HHS Public Access

Author manuscript

Hypertens Res. Author manuscript; available in PMC 2018 June 22.

Published in final edited form as:

Hypertens Res. 2018 March ; 41(3): 183–192. doi:10.1038/s41440-017-0009-x.

Marinobufagenin is related to elevated central and 24-h systolic blood pressures in young black women: the African-PREDICT Study

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Abstract

Marinobufagenin (MBG) is an endogenous steroidal α1-Na^+K^+-ATPase inhibitor. Because of its role in sodium handling, MBG has been associated with both antihypertensive and prohypertensive effects in normal physiology and pathology. MBG is positively associated with blood pressure in Dahl salt-sensitive rats exhibiting a similar hypertensive phenotype to black populations, characterized by impaired urinary Na^+ excretion. However, clinical studies exploring blood pressure (BP)-related effects of MBG in black populations are scant. We determined whether the MBG/Na^+ ratio (assessing the effectiveness of Na^+ excretion resistance to MBG) is related to systolic BP (SBP) in young black men and women, compared to whites. We included 331 apparently healthy participants (20–30 years) (42.9% black, 43.8% men) on a habitual diet. We obtained 24-h and central SBP, and 24-h urinary Na^+ and MBG levels. We found no ethnic differences in MBG, Na^+ or MBG/Na^+. MBG excretion correlated positively with Na^+ excretion in all groups and to SBP in white men and black women ($p \leq 0.011$). In black women only SBP related positively to MBG/Na^+ in single and multi-variable adjusted regression models: central SBP ($R^2 = 0.26$; $ß = 0.28$; $p = 0.039$), 24-h SBP ($R^2 = 0.46$; $ß = 0.30$; $p = 0.011$), daytime ($R^2 = 0.38$; $ß = 0.28$; $p = 0.023$) and nighttime SBP ($R^2 = 0.38$; $ß = 0.33$; $p = 0.009$). In contrast, inverse associations of MBG/Na^+ with nighttime SBP were evident in white women ($r = -0.20$; $p = 0.038$) but lost significance after multiple adjustments ($R^2 = 0.36$; $ß = -0.13$; $p = 0.12$). We found independent positive associations of SBP with MBG/Na^+ in black women. This data supports the concept that reduced MBG-mediated Na^+ excretion can contribute to adverse hemodynamics.

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Electronic supplementary material The online version of this article (https://doi.org/10.1038/s41440-017-0009-x) contains supplementary material, which is available to authorized users.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Introduction

Hypertension contributes to an increased risk of cardiovascular morbidity and mortality [1]. Black populations are particularly predisposed to hypertension in part due to a genetic susceptibility to retain more sodium [2–4] with concurrent volume expansion [5]. Indeed, excessive sodium has been shown to influence central hemodynamics [6]. Sodium-induced plasma volume expansion stimulates the production of the endogenous steroidal sodium pump ligand, marinobufagenin (MBG) [7], as a compensatory natriuretic mechanism [8]. MBG has been implicated in the well-known relationship between sodium handling and blood pressure (BP) regulation via the inhibition of both renal and cardiovascular α1-Na^+K^+-ATPase [8–11].

A sustained increase in MBG production, along with an elevated BP and diminished natriuresis, have been noted in Dahl salt-sensitive rats with genetically impaired pressure-natriuresis [8]. At the scenario of salt-sensitivity, a blunted natriuretic response of the kidneys stimulates the production of the natriuretic hormone MBG; excessive plasma MBG levels add to an increase in the tone of vascular smooth muscle cells [8, 11], thereby increasing total peripheral vascular resistance (TPR). The aforementioned is consistent with findings that excessive MBG is related to increased BP in middle-aged individuals ($n = 20$) [12] as well as arterial stiffness in older salt-sensitive hypertensive subjects ($n = 11$) [9]. Dahl salt-sensitive rats exhibit a similar genetic predisposition to the hypertension phenotype characterized by a low fractional sodium excretion [8, 11] also observed in black populations [2–4]. Essentially the genetic predisposition of blacks to reabsorb more sodium in the proximal tube [4] might override the compensatory natriuretic activity of MBG [8, 11]. Notably, prior studies conducted in small cohorts of middle-aged white individuals reported contrasting sex-specific relationships of MBG with systolic BP (SBP) [10, 12]. They demonstrated that MBG was positively related to SBP in men [12], whereas a negative association was observed in women ($n = 28$) [10]. These sex-specific patterns have yet to be investigated in a young black population.

This study presents the first findings on 24-h urinary MBG, central SBP (cSBP), 24-h BP and hemodynamic parameters in a young black ($n = 142$) population, in comparison to a homogenous white population ($n = 189$). We hypothesized that due to less efficient sodium handling in blacks [2–4], higher levels of sodium will continue to drive MBG production with a resultant higher MBG/Na^+ ratio. This prompts an increase in BP due to MBG's vaso-constrictive effect at high levels. To address this hypothesis we measured 24-h urinary sodium and MBG excretion, as well as cSBP and 24-h SBP in young black and white adults. Our investigation of MBG in this young bi-ethnic population sample ($n = 331$) might provide insight into the role of MBG prior to the onset of pathology. We calculated the MBG/Na^+ excretion ratio as an estimate of the effectiveness of MBG in Na^+ excretion, i.e., Na^+ excretion resistance to higher levels of urinary MBG.

Methods

Protocol and participants

The protocol of the African Prospective study on the Early Detection and Identification of Cardiovascular disease and Hypertension (African-PREDICT) study was approved by the Health Research Ethics Committee of the North-West University in South Africa. All procedures were in adherence with institutional guidelines and the Declaration of Helsinki.

The African-PREDICT study is an ongoing prospective study that will recruit 1200 black and white, men and women (20–30 years of age) and perform follow-up measurements for 10–20 years. For the purpose of this sub-study, the data of the first 331 consecutively enrolled participants with complete 24-h urinary collection were analyzed cross-sectionally. Participant recruitment took place on a voluntary basis, where black and white individuals from communities in and around the Potchefstroom area were invited to take part in the study. Participants were informed about the objectives and procedures of the study before enrollment, after which all participants gave informed consent. Individuals were thereafter screened for inclusion into the African-PREDICT study. Participants were included into the study provided they were normotensive based on clinic BP (<140/90), HIV uninfected and self-reported not previously diagnosed with any chronic diseases (diabetes, tuberculosis, cancer, cardiac or renal disease). Additional inclusion criteria were as follow: glucose <5.6 mmol/L, ear temperature ≤37.5° and spot urine microalbuminuria <30 mg/ml. Participants who were enrolled in the study were not on any antihypertensive medication or any other medication for chronic disease. None of the women included into the study were breast feeding or pregnant.

Organizational procedures

Information leaflets were provided and discussed with participants prior to the day on which the study measurements commenced. Participants willing to take part in the African-PREDICT study were transported to the Hypertension Clinic, arriving at approximately 08:00, where they were familiarized with the research environment and experimental setup. All measurements were explained and performed by trained researchers. After the informed consent was given, general health questionnaires were completed to obtain information on socio–economic status, smoking and alcohol intake, as well as the self reported use of oral contraceptives or the contraceptive injection-depot medroxyprogesterone acetate.

Anthropometric measurements

Body height (SECA 213 Portable Stadiometer (SECA, Hamburg, Germany)), weight (SECA 813 Electronic Scales (SECA, Hamburg, Germany)) and waist circumference (Lufkin Steel Tape; W606PM; Lufkin, Apex, USA) were measured according to the guidelines of the International Society for the Advancement of Kinanthropometry by an anthropometrist using calibrated instruments [13]. We calculated body mass index (BMI) (weight (kg)/height (m^2)) and waist:height ratio.

Cardiovascular measurements

cSBP was measured in duplicate, non-invasively using the Sphygmocor XCEL device (AtCor Medical Pty. Ltd., Sydney, Australia). Participants were fitted with an appropriate sized brachial cuff and requested to lie in a supine position for approximately 5 min before commencement of the measurement.

Beat-to-beat hemodynamic measurements were taken non-invasively using a validated Finometer device (FMS, Finapres Measurement Systems, Amsterdam, Netherlands), computing precise values based on the non-linear three element model [14]. These measurements included heart rate, stroke volume and total peripheral resistance. Participants lay in the Fowler's position with their arm at heart level, connected to a brachial BP cuff as well as a finger cuff. Following a resting period the apparatus was calibrated for a 2-min period to provide a subject-level individual adjustment of the finger arterial pressure with the brachial artery pressure, after which continuous hemodynamic measurements were taken over a 5-min period.

CardioXplore devices (Meditech, Budapest, Hungary, British Hypertension Society validated) were used to obtain ambulatory BP (ABPM) data over a 24 h time period. Participants were fitted with an appropriate sized cuff and instructed to be relaxed while measurements were taken. BP readings were recorded in 30-min intervals during the day (08:00–22:00) and hourly at night (22:00–06:00). An ambulatory diary card was distributed and completed during the duration of the measurements to report any abnormalities. ABPM was repeated if measurements were not successful and readings did not meet the pre-specified parameter criteria, with at least 70% of the measurements being successful or having at least 20 valid daytime together with 7 nighttime measures [15].

Biological sample collection and biochemical analyses

All participants were on a habitual diet. Participants refrained from eating or drinking, except for water, approximately 8–10 h prior to biological sampling. Participants were then asked to collect all urine that they passed over a 24-h time period. To verify the completeness of the 24-h urine samples we used the following cut-off points: the volume of the 24-h urine collections <500 ml and urinary creatinine <4.0 mmol/day for women or <6.0 mmol/day for men [16]. Biological samples (serum, plasma, whole blood and urine) were stored in cryovials and kept in biofreezers at −80 °C. High-density lipoprotein cholesterol (HDL-C), total cholesterol (TC), and γ-glutamyltransferase (GGT) were determined in serum whereas the percentage of glycated hemoglobin (HbA1c) was analyzed in EDTA whole blood. Twenty-four-hour urinary sodium and potassium were determined using ion-selective electrodes. Estimated NaCl intake was subsequently derived from the 24-h urinary sodium excretion. All of the aforementioned analyses were performed using the Cobas Integra 400plus (Roche, Basel, Switzerland). Aldosterone was determined using the Radioimmunoassay Aldosterone Kit (Beckman Coulter, Immunotech, Radiova, Czech Republic). Twenty-four-hour urinary MBG concentrations were measured using a solid-phase DELFIA (Dissociation-Enhanced Lanthanide Fluorescent Immunoassay) fluoroimmunoassay based on 4G4 anti-MBG mouse monoclonal antibody, as reported in detail by Fedorova et al. [9, 17].

Statistical analyses

Statistical analyses were performed using Statistica v13.0 (Statsoft Inc., Tulsa, USA, 2010). Normally distributed data was presented as mean ± standard deviation. Non-Gaussian distributed data were logarithmically transformed, with the central tendency and spread of these variables represented as geometric means with 5th and 95th percentile intervals. We performed interaction testing using multiple regression analysis to determine the potential influence of sex and ethnicity on the relationship between MBG/Na^+ and SBP. Subsequent group divisions were made; where means and proportions were compared between groups using independent *t*-tests and χ^2 tests, respectively. Analyses of covariance were performed to determine significant differences in BP across MBG/Na^+ quartiles within groups, adjusting for age and waist:height ratio. We determined the relationship between urinary MBG/Na^+ ratio and hemodynamic measurements using Pearson-regression analysis, partial-regression analysis and multiple regression analysis with MBG/Na^+ as the main independent variable. We included various cardiovascular variables including cSBP, 24-h ABPM, stroke volume and TPR as dependent variables in separate models. Several covariates were considered as possible independent variables based on the strongest bivariate associations with the dependent and independent variables. We finally included age, waist:height ratio, GGT, HbA1c, and TC:HDL in multiple regression models. $P < 0.05$ was considered as statistically significant. We additionally performed several sensitivity analyses with socioeconomic status, contraceptive use, aldosterone and potassium [18] included into multiple regression models, so to determine whether associations with the MBG/Na^+ ratio were confounded by these factors.

Results

Participant characteristics

We found an interaction of sex on the relationship between cSBP and MBG/Na^+ excretion ratio in the total group ($N = 331$; $p = 0.027$). In all women ($N = 186$) we also found an interaction of ethnicity on the associations between cSBP or 24-h SBP with MBG excretion ($p = 0.047$ and $p = 0.035$) as well as urinary MBG/Na^+ ratio ($p = 0.010$ and $p = 0.012$). These interactions of ethnicity were absent in black and white men ($p = 0.49$ and $p = 0.89$), respectively. Based on the focus of this paper, participants were therefore subsequently stratified by sex and ethnicity. Table 1 provides the characteristics of the study population. All anthropometric measures were higher in white men compared to black men, whereas black women had a larger BMI, waist circumference and waist:height ratio than white women. In terms of the cardiovascular profile, black men and women displayed a higher cSBP and TPR, and lower stroke volume than whites. However, black men displayed lower systolic ABPM measurements than white men. A collective mean 24-h salt intake of 8.44 g/day, calculated as a total excreted 24 h NaCl, was found in all four groups which exceeds the World Health Organization's daily recommendation of less than 5 g/day [19]. There were no apparent ethnic differences in MBG excretion ($p \geq 0.16$) or MBG/Na^+ ($p \geq 0.067$) in men or women, but both black ($p = 0.005$) and white men ($p < 0.001$) had a higher salt intake as well as MBG excretion compared to women. Evidently, black men and women had a greater Na^+/K^+ ratio when compared to their white counterparts ($p < 0.001$), as a result of

significantly lower potassium excretion. Both black men and women had significantly lower aldosterone levels compared to their white counterparts.

Regression analyses

Single regression analysis for women and men is shown in the Supplementary Table 1 and Supplementary Table 2, with the main findings with regards to MBG and the MBG/ Na^+ ratio presented in Fig. 1a–f. As expected, 24-h urinary MBG excretion correlated positively with Na^+ excretion in men and women indicative of its natriuretic function (Fig. 1a, b). However, no significant correlation existed between 24-h SBP and Na^+ excretion in black and white, men ($r = 0.19$; $p = 0.13$)($r = 0.21$; $p = 0.068$) and women ($r = -0.03$; $p = 0.79$)($r = 0.09$; $p = 0.37$)(not shown). Twenty-four-hour SBP ($r = 0.24$; $p = 0.038$) and daytime SBP ($r = 0.23$; $p = 0.050$) correlated significantly with MBG excretion alone in white men and black women, respectively. We found no relationship between 24 h urinary MBG excretion and aldosterone in women or in white men, although a correlation existed in young black men ($r = 0.35$; $p = 0.011$). This finding merits further investigation, it may indicate deep relations between these two steroids in their pro-hypertensive action [20]. The results of the regression analyses with regards to the MBG/Na^+ excretion ratio will mainly be discussed in young women as we found no statistically significant associations with cardiovascular variables in either black or white young men. Only in young black women the urinary MBG/Na^+ ratio was positively related to cSBP ($r = 0.25$; $p = 0.034$), daytime ($r = 0.23$; $p = 0.047$) and nighttime ($r = 0.24$; $p = 0.045$) systolic ABPM (Supplementary Table 1). In young white women there was a negative association of MBG/Na^+ excretion ratio with nighttime SBP ($r = -0.20$; $p = 0.038$).

When comparing SBP according to MBG/Na^+ quartiles (Fig. 2), black women exhibited a significant positive trend in cSBP ($p = 0.003$) as well as nighttime systolic ABPM ($p = 0.013$) with increasing MBG/Na^+ quartiles. Additionally all the SBP measurements of young black women were higher within the highest urinary MBG/Na^+ ratio quartile when compared to white women, as seen in Fig. 2. We found no significant trends in men. Adjusting for age and waist:height ratio we compared 24-h urinary MBG excretion across increasing quartiles of NaCl intake. It was evident that MBG excretion increased significantly along with an increase in NaCl intake within black and white, men and women (Supplementary Figure 1). Regardless, no correlation between NaCl intake and SBP was demonstrated in this study, with the exception of night-time ABPM and NaCl intake correlations in white men.

To determine the independent associations between SBP and MBG/Na^+ excretion ratio in women we performed multiple regression analyses adjusting for potential con-founders, as shown in Table 2. Only in black women we found independent positive associations of cSBP ($R^2 = 0.26$; $ß = 0.28$; $p = 0.039$), 24 h SBP ($R^2 = 0.46$; $ß = 0.30$; $p = 0.011$), daytime ($R^2 = 0.38$; $ß = 0.28$; $p = 0.023$) and nighttime SBP ($R^2 = 0.38$; $ß = 0.33$; $p = 0.009$) with MBG/Na^+ excretion ratio, confirming previous unadjusted associations. In addition, TPR ($ß = -0.33$; $p = 0.018$) and stroke volume ($ß = 0.29$; $p = 0.036$) were significantly explained by MBG/Na^+. In contrast, non-significant inverse associations of all SBP measures with the MBG/Na^+ excretion ratio were evident in white women. Nonetheless independent

associations of all hemodynamic measures with MBG/Na^+ were absent in young black and white men (not shown).

Sensitivity analyses

We performed a sensitivity analyses to investigate whether the associations between cSBP and 24 h SBP with the MBG/Na^+ excretion ratio were confounded by socioeconomic status of participants. In doing so, these results confirmed the robustness of our associations, which remained significant in black women (Supplementary Table 3). Due to the steroidal nature of MBG, additional sensitivity analyses were performed for the use of contraceptives in black and white young women. The association of nighttime SBP with the MBG/Na^+ excretion ratio remained significant in black women (R^2 = 0.38, ß = 0.30, p = 0.026), and confirmed no association in white women (R^2 = 0.36, ß = −0.16, p = 0.11). After repeating the same analysis with the cSBP, the association with MBG/Na^+ became borderline significant in black women (R^2 = 0.26, ß = 0.24, p = 0.082) and remained non-significant in white women. Moreover, potassium and aldosterone are known factors influencing BP. Therefore, in order to corroborate the independent association of SBP with MBG/Na^+ excretion in black women, sensitivity analyses were done with potassium and aldosterone, respectively, included as independent variables in multiple regression models with cSBP (p = 0.59)(p = 0.63) or 24 h SBP (p = 0.75)(p = 0.10) as dependent variables. Evidently, associations of cSBP (R^2 = 0.25, ß = 0.27, p = 0.046)(R^2 = 0.25, ß = 0.29, p = 0.037) and 24 h SBP (R^2 = 0.45, ß = 0.30, p = 0.012)(R^2 = 0.48, ß = 0.27, p = 0.018) with MBG/Na^+ excretion remained significant.

Discussion

We found that cSBP, as well as daytime and nighttime SBP, related positively and independently with a proposed estimate of Na^+ excretion resistance to elevated MBG levels, namely the 24-h urinary MBG/Na^+ ratio, in young black, but not white women. Additionally, we found no significant relationship between different measures of SBP and MBG/ Na^+ ratio in either black or white men. Ours is the first known study highlighting ethnic as well as sex differences with associations between MBG/Na^+, SBP, stroke volume and vascular resistance in a large young bi-ethnic population.

Differences observed in the etiology of cardiovascular pathophysiology between black and white populations are complex. We have recently published a detailed review on the advances in understanding the distinct physiological, lifestyle and demographic differences between black and white populations, including dietary, cultural and physiological differences [21] (e.g., suppressed renin [22], increased arterial stiffness [23–25], sympathetic nerve activity [26, 27] and an increased inflammatory profile [28, 29] in black populations). Thus, the results from the present study reflect one aspect of an entire array of factors collectively contributing to an adverse cardiovascular pro-file, specifically observed in the black women of this study, and should therefore be interpreted accordingly. The potential role of salt-sensitivity in the contribution to SBP in black populations, however, remains a pivotal physiological area where an increased understanding is required. Therefore, these

new findings on an independent association between SBP and MBG, only in black women, are significant in this field.

To our best knowledge, a single reference to MBG and ethnicity was published in 2001, suggesting that higher urinary MBG was indicative of volume expansion in black women [30]. Our results add to these findings in black women [30], suggesting that a blunted natriuretic response to MBG in the presence of relatively high salt intake could initiate a volume-related increase in SBP. The positive association between stroke volume and MBG/Na^+ ratio in black women, may reflect the initiation of relative sodium retention and sodium-induced volume expansion. Accordingly, blood vessels distend as a compensatory homeostatic mechanism, and although speculative, may explain the inverse association observed with MBG excretion ($R^2 = 0.19$, $ß = -0.28$, $p = 0.039$) or MBG/Na^+ and TPR in black women. Thus, despite our initial hypothesis, a higher TPR in black women compared to white women may not be attributed to MBG/Na^+ at this young age. Over time however, this ability to preserve homeostasis is lost.

We found clear contrasting results in white women (Figs. 1c, e). A significant inverse association existed between nighttime SBP and MBG/Na^+ in single regression, that became weaker after adjusting for various covariates ($\beta = -0.13$, $p = 0.12$). This natriuretic tendency is in accordance with the normal physiological response expected from a renal α1-Na^+K^+-ATPase inhibitor, similarly demonstrated by Anderson et al. [10]. In addition, white women consume more potassium compared to black women as indicated by the potassium excretion and lower Na^+/K^+ excretion ratio, likely due to greater dietary fruit and vegetables intake [31]. Potassium has a known BP attenuating effect counterbalancing the effect of a high salt diet [32]. Another reasoning for different relationships with MBG/Na^+ in black and white women may be due to the contribution of a more adverse metabolic profile observed in black women in our study (increased adiposity, glycated hemoglobin and GGT compared to white women). Why our findings are mainly prevalent in women, is yet unclear.

Our study supports the confounding role of sex hormones in the relationship between MBG/Na^+ and SBP, indicated by the sensitivity analyses for contraceptive use. As this study did not include an exploration of the relationship between MBG and sex hormones, but only observed sex differences in the association of MBG/Na and SBP, we cannot provide a precise physiological reason, even if speculative for these differences. However, the results from this study substantiate the need to investigate the relationship between MBG and sex hormones in future studies.

Apart from these findings in women, based on the literature we did expect a strong relationship between sodium, MBG and SBP [8, 11, 33] in men [9, 12]. A positive correlation (single regression analyses) between 24 h SBP and MBG excretion ($r = 0.24$; $p = 0.038$) was evident in the young white men from our study, indicative of the contribution of MBG to increased SBP in white men (Fig. 1d). The absence of associations between MBG/Na^+, SBP and hemodynamic measures in fully adjusted models were surprising since both black and white men had a significantly higher MBG excretion compared to women, likely due to greater salt intake or higher body weight.

A strength of our study was the parallel tendencies observed in cSBP and 24-h SBP and supported by stroke volume and TPR hemodynamics, as indicated by three independent BP devices. As this is a cross-sectional study, it limits our ability to examine the physiological sequence of hemodynamic changes as a result of the variability in the levels of MBG in a young healthy population. Therefore, our results reflect only associations between the MBG/Na^+ excretion ratio and hemodynamic variables and should be interpreted as such. Albeit, the longitudinal design of the African-PREDICT study will allow us to monitor the progression of cardiovascular changes associated with MBG/ Na^+ in black and white, men and women. We were not able to take into account salt-sensitivity within each group, since this was not an intervention study. Because the positive correlation between MBG and salt intake was demonstrated in all groups, we acknowledge that it might be of interest to measure plasma MBG in addition to urinary MBG.

To conclude, in young black women the MBG/Na^+ excretion ratio associated with an increase in cSBP, 24-h SBP and stroke volume, which might reflect an increased cardiovascular risk due to abnormal sodium handling. Conversely, the inverse correlation between MBG/Na^+ and nighttime SBP in young white women supports the concept of the expected natriuretic effect of MBG in a young healthy population. Future studies should further investigate the potential role of MBG or the MBG/Na^+ excretion ratio as an alternative mechanism possibly contributing to the prevalence of salt-sensitive hypertension especially in black populations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors of this study are grateful towards all individuals participating voluntarily in the study. The dedication of the support and research staff as well as students at the Hypertension Research and Training Clinic at the North-West University is also duly acknowledged.

Funding The research funded in this manuscript is part of an ongoing research project financially supported by the South African Medical Research Council (SAMRC) with funds from National Treasury under its Economic Competitiveness and Support Package; the South Afri-can Research Chairs Initiative (SARChI) of the Department of Science and Technology and National Research Foundation (NRF) of South Africa; as well as corporate social investment grants from Pfizer (South Africa), Boehringer Ingelheim (South Africa), Novartis (South Africa), the Medi Clinic Hospital Group (South Africa) and in kind contributions of Roche Diagnostics (SA). Any opinion, findings, and conclusions or recommendations expressed in this material are those of the authors, and therefore, the NRF does not accept any liability in regard. This study was supported in part by the Intramural Research program, National Institute on Aging, National Institutes of Health, USA.

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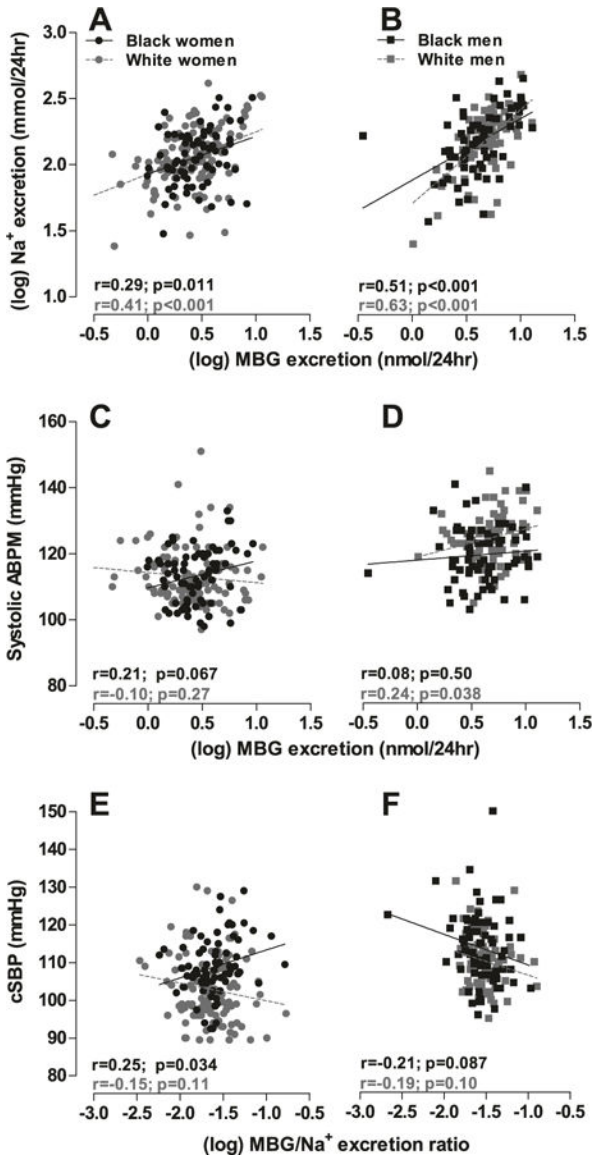

Fig. 1.

Ethnic and sex differences for the relationships between Na^+ and MBG excretion (a, b); 24 h SBP and MBG excretion (c, d); and cSBP and MBG/Na^+ excretion ratio (e, f)

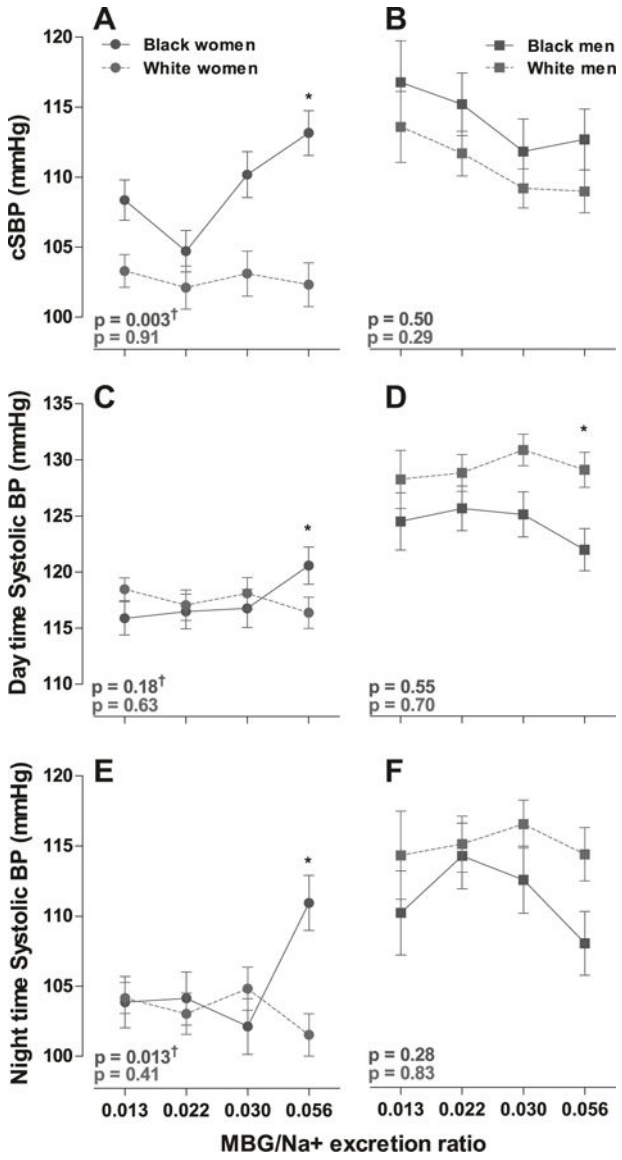

Fig. 2.

Ethnic differences in cSBP (a, b); daytime systolic BP (c, d) and nighttime systolic BP (e, f) within MBG/Na^+ quartiles (adjusted for age and waist:height ratio). *Significant differences ($P < 0.05$) between black and white participants within the same MBG/Na^+ quartile. †Significant differences ($P < 0.05$) between 1st and 4th quartiles within group

Table 1

Basic characteristics of young black and white, men and women

	Men			**Women**		
	***N* = 145**			***N* = 186**		
	Black	**White**	***P***	**Black**	**White**	***P***
N (%)	68 (46.9)	77 (53.1)		74 (39.8)	112 (60.2)	
Age (years)	24.1 ± 3.24	25.7 ± 2.76	0.002	24.3 ± 3.64	25.6 ± 2.78	0.008
Socio economic status, *N*(%)			<0.001			<0.001
Low	48 (70.6)	9 (11.7)		47 (63.5)	6 (5.4)	
Middle	9 (13.2)	11 (14.3)		21 (28.4)	25 (22.3)	
High	11 (16.2)	57 (74)		6 (8.1)	81 (72.3)	
Smoking, *N*(%)	26 (48.2)	15 (21.7)	0.002	9 (14.5)	12 (12)	0.64
Alcohol intake, *N*(%)	37 (68.5)	47 (68.1)	0.26	33 (53.2)	64 (64)	0.025
Contraception, *N*(%)	–	–	–	31 (41.9)	41 (36.6)	0.47
Anthropometric measurements						
Height (m)	1.69 ± 0.06	1.79 ± 0.06	<0.001	1.59 ± 0.07	1.68 ± 0.06	<0.001
Weight (kg)	62.1 (47.8; 88.0)	87.4 (63.2; 124)	<0.001	65.8 (46.7; 96.3)	66.9 (52.9; 102)	0.59
Body mass index (kg/m^2)	21.7 (17.1; 30.1)	27.3 (20.2; 40.4)	<0.001	25.9 (18.5; 39.9)	23.7 (18.3; 34.9)	0.004
Waist circumference (m)	0.73 (0.63; 0.95)	0.90 (0.70; 1.22)	<0.001	0.78 (0.63; 1.03)	0.75 (0.63; 1.03)	0.029
Waist:Height ratio	0.44 (0.38; 0.55)	0.50 (0.40; 0.67)	<0.001	0.49 (0.39; 0.64)	0.44 (0.38; 0.62)	<0.001
Clinic blood pressure						
bSBP (mmHg)	125 ± 11.6	125 ± 8.32	0.72	115 ± 9.32	109 ± 9.85	<0.001
bDBP (mmHg)	81.1 ± 8.25	80.2 ± 6.76	0.45	78.5 ± 7.29	74.9 ± 6.91	<0.001
cSBP (mmHg)	113 ± 9.63	110 ± 7.24	0.013	108 ± 7.56	103 ± 8.67	<0.001
24-h Blood pressure						
SBP (mmHg)	119 ± 8.40	125 ± 7.28	<0.001	113 ± 8.17	113 ± 8.46	0.83
Day	124 ± 8.69	129 ± 7.78	<0.001	117 ± 8.17	117 ± 8.75	0.66
Night	111 ± 10.4	115 ± 9.47	0.014	105 ± 9.53	103 ± 8.93	0.26
DBP (mmHg)	69.1 ± 6.27	70.9 ± 6.09	0.098	68.8 ± 5.07	68.3 ± 5.56	0.53
Day	73.9 ± 6.62	75.7 ± 6.73	0.12	72.8 ± 5.28	73.2 ± 5.84	0.62
Night	59.6 ± 7.39	61.4 ± 7.55	0.16	60.7 ± 6.33	58.6 ± 6.03	0.027

	Men			Women		
	N = 145			*N* = 186		
	Black	**White**	***P***	**Black**	**White**	***P***
Beat-to-beat cardiovascular measurements						
Heart rate (bpm)	57.7 ± 7.55	62.3 ± 9.94	0.002	69.7 ± 9.08	66.9 ± 10.1	0.063
Stroke volume (ml)	81.4 (52.5; 119)	109 (82.3; 157)	<0.001	75.1 (49.2; 103)	85.8 (62.7; 129)	<0.001
TPR (mmHg/ml/s)	1.34 (0.88; 2.04)	0.88 (0.54; 1.36)	<0.001	1.19 (0.82; 1.80)	1.01 (0.67; 1.56)	<0.001
24-h Urinary profile						
Volume (L/24 h)	1.19 (0.55; 2.46)	1.34 (0.56; 3.06)	0.12	1.12 (0.55; 2.06)	1.13 (0.56; 2.95)	0.82
MBG conc. (nmol/L)	3.36 (1.58; 7.49)	3.49 (1.59; 6.94)	0.65	2.53 (0.88; 5.93)	2.28 (0.81; 5.93)	0.16
MBG exc. (nmol/24 h)	3.99 (1.92; 10.2)	4.69 (1.87; 10.1)	0.067	2.82 (1.29; 6.45)	2.52 (0.81; 7.86)	0.20
Na^+ excretion (mmol/24 h)	146 (54.8; 324)	152 (51.7; 287)	0.61	114 (50.5; 270)	114 (47.6; 257)	0.95
MBG/Na^+ excretion ratio	0.03 (0.01; 0.06)	0.03 (0.02; 0.07)	0.16	0.02 (0.01; 0.07)	0.02 (0.01; 0.07)	0.24
NaCl intake (g/24 h)	8.52 (3.20; 18.6)	8.91 (3.02; 16.8)	0.61	6.65 (2.78; 15.0)	6.68 (2.95; 15.8)	0.95
K^+ excretion (mmol/24 h)	34.2 (12.6; 83.0)	49.7 (15.6; 105)	<0.001	30.7 (14.3; 67.6)	39.3 (17.3; 81.0)	0.001
Na:K ratio	4.26 (2.17; 7.14)	3.10 (1.21; 6.60)	<0.001	3.72 (1.73; 7.62)	2.86 (1.41; 5.68)	<0.001
Albumin (mg/24 h)	4.19 (1.53; 16.2)	3.87 (1.58; 10.4)	0.49	4.72 (1.98; 11.8)	3.98 (1,19; 12.3)	0.11
Biochemical profile						
Total cholesterol (mmol/L)	3.77 (2.80; 5.10)	4.58 (3.29; 5.95)	<0.001	3.84 (2.70; 5.35)	4.68 (3.42; 6.50)	<0.001
HDL-C (mmol/L)	1.33 (0.84; 1.82)	1.09 (0.77; 1.57)	<0.001	1.81 (0.79; 1.88)	1.57 (1.03; 2.48)	<0.001
HbA1c (%)	5.48 ± 0.28	5.28 ± 0.24	<0.001	5.52 ± 0.31	5.29 ± 0.27	<0.001
γ-GT (U/L)	27.5 (13.0; 76.6)	24.3 (10.8; 65.4)	0.28	24.3 (10.5; 57.4)	14.1 (6.70; 40.7)	<0.001
Aldosterone (pg/ml)	61.3 (16.8; 155)	88.5 (30.8; 308)	0.004	70.1 (28.1; 224)	104 (28.4; 614)	0.003

Mean ± standard deviation; geometric mean (5 percentile; 95 percentile)

bSBP brachial systolic blood pressure, *bDBP* brachial diastolic blood pressure, *cSBP* central systolic blood pressure, *DBP* diastolic blood pressure, *HbA1c* glycated hemoglobin, *HDL-C* high-density lipoprotein cholesterol, K^+ Potassium, *MBG* marinobufagenin, Na^+ sodium, *SBP* systolic blood pressure, *TPR* total peripheral resistance, *conc.* concentration, *exc.* excretion, γ*-GT* γ-glutamyl transferase

Table 2

Respective multiple regression analyses of blood pressure and hemodynamic variables with MBG/Na^+ excretion ratio as the main independent variable in black and white women

	Independent variable: MBG/Na^+ excretion ratio					
	Black women			White women		
Dependent variable	**Adj. R^2**	**ß (95% CI)**	***P***	**Adj. R^2**	**ß (95% CI)**	***P***
cSBP (mmHg)	0.259	0.279 (0.02; 0.54)	0.039	0.251	−0.112 (−0.29; 0.07)	0.23
24-h SBP (mmHg)	0.458	0.298 (0.08; 0.52)	0.011	0.412	−0.082 (−0.24; 0.08)	0.31
Day	0.379	0.283 (0.05; 0.52)	0.023	0.392	−0.053 (−0.21; 0.11)	0.52
Night	0.381	0.334 (0.09; 0.57)	0.009	0.361	−0.134 (−0.30; 0.03)	0.12
24-h DBP (mmHg)	0.192	0.223 (−0.05; 0.49)	0.11	0.117	−0.03 (−0.23; 0.17)	0.75
Day	0.112	0.262 (−0.02; 0.54)	0.075	0.076	−0.030 (−0.23; 0.15)	0.77
Night	0.099	0.222 (−0.07; 0.51)	0.14	0.165	−0.037 (−0.17; 0.21)	0.71
Stroke volume (ml)	0.259	0.286 (0.03; 0.55)	0.036	0.171	0.023 (−0.17; 0.21)	0.81
TPR (mmHg/ml/s)	0.214	−0.334 (−0.60; −0.07)	0.018	0.164	0.115 (−0.07; 0.30)	0.24

Each dependent variable represents a separate multiple regression analysis. All models included the following covariates: age; waist:height ratio; γ-glutamyl transferase; glycated hemoglobin; total cholesterol: high density lipoprotein cholesterol ratio

cSBP central systolic blood pressure, *DBP* diastolic blood pressure, *MBG* marinobufagenin, *Na^+* sodium, *SBP*, systolic blood pressure, *TPR* total peripheral resistance