

ORIGINAL PAPER

Microvascular function in non-dippers: Potential involvement of the salt sensitivity biomarker, marinobufagenin—The African-PREDICT study

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Funding information

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 African Research Chairs Initiative (SARChI) of the Department of Science and Technology and National Research Foundation (NRF) of South Africa (Grant numbers: UID86895; 111862); the SAMRC with funds received from the South African National Department of Health; GlaxoSmithKline R&D (Africa Non-Communicable Disease Open Lab grant), the UK Medical Research Council and with funds from the UK Government's Newton Fund; as well as corporate social investment grants from Pfizer (SA), Boehringer Ingelheim (SA), Novartis (SA), the Medi-Clinic Hospital Group (SA), and in-kind contributions of Roche Diagnostics (SA). This work was supported in part by the Intramural Research Program, National Institute on Aging, National Institutes of Health, USA. 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.

Abstract

Suppressed nighttime blood pressure dipping is associated with salt sensitivity and may increase the hemodynamic load on the microvasculature. The mechanism remains unknown whereby salt sensitivity may increase the cardiovascular risk of non-dippers. Marinobufagenin, a novel steroidal biomarker, is associated with salt sensitivity and other cardiovascular risk factors independent of blood pressure. The authors investigated whether microvascular function in non-dippers is associated with marinobufagenin. The authors included 220 dippers and 154 non-dippers (aged 20–30 years) from the African-PREDICT study, with complete 24-hour urinary marinobufagenin and sodium data. The authors determined dipping status using 24-hour blood pressure monitoring and defined nighttime non-dipping <10%. The authors measured microvascular reactivity as retinal artery dilation in response to light flicker provocation. Young healthy non-dippers and dippers presented with similar peak retinal artery dilation, urinary sodium, and MBG excretion ($P > .05$). However, only in non-dippers did peak retinal artery dilation relate negatively to marinobufagenin excretion after single ($r = -0.20$; $P = .012$), partial ($r = -0.23$; $P = .004$), and multivariate-adjusted regression analyses (Adj. $R^2 = 0.34$; $\beta = -0.26$; $P < .001$). The authors also noted a relationship between peak artery dilation and estimated salt intake (Adj. $R^2 = 0.30$; $\beta = -0.14$; $P = .051$), but it was lost upon inclusion of marinobufagenin (Adj. $R^2 = 0.33$; $\beta = -0.015$; $P = .86$). No relationship between microvascular reactivity and marinobufagenin was evident in dippers ($P = .77$). Marinobufagenin, representing salt sensitivity, may be involved in early microvascular functional changes in young non-dippers and thus contributes to the development of hypertension and cardiovascular disease later in life.

1 | INTRODUCTION

Nighttime blood pressure dipping forms part of the normal circadian rhythm where blood pressure is elevated during the daytime and lowered with more than 10% during the night.¹ This physiological rhythm, however, is impaired in individuals who are classified as non-dippers (BP dipping <10%).¹ The non-dipping phenotype is associated with salt sensitivity,^{2,3} commonly defined as a meaningful change in an individual's BP response to a salt intervention (Δ MAP > 10mm Hg in response to a sodium intervention, or a >10% Δ in MAP). Soltysiak² and Uzu et al⁴ demonstrated that the non-dipping phenotype was more frequent in salt-sensitive adults on a high salt diet and that nocturnal dipping was restored with sodium restriction in some cases. The American Heart Association (AHA) also recognized salt sensitivity as a cardiovascular risk factor independent of blood pressure.⁵ It is reported that approximately 30%-50% of hypertensive⁶ and one in four normotensive individuals⁷ are salt-sensitive. Salt sensitivity is associated with increased mortality not only in hypertensive adults but also in normotensive adults.⁸ Both the AHA⁵ and Weinberger et al⁸ noted that continued research is needed to investigate possible mechanisms whereby salt sensitivity increases cardiovascular risk beyond blood pressure, especially in normotensive individuals. It is possible that individuals who demonstrate increased salt sensitivity of blood pressure may also be more sensitive to the effects of salt intake on endothelial, and micro- and macrovascular level—key role players in cardiovascular health.

The precursory role of microvascular dysfunction in the development of hypertension and cardiovascular disease has been recognized.⁹ Although there is little information on dipping status and microvascular function, salt sensitivity was indeed associated with impaired microvascular function.¹⁰ Past examinations of the microvasculature were limited and invasive; however, technological advancements have made it possible to now gain valuable information via methods including retinal microvascular imaging¹¹—reflective of the systemic microvasculature state.¹² Structural changes in the retinal microvascular calibers including small artery narrowing and vein widening have been consistently associated with increased blood pressure and inflammation, respectively.¹³⁻¹⁵ In addition, retinal microvascular responses to a light flicker provocation (function changes) may be indicative of microvascular endothelial function,¹² as reduced retinal artery dilation was related to hypertension,¹⁶ diabetes mellitus,¹⁶ obesity,¹⁷ and coronary artery disease.¹⁸

The novel steroidal biomarker, cardiotonic steroid marinobufagenin (MBG), shown to markedly increase with increased salt intake,^{19,20} strongly associates with salt sensitivity.²¹⁻²³ In vitro investigation of the adverse role of MBG on the microvasculature indicated elevated MBG to promote endothelial damage of human brain microvascular endothelial cells²⁴; however, in vivo studies investigating relationships between MBG and microvascular function are scarce. We previously demonstrated that MBG associates with increased large artery stiffness, left ventricular mass, and autonomic activity in young normotensive adults consuming excessive

amounts of salt, independent of blood pressure.^{19,25,26} The latter was predominantly demonstrated in women, who are reportedly more salt-sensitive,²⁷ despite men having higher levels of MBG. It is, therefore, possible that MBG may play a harmful role in early microvascular function, independent of blood pressure, in individuals who are salt-sensitive—including those with a non-dipping nighttime blood pressure profile. We therefore investigated the relationship of microvascular function with MBG excretion in young normotensive non-dippers, when compared to dippers.

2 | METHODS

This study forms part of the African Prospective study on the Early Detection and Identification of Cardiovascular disease and Hypertension (African-PREDICT) and included the data of the first 374 consecutively enrolled participants with complete 24-hour urinary, 24-hour blood pressure, and dynamic retinal vessel analysis data. The African-PREDICT study aims to investigate novel markers of early cardiovascular risk, while identifying potential strategies for the prevention of adverse cardiovascular outcomes.²⁸ All procedures for the African-PREDICT study adhered to institutional guidelines and the Declaration of Helsinki, and were approved by the Health Research Ethics Committee of the North-West University (NWU-00001-12-A1). The study is registered at Clinical Trials.gov (Nr NCT03292094).

The African-PREDICT study enrolled young apparently healthy black and white, men and women (between the ages of 20-30 years), from communities near Potchefstroom, in the North West Province of South Africa. Participant recruitment and data collection took place from 2012 to 2017. Community members took part in screening to determine eligibility for inclusion into the study based on the following criteria: office blood pressure <140/90 mm Hg,²⁹ microalbuminuria <30 mg/mL, HIV-uninfected, no self-reported previous diagnosis of chronic illnesses and did not make use of antihypertensive or chronic disease medication. Women included in the study were not pregnant or lactating at the time of participation.

Participants who met these inclusion criteria were invited back for additional measurements at the Hypertension Research Clinic on the North-West University campus. Participation in the study was voluntary, and all participants completed written informed consent prior to the screening and study measurements.

2.1 | Questionnaire and anthropometric data

General health and demographic questionnaires were completed by each participant to collect data on ethnicity, sex, age, self-reported alcohol use, and smoking.

The body height (m; SECA 213 Portable Stadiometer; SECA), weight (kg; SECA 813 Electronic Scales), and waist circumference (cm; Lufkin Steel Anthropometric Tape; W606PM; Lufkin, Apex) of

participants were measured, and body mass index (BMI; weight (kg)/height (m²))³⁰ and waist-to-height ratio (WHtR) were calculated.

2.2 | Cardiovascular measurements

2.2.1 | Ambulatory blood pressure

Ambulatory blood pressure (ABPM) was used to identify dipper status of participants. Each participant was fitted with a Card(X)plore device (Meditech) following the European Society of Hypertension practice guidelines.¹ We programmed ABPM devices to measure daytime blood pressure in 30-minute intervals (06:00-22:00) and nighttime blood pressure every hour (22:00-06:00). Participants included in this study had more than 70% successful ABPM recordings, or 20 daytime and five nighttime measurements.³¹ Non-dipping was defined as nighttime systolic blood pressure dipping <10%.¹

2.2.2 | Microvascular reactivity

Participants refrained from eating at least one hour before the retinal microvascular measurements were performed. A registered nurse measured the intraocular pressure (Tonopen, Avia, Reichert Technologies) prior to retinal microvascular measurements, and those with an intraocular pressure exceeding 24 mm Hg did not participate in further retinal microvascular assessments. Mydriasis was

induced by administering a drop of tropicamide (1% Alcon) to the right eye 15-30 minutes before the measurement commenced.

Microvascular reactivity in response to light flicker provocation was measured non-invasively using the Dynamic Retinal Vessel Analyzer (Imedos), fitted with a Zeiss Fundus camera FF-450plus set at a 30° angle.³² The dynamic retinal vessel analyses were performed using the standard flicker protocol of the Imedos Systems. Using RVA version 4.50 software, segments of both the artery and vein branches, between 0.5 and 2.0 optic disk diameter from the optic disk, were selected for analysis. The first light flicker stimulus was applied for 20 seconds after a 50-second baseline phase. The 20-second flicker stimulus was repeated for three cycles, each interrupted by a 80-second recovery period. The quality of each measurement was assessed as previously described.³³ Raw data were exported to Excel sheets with built-in macros. The maximum retinal artery and vein dilation in response to FLIP were calculated as a percentage of baseline previously described by Kotliar et al¹⁷ Figure 1 demonstrates the expected retinal artery dilation in response to FLIP (Figure 1A), in comparison with suppressed retinal artery dilation (Figure 1B).

2.2.3 | Microvascular calibers

The Dynamic Retinal Vessel Analyzer was also used to capture retinal images, so the central retinal artery equivalent (CRAE) and central retinal vein equivalent (CRVE) were determined, as previously

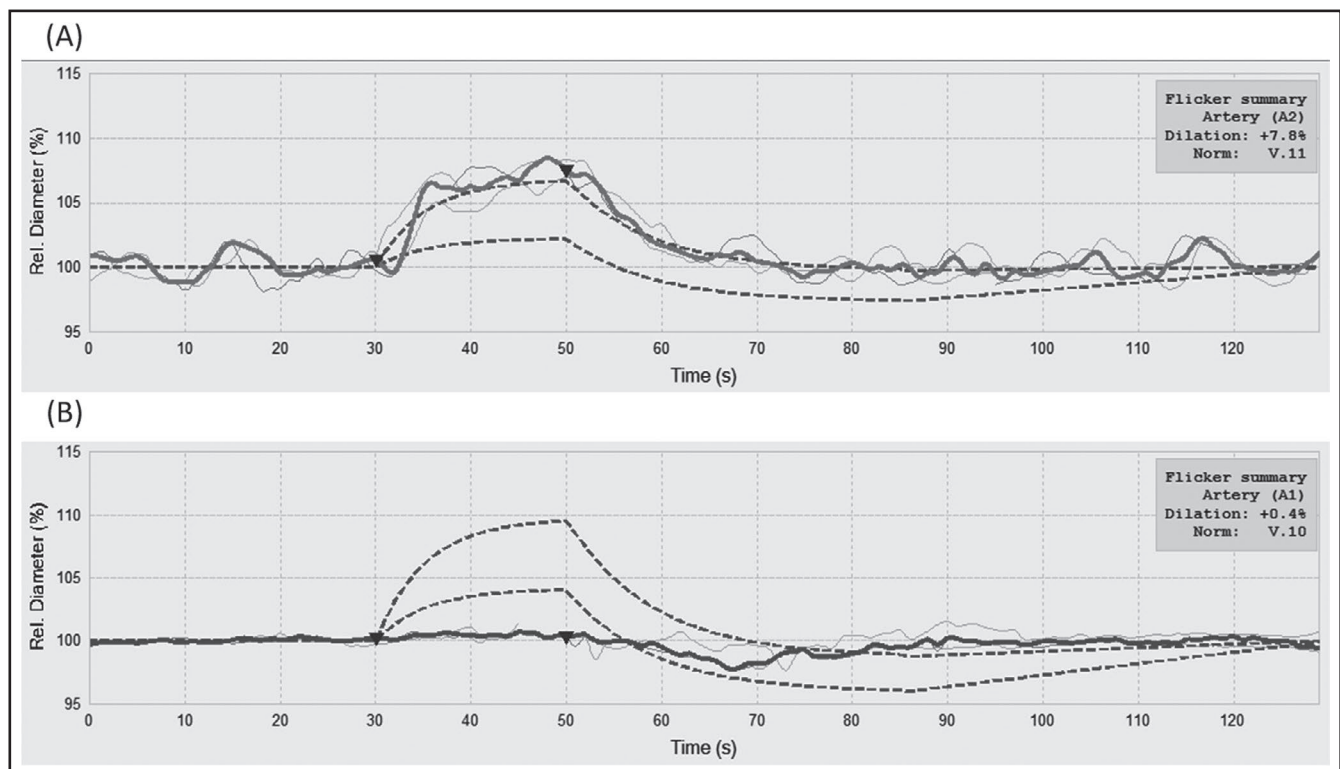


FIGURE 1 Retinal arterial responses to a light flicker provocation in individuals with (A) normal retinal arterial dilation and (B) suppressed arterial dilation

described.³⁴ Retinal images were captured with the Fundus camera angled at 50° and individual data extracted using Visualis software. Vessels located between 0.5 and 1.5 optic disk diameters from the optic disk were selected as either arteries or veins, and the CRAE and CRVE were subsequently calculated using the revised formulas.³⁵

2.3 | Biological sampling and biochemical analyses

All participants were requested to fast from 22:00 the night before the study measurements. Early morning blood samples were collected at approximately the same time every morning by trained research nurse. We have previously published a detailed description on the handling of the biological samples.²⁵ The 24-hour urine sampling protocol for this study followed standard protocols of the Pan American Health Organization/World Health Organization (PAHO/WHO).³⁶

Twenty-four-hour urinary MBG was analyzed 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.³⁷ We measured 24-hour urinary sodium and potassium, serum low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides, C-reactive protein (CRP), glucose, and gamma glutamyltransferase (GGT) using the Cobas Integra 400 plus (Roche). Serum cotinine was determined using the chemiluminescence method (Immulite, Siemens) and IL-6 using the high-sensitivity Quantikine ELISA kit (R & D Systems). Estimated salt intake was calculated from 24-hour urinary sodium using the Equation³⁸:

$$\text{Estimate NaCl (g/day)} = \frac{(24 \text{ hr Urinary Na (mmol/L)} * \text{Urinary volume (L)}) * 58.44}{1000}$$

2.4 | Statistical analyses

All statistical analyses were performed with Statistica version 13 (TIBCO Software Inc). Normally distributed data were presented as the arithmetic mean and standard deviation, with non-Gaussian-distributed data presented as the geometric mean, and 5th and 95th percentiles. Interaction testing was done to determine the potential influence of sex or ethnicity on the relationship between MBG excretion and microvascular function in dippers and non-dippers. We performed independent *t* tests to compare continuous data, and the chi-square test for categorical data, between dippers and non-dippers. Pearson, partial, and multiple regression analyses were conducted to investigate the relationships of peak artery dilation with MBG excretion and estimated salt intake, respectively, in dippers and non-dippers. Covariates included into multiple regression models were included based on the strongest bivariate associations with peak artery dilation, MBG excretion, or estimated salt intake. The model included waist-to-height ratio

(WHTR), 24-hour systolic blood pressure, IL-6, LDL-C, cotinine, and glucose. Multiple regression analyses with peak artery dilation as dependent variable additionally included artery segment diameter as a covariate.

3 | RESULTS

We found no interaction of sex or ethnicity on the relationship between MBG excretion and microvascular function in dippers or non-dippers. Table 1 demonstrates the basic characteristic of this young adult population according to their nocturnal dipping status. Of those exhibiting a normal nighttime blood pressure dipping pattern, the proportion of black (35%) compared to white adults (65%) was significantly lower. Although 24-hour systolic and diastolic blood pressure did not differ between dippers and non-dippers, non-dippers had lower daytime blood pressure ($P = .055$) and expectantly higher nighttime blood pressure ($P < .001$). Accordingly, nighttime pulse pressure was higher in non-dippers ($P < .001$). While we observed no differences in the microvascular reactivity between dippers and non-dippers, CRAE was narrower in non-dippers ($P = .050$). Also, there were no differences in the 24-hour urinary volume, sodium, potassium, or MBG excretion.

3.1 | Regression analyses

We firstly performed Pearson correlations of nighttime dipping with MBG and peak artery dilation. Only in non-dippers did we find a borderline negative correlation between nighttime dipping and MBG excretion ($r = -0.15$; $P = .064$)—but not between nighttime dipping and peak artery dilation ($r = -0.036$; $P = .66$; Table 2). We furthermore found a negative correlation between peak artery dilation and MBG excretion only in non-dippers ($r = -0.20$; $P = .012$; Figure 2A), which remained significant after partial adjustment for age, sex, ethnicity, and WHTR ($r = -0.23$; $P = .004$; Table 3). With partial correlations, we also found that MBG positively related to nighttime pulse pressure, only in non-dippers ($r = 0.18$; $P = .027$).

We determined whether estimated salt intake correlates with peak artery dilation, and found a weak relationship after partial adjustments ($r = -0.14$, $P = .091$; Table 3). When we performed multivariate-adjusted regression analyses in non-dippers (Table 4), a borderline significant association between peak artery dilation and estimated salt intake persisted (Adj. $R^2 = 0.30$; $\beta = -0.14$; $P = .051$). However, this association was altered after adding MBG excretion into the multiple regression model (Adj. $R^2 = 0.33$; $\beta = -0.015$; $P = .86$). Conversely, the negative association between peak artery dilation and MBG excretion remained robust (Adj. $R^2 = 0.34$; $\beta = -0.26$; $P < .001$) before and after including estimated salt intake into the model (Adj. $R^2 = 0.33$; $\beta = -0.25$; $P = .006$). No relationships were evident between peak artery dilation and MBG excretion (Adj. $R^2 = 0.21$; $\beta = 0.02$; $P = .77$) or estimated salt intake (Adj. $R^2 = 0.21$; $\beta = -0.06$; $P = .32$) in dippers (Table 3 & Table S1).

TABLE 1 Basic characteristics of dippers and non-dippers

	Dippers N = 220	Non-dippers N = 154	P
Sex, men, N (%)	83 (37.7)	68 (44.2)	.21
Ethnicity, black, N (%)	77 (35.0)	76 (49.7)	.005
Cardiovascular profile			
24-h SBP (mm Hg)	116 ± 9.68	117 ± 8.79	.14
Day	122 ± 10.2	120 ± 8.88	.055
Night	105 ± 8.99	113 ± 9.01	<.001
24-h DBP (mm Hg)	68.6 ± 5.93	69.2 ± 5.90	.36
Day	74.0 ± 6.31	72.5 ± 6.13	.025
Night	58.0 ± 5.92	62.7 ± 6.68	<.001
Nighttime Dipping (%)	14.1 ± 2.57	5.92 ± 2.66	<.001
Pulse pressure (mm Hg)	47.3 ± 7.44	48.2 ± 6.91	.24
Day	47.8 ± 7.81	47.4 ± 6.87	.53
Night	46.5 ± 7.33	50.1 ± 7.90	<.001
24-h Heart rate (bpm)	74.4 ± 10.4	74.9 ± 10.9	.62
Day	79.0 ± 11.2	79.3 ± 11.1	.76
Night	65.8 ± 10.5	67.1 ± 11.4	.24
Retinal microvascular calibers			
CRAE (MU)	160 ± 11.6	158 ± 12.3	.050
CRVE (MU)	249 ± 17.7	248 ± 16.8	.46
Microvascular reactivity to an acute stressor			
Retinal peak artery dilation (%)	4.30 ± 2.30	4.45 ± 2.26	.53
24-h Urinary profile			
Volume (L/24 h)	1.43 ± 0.81	1.39 ± 0.78	.63
MBG excretion (nmol/d)	3.28 (1.04; 9.37)	3.40 (1.03; 10.1)	.60
Na ⁺ excretion (nmol/d)	124 (47.9; 291)	128 (54.2; 326)	.46
K ⁺ excretion (mmol/d)	40.5 (14.3; 99.2)	42.7 (16.7; 104)	.40
Na ⁺ /K ⁺ ratio	3.11 (1.45; 6.65)	3.13 (1.17; 6.04)	.94
Salt intake (g/d)	7.33 (2.82; 17.2)	7.66 (3.19; 19.2)	.46
Biochemical profile			
Aldosterone (pg/mL)	77.6 (20.7; 405)	79.8 (20.4; 336)	.75
Glucose (mmol/L)	4.69 ± 0.68	4.64 ± 0.76	.47
HDL-C (mmol/L)	1.32 (0.82; 2.24)	1.31 (0.82; 2.11)	.90
LDL-C (mmol/L)	2.72 (1.60; 4.37)	2.59 (1.39; 4.32)	.17
Triglycerides (mmol/L)	0.87 (0.42; 2.05)	0.87 (0.41; 1.90)	.95
C-reactive protein (mg/L)	1.00 (0.12; 10.9)	1.00 (0.10; 9.37)	.97
Interleukin-6 (pg/mL)	0.88 (0.29; 3.03)	0.93 (0.30; 3.21)	.44
Lifestyle			
Smoking, N (%)	52 (23.6)	27 (17.5)	.15
Cotinine, N (%) >10 (ng/mL)	59 (27.1)	29 (19.5)	.094
Alcohol, N (%)	139 (63.2)	88 (58.7)	.38
GGT (U/L)	19.9 (8.10; 53.3)	22.0 (8.80; 65.4)	.014

Note: Arithmetic mean ± standard deviation; geometric mean (5th percentile; 95th percentile intervals).

Abbreviations: CRAE, central retinal artery equivalent; CRVE, central retinal vein equivalent; DBP, diastolic blood pressure; GGT, γ -glutamyltransferase; HDL-C, high-density lipoprotein cholesterol; K⁺, potassium; LDL-C, low-density lipoprotein cholesterol; MBG, marinobufagenin; Na⁺, sodium; SBP, systolic blood pressure.

TABLE 2 Correlations of MBG excretion and peak artery dilation with nighttime dipping percentage

	Nighttime dipping (%)	
	Dippers N = 220	Non-dippers N = 154
Peak artery dilation (%)	$r = -0.003$; $P = .97$	$r = -0.036$; $P = .66$
MBG excretion (nmol/d)	$r = 0.024$; $P = .72$	$r = -0.15$; $P = .064$

Abbreviation: MBG, marinobufagenin.

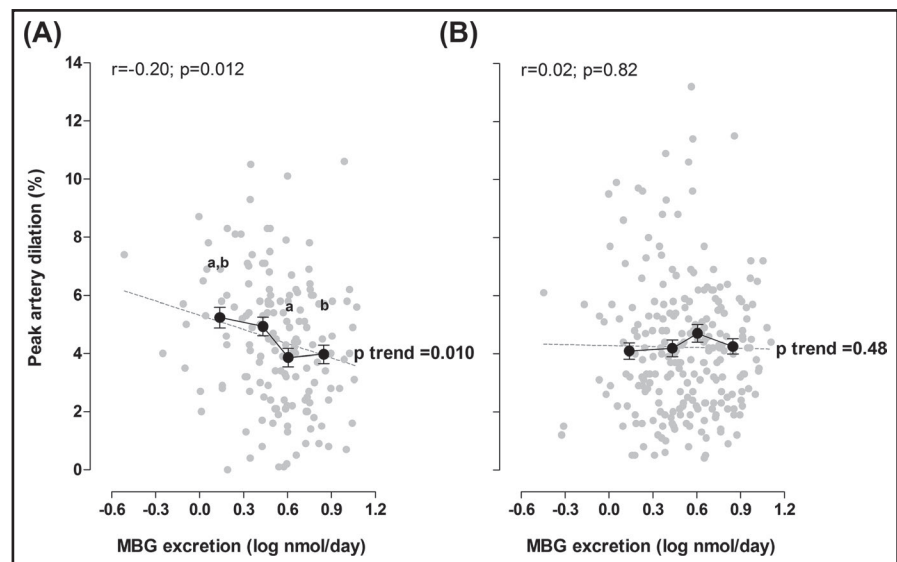
4 | DISCUSSION

In young adults with suppressed nighttime blood pressure dipping, we found that their acute microvascular dilatory responses were independently and negatively associated with a biomarker of salt sensitivity, namely MBG. This was not found in those with normal dipping.

Nighttime blood pressure dipping forms part of the normal physiological circadian rhythm that plays an important role in lowering unnecessary cardiovascular hemodynamic load while sleeping. The lack of a decrease in blood pressure from day to nighttime increases cardiovascular load and concurrently cardiovascular risk.

Indeed, Hermida et al showed that non-dippers with a normal 24-hour blood pressure (<135/90 mm Hg) demonstrated similar hazard ratios of total cardiovascular events compared to hypertensive dippers.³⁹ Also, non-dipping blood pressure is associated with increased mortality, even in those with normotensive blood pressures.⁴⁰ Accordingly, the non-dippers in our study population had a narrower retinal artery equivalent, which itself is associated with increased risk of hypertension¹⁴ and cardiovascular mortality.⁴¹ The smaller CRAE in non-dippers may reflect the functional narrowing of arterioles due to a myogenic response to increased nighttime blood pressure (Bayliss effect).⁴² Still, the questions regarding the physiological mechanisms promoting early cardiovascular risk independent of blood pressure in these individuals remain. One possibility is that increased salt sensitivity,^{2,3} a recognized cardiovascular risk factor,⁵ may increase the cardiovascular risk of non-dippers.

An endogenous inhibitor of $\text{Na}^+\text{K}^+\text{-ATPase}$, the cardiotonic steroid MBG, is strongly associated with salt sensitivity.²¹⁻²³ The interaction of MBG with $\text{Na}^+\text{K}^+\text{-ATPase}$ via either the inhibitory or signaling pathway,⁴³ has been shown to promote vasoconstriction⁴⁴ and vascular fibrosis,^{45,46} respectively, ultimately impairing vasorelaxation. In support, Fedorova et al have demonstrated impaired sodium nitroprusside-induced vasorelaxation of rat aortic explants pretreated with MBG.⁴⁵ The effect of MBG on vascular

FIGURE 2 Unadjusted (●) and adjusted (●) relationship between peak artery dilation and MBG excretion in (A) non-dippers and (B) dippers. *Adjusted for age, sex, ethnicity, and waist-to-height ratio. ^{a,b} $P = .025$ **TABLE 3** Pearson and partial correlations

	MBG excretion (nmol/d)		Salt intake (g/d)	
	Dippers N = 220	Non-dippers N = 154	Dippers N = 220	Non-dippers N = 154
Peak artery dilation (%)	$r = 0.02$; $P = .82$	$r = -0.20$; $P = .012$	$r = -0.01$; $P = .85$	$r = -0.11$; $P = .16$
Adjusted for sex, age, ethnicity, and waist-to-height ratio				
Peak artery dilation (%)	$r = 0.03$; $P = .71$	$r = -0.23$; $P = .004$	$r = -0.05$; $P = .45$	$r = -0.14$; $P = .091$

Note: Bold values denote $P < .05$.

Abbreviation: MBG, marinobufagenin.

TABLE 4 Multiple regression analyses in non-dippers

	Retinal peak artery dilation (%)					
	Salt model N = 144		MBG model N = 145		Salt and MBG model N = 144	
	R^2		R^2		R^2	
	0.30		0.34		0.33	
	β	P	β	P	β	P
MBG excretion (nmol/d)	N/A		-0.260 (0.075)	<.001	-0.250 (0.090)	.006
Salt intake (g/d)	-0.144 (0.073)	.051	N/A		-0.015 (0.085)	.86
Age (y)	0.070 (0.076)	.36	0.066 (0.073)	.37	0.064 (0.074)	.39
Sex (women/men)	-0.074 (0.092)	.42	0.003 (0.093)	.98	0.002 (0.094)	.98
Ethnicity (black/white)	-0.362 (0.085)	<.001	-0.348 (0.083)	<.001	-0.348 (0.083)	<.001
WHtR	0.196 (0.095)	.041	0.194 (0.092)	.036	0.196 (0.093)	.037
24-h SBP (mm Hg)	0.092 (0.101)	.37	0.110 (0.098)	.27	0.107 (0.099)	.28
Interleukin-6 (pg/mL)	-0.192 (0.081)	.019	-0.184 (0.079)	.020	-0.186 (0.079)	.021
Cotinine (ng/mL)	0.173 (0.077)	.027	0.158 (0.075)	.037	0.158 (0.076)	.039
LDL-C (mmol/L)	-0.225 (0.080)	.006	-0.228 (0.077)	.004	-0.229 (0.078)	.004
Glucose (mmol/L)	-0.126 (0.083)	.13	-0.153 (0.080)	.059	-0.150 (0.082)	.070

Bold values denote significance of $P < .05$.

Abbreviation: LDL-C, low-density lipoprotein cholesterol; MBG, marinobufagenin; SBP, systolic blood pressure; WHtR, waist-to-height ratio.

Na^+K^+ -ATPase can be potentiated by the mechanisms related to the salt sensitivity,⁴⁷ which may in part support our finding of a positive correlation between MBG and increased nighttime pulse pressure, a indices of arterial stiffness, in the non-dippers only. In vitro examination of the effect of MBG on human brain,²⁴ as well as rat lung microvascular endothelial cells,⁴⁸ has also provided evidence of a direct adverse effect of MBG on the microvascular endothelium—an essential deterrent of microvascular function. Both studies demonstrate increased endothelial cell disruption and concurrent microvascular permeability in response to MBG exposure.

Our findings—albeit based on cross-sectional association studies—suggest that MBG may play an adverse role in altering microvascular function in non-dipping normotensive adults, thereby increasing their cardiovascular risk independent of blood pressure. Although microvascular reactivity in this healthy population did not differ between dippers and non-dippers at this young age, the clear difference of the relationship of MBG with microvascular function in the respective groups may be vital. The association of MBG with reduced peak artery dilation in non-dippers suggests that MBG could contribute to microvascular dysfunction in the “at-risk” non-dipping group—and may give rise to a discernible attenuation in microvascular function compared to dippers later on.

In addition, salt sensitivity is not characterized by an altered salt balance, but rather abnormal sodium handling and the concurrent hypertensive responses in these individuals.⁵ Therefore, although salt intake and MBG did not differ between dippers and non-dippers, the negative relationship observed between microvascular function and MBG only in non-dippers suggests differential sodium

handling and MBG functionality. The inverse association observed between estimated salt intake and microvascular reactivity, confounded by MBG excretion, suggests that the salt-sensitive phenotype associated with non-dipping may likely be resultant of MBG. In support, Fedorova et al previously demonstrated distinct patterns of renal and vascular Na^+K^+ -ATPase inhibition in normotensive and salt-sensitive rats—despite similar increases in MBG in response to salt loading. Normotensive rats exhibited greater inhibition of renal Na^+K^+ -ATPase to promote natriuresis, while vascular Na^+K^+ -ATPase was only inhibited in salt-sensitive rats.⁴⁷

Microvasculature functionality is crucial in terms of regulating the exposure of capillaries to alterations in pulsatile pressure.⁹ The question of whether microvascular dysfunction precedes macrovascular dysfunction, or vice versa, remains subjective.⁹ In our study, however, it was evident that an association between MBG excretion and attenuated microvascular reactivity was prominent in these young adults. Relationships between MBG and macrovascular reactivity at a later stage remain possible as MBG is associated with large artery stiffness.¹⁹

This study is limited by its cross-sectional design, and therefore, the results should be interpreted within the appropriate context. Also, while MBG is strongly associated with salt intake in normotensive rats⁴⁶ and humans,¹⁹ and Dahl salt-sensitive hypertension,²¹⁻²³ more studies are needed to establish MBG as a marker of salt sensitivity in humans. Strengths of this study include the use of gold standard measurements and high-quality data from a unique healthy population sample of black and white adults in Africa. The young age of this study population allowed researchers to identify early

associations of MBG with established risk factors prior to the onset of cardiovascular disease that might be exaggerated over time and contribute to cardiovascular disease development.

We conclude that MBG is associated with reduced retinal microvascular artery dilation in young healthy normotensive adults, exhibiting a non-dipping blood pressure pattern. Salt intake, with resultant elevation in circulating MBG, may have profound effects in those with salt sensitivity and non-dipping nighttime pressures. It is possible that MBG may play a pathophysiological role contributing to increased cardiovascular risk, independent of blood pressure, observed in those with impaired nighttime blood pressure dipping.

ACKNOWLEDGMENTS

The authors of this study are grateful toward 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.

AUTHOR CONTRIBUTIONS

MS, WS, OVF, and AES contributed to the concept and design of the study. MS, WS, WW, AYB, OVF, and AES contributed to the acquisition, analyses, and interpretation of data. MS drafted the manuscript. WS, WW, AYB, OVF, and AES critically revised the manuscript.

DISCLOSURES

Prof. Schutte reports personal fees from Omron Healthcare, personal fees from Servier, personal fees from Takeda, personal fees from Abbott, and personal fees from Novartis, outside the submitted work.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Strauss-Kruger M, Smith W, Wei W, Bagrov AY, Fedorova OV, Schutte AE. Microvascular function in non-dippers: Potential involvement of the salt sensitivity biomarker, marinobufagenin—The African-PREDICT study. *J Clin Hypertens*. 2020;22:86-94. <https://doi.org/10.1111/jch.13767>