21; 8.13)	4.15 (2.32; 11.1)	<0.001
Creatinine (mmol/l)	9.85 (4.55; 20.2)	7.72 (3.08; 16.3)	<0.001

	Men (N = 296)	Women (N = 415)	P
Biochemical profile			
Glucose (mmol/l)	4.82 ± 0.78	4.64 ± 0.66	0.002
HDL-C (mmol/l)	1.15(0.77; 1.74)	1.33 (0.81; 2.13)	<0.001
LDL-C (mmol/l)	2.75 (1.54; 4.57)	2.61 (1.50; 4.25)	0.070
Trig (mmol/l)	0.90 (0.45; 2.05)	0.76 (0.39; 1.67)	<0.001
CRP (mg/l)	0.73 (0.10; 5.94)	1.38 (0.13; 12.3)	<0.001
Interleukin-6 (pg/ml)	0.92 (0.34; 3.29)	1.13 (0.35; 3.98)	<0.001
Aldosterone (pg/ml)	63.7 (17.5; 203)	73.1 (17.1; 425)	0.055
Lifestyle measures			
Smoking, N(%)	101 (34.2)	57 (13.7)	<0.001
Cotinine >10 (ng/ml)	85 (35.3)	54 (16.3)	<0.001
Alcohol intake, N(%)	181 (61.8)	207 (50.4)	0.003
GGT (U/l)	26.0 (12.8; 66.2)	18.1 (7.80; 54.0)	<0.001
Hormonal contraception, N(%)	—	163 (40.3)	

Arithmetic mean ± standard deviation; geometric mean (5th percentile; 95th percentile intervals). cPWV, carotid-femoral pulse wave velocity; CRP, C-reactive protein; GGT, γ -glutamyl transferase; HDL-

C, high-density lipoprotein cholesterol; K⁺, potassium; MAP, mean arterial pressure; MBG, marinobufagenin; Na⁺, sodium; Trig, triglycerides; WC, waist circumference.

^a Adjusted for MAP.

TABLE 4.

Single and partial regression analyses with carotid–femoral pulse wave velocity as dependent variable

	MBG excretion (nmol/day)	
	Women	Men
Age (years)	$r = -0.11$; $P = 0.03$	$r = -0.03$; $P = 0.64$
Waist-to-height ratio	$r = -0.06$; $P = 0.20$	$r = 0.13$; $P = 0.024$
Cardiovascular measures		
cfPWV (m/s) ^a	$r = 0.17$; $P = 0.003$	$r = 0.04$; $P = 0.46$
Adjusted for ethnicity, age and waist to height ratio cfPWV (m/s) ^a	$r = 0.19$; $P < 0.001$	$r = 0.07$; $P = 0.26$

cfPWV, carotid–femoral pulse wave velocity; MBG, marinobufagenin.

^aAdjusted for mean arterial pressure.

TABLE 5.
Multiple regression analyses with carotid–femoral pulse wave velocity as dependent variable

	Carotid–femoral pulse wave velocity (m/s)			
	Women		Men	
	β (SE)	P	β (SE)	P
Adj. R^2	0.23		0.23	
MBG (nmol/day)	0.150 (0.048)	0.002	0.062 (0.061)	0.31
Age (years)	0.259 (0.049)	<0.001	0.225 (0.062)	<0.001
MAP (mmHg)	0.441 (0.052)	<0.001	0.367 (0.064)	<0.001
Waist-to-height ratio	−0.232 (0.058)	<0.001	−0.158 (0.080)	0.048
Ethnicity (black/white)	0.063 (0.055)	0.23	0.011 (0.075)	0.88
CRP (mg/l)	−0.059 (0.055)	0.28	0.070 (0.065)	0.28
GGT (U/l)	0.047 (0.053)	0.38	0.096 (0.064)	0.14
HDL-C (mmol/l)	−0.043 (0.051)	0.40	−0.050 (0.063)	0.43
Glucose (mmol/l)	0.034 (0.049)	0.48	0.007 (0.069)	0.92
Sensitivity analyses for hormonal contraceptive use ^a				
Adj. R^2	0.21			
MBG (nmol/day)	0.139 (0.050)	0.005	—	
Hormonal contraceptives (yes/no)	0.090 (0.049)	0.067	—	
Sensitivity analyses for estimate NaCl intake ^a				
Adj. R^2	0.23		0.16	
MBG (nmol/day)	0.142 (0.056)	0.012	0.033 (0.075)	0.66
NaCl intake (g/day)	0.021 (0.056)	0.70	0.045 (0.073)	0.74

Adj., adjusted; cPWV, carotid–femoral pulse wave velocity; CRP, C-reactive protein; GGT, γ -glutamyl transferase; HDL-C, high-density lipoprotein cholesterol; MAP, mean arterial pressure; MBG, marinobufagenin.

^aMultiple regression models for sensitivity analyses are additionally adjusted for age, ethnicity, MAP, waist-to-height ratio, HDL, CRP, GGT, and glucose.