



Research Article

Amiloride Ameliorates Response of Forearm Vascular Resistance to Sympathetic Activation in Normotensive and Hypertensive Adults after Salt-Loading

Elias Simiat O^{*1} and Sofola Olusoga A²

¹Department of Physiology, Lagos State University College of Medicine, Ikeja, Nigeria

²Department of Physiology, College of Medicine of the University of Lagos, Idiaraba, Nigeria

**Author for Correspondence:*

Elias Simiat O

simiat.elias@lasucom.edu.ng

Keywords:

hypertension; cold pressor test; forearm vascular resistance; amiloride; salt-loading

ABSTRACT

Background: Forearm Vascular Resistance is an indicator of endothelial wellbeing. When salt is combined with amiloride, a specific β -Epithelial Sodium Channel blocker, we hypothesized that the vascular effect of cold pressor test (CPT) will be moderated.

Methods: The effect of CPT on 39 normotensive (NT) and 52 hypertensive (HT) participants was investigated after ethical approval from the College of Medicine, Idiaraba's Research Grants Experimentation Ethics Committee. Baseline measurement of blood pressure and forearm blood flow (FBF) were carried out with or without exposure to the CPT. These were repeated after each intervention. Thereafter, the participants were given 200 mmol of NaCl (Analar Grade) to be ingested in two-divided doses. Following a 7-day washout period, they were given 200 mmol of salt and 5mg amiloride to take daily.

Results: Forearm blood flow was reduced in both groups following salt-loading and salt+amiloride ingestion. Exposure to the CPT led to significant decrease in FBF in the two groups at baseline and following salt-loading and salt+amiloride. However, these decreases were not significantly different across group. Forearm vascular Resistance (FVR) was significantly higher among NT compared to the HT. Ingestion of salt+amiloride led to a significant reduction in FVR among NT but a slight increase among HT from the salt-loading level. However, in both groups FVR was brought back towards baseline value by amiloride.

Conclusion: We conclude that in addition to inhibition of ENaC activity in the renal tubules, Amiloride may also be lowering blood pressure by reduction in sympathetic neurally-mediated vascular responses

INTRODUCTION

Hypertension is a major cause of morbidity and mortality; it has the highest worldwide incidence of non-communicable diseases. According to the World Health Organization,[1] about 1.13 billion people worldwide suffer from this disease, of which two-thirds reside in the low- and middle-income countries. The incidence of hypertension is higher in Africa at about 46% compared with the United States of America at about 35%.[1] The prevalence in South-West Nigeria has recently been reported to be above 55%.[2] The most prevalent cause of vascular events is hypertension [3] being responsible for about 50% of risk.[4] Patients with only episodic hypertension have a high risk for vascular events.[5] Resistance arteries are the primary determinants of blood flow and blood pressure within the capillary beds. Resistance to blood flow within a network of vessels depends mainly on the size of the individual vessels; changes in diameter especially in precapillary arteries maintain adequate blood flow and blood pressure within the capillary beds thereby promoting maximal gas and nutrient exchange. Optimal pressure and flow relationship are maintained primarily by small resistance artery pressure-dependent myogenic reactivity.[6] Myogenic component of vascular

tone refers to the correlation between intraluminal pressure and pressure-dependent tone; it is commonly exhibited by small arteries with lumen diameter less than 150 μ m. Myogenic reactivity is a very important determinant of vascular resistance and a regulator of regional blood flow.[6] High dietary salt ingestion, quite apart from causing hypertension, also leads to impaired endothelial function. This is because high salt ingestion leads to oxidative stress, increased endothelial cell stiffening and increased arterial stiffness.[7]

Vasoconstriction can be induced by the Cold Pressor Test through activation of the sympathetic nervous system.[8,9] The sympathetic nervous system is a major driver of blood pressure regulation.[10] When there is an increase in sympathetic outflow to the heart and blood vessels, there is increased vasoconstriction leading to increased peripheral resistance; and cardiac contractility also increases while the accompanying decrease in parasympathetic activation of the heart results in an increase in heart rate. It has been reported that black individuals show a higher vascular response to the cold pressor test [11] and this response could be an indicator of future cardiovascular events. The increased systemic vascular resistance,

characteristic of essential hypertension, lies at the resistance-vessel microcirculation.[12] Reactive hyperaemia in the forearm may be due to remodelling within the small arteries which in turn may be significant in the pathogenesis of high blood pressure since it may result in lumen narrowing.[13] Hypertension is more prevalent in Blacks than in Caucasians, some due to dysfunction of the Epithelial Sodium Channel (ENaC) causing sodium retention. High salt ingestion can lead to elevated blood pressure. Some of the mechanisms involved include enhanced constrictor response to agonists.[14] Amiloride blocks ENaC activity in the kidney and so can result in reduction of blood pressure.[15] This study was therefore designed to determine the effect of salt-loading and blockage of the ENaC by amiloride on Forearm Vascular Resistance and its response to an autonomic sympathetic stress by way of the cold pressor test among normotensive and hypertensive adults.

METHODS

Ethical Consideration and Approval

Ethical approval was sought and obtained (CM/COM/8/VOL XXI) from the Research Grants and Experimentation Ethics Committee, College of Medicine of the University of Lagos, Idiaraba. Thereafter, informed verbal and written consent was obtained from each volunteer before being enrolled in the study. The experiments were carried out in accordance with the Helsinki Declaration with regards to human experiments and they were well-tolerated by the participants.

Participants

There were 39 normotensive (blood pressure <140/90 mmHg) and 52 hypertensive (blood pressure $\geq$ 140/90 mmHg) participants. They were volunteers from the community surrounding the Lagos University College of Medicine and Teaching Hospital, Idiaraba. The sample size was calculated from the equation for determining sample size for a comparative research: $N = 4\sigma^2(Z_{crit} + Z_{pwr})^2 / D^2$ [16] where N = total sample size for the two groups, normotensive and hypertensive participants; σ = the assumed standard deviation for each group (assumed to be equal for both groups); Z_{crit} = standard normal deviate at the chosen level of significance (α) of 0.05; Z_{pwr} = desired statistical power for the study and D = minimum expected difference between the two means. From a similar work,[17] we assumed a standard deviation of 15.1 for this study.

Participant were included in the study and categorized based on the blood pressure characteristics as earlier defined.[18] Patients with ischaemic heart disease, cerebrovascular disease, renal impairment, diabetes mellitus, secondary causes of hypertension or other concurrent illness were excluded from the study population. Participants were also excluded if plasma creatinine was greater than 150 μ mol/L or serum potassium concentration greater than 5mmol/L *ab initio*. Pregnant females were excluded for ethical reasons. Participants were allowed to withdraw if they did not feel able to continue at any time, or if their plasma creatinine exceeded 200 μ mol/L; or serum potassium became higher than 5.5mmol/L or if they had a blood pressure consistently greater than 180mmHg systolic and 110mmHg diastolic at any time. All these factors indicated worsening of the individuals' condition.

Specifically, participants with systolic blood pressure < 110mm Hg or diastolic blood pressure < 65mmHg before the amiloride tests were withdrawn; this was to prevent the possibility of hypotension in these participants [17] during the amiloride part of the experiments.

Anthropometric Data and Baseline Measurements

The anthropometric characteristics of the participants such as age (years), weight (kg), height (m²) and blood pressure (mmHg) were obtained at baseline.

Determination of Forearm Vascular Resistance

Forearm vascular resistance (FVR) was determined from the haemodynamic equivalent of Ohm's Law by dividing mean arterial blood pressure (MABP) by blood flow. Blood pressure of the participants was measured using the auscultative method. This was followed by the measurement of their forearm blood flow (FBF) by means of Venous Occlusion Plethysmography. The plethysmograph comprised a Perspex chamber sealed with a rubber cuff but with space through which participants' forearm could be inserted. The plethysmograph was coupled to a Grass polygraph (Model 7D, Grass instruments, Mass, USA) via a volume transducer (Model PT5A) inserted in a side vent. The transducer was connected to the polygraph through a pre-amplifier and driver amplifier to an ink stylus. The transducer was calibrated every time before the plethysmograph was put to use. The polygraph and its writer amplifier were calibrated as well; a sensitivity level of 1 mV/cm was selected. The subject's forearm was inserted into the plethysmograph and the cuff of the sphygmomanometer was then wrapped around the upper arm. With the polygraph set at a speed of 10mm/s and patient at rest, the sphygmomanometer was inflated to 60mmHg to cause venous occlusion. It was kept there until the rate of rise of the stylus got to a plateau. Then the polygraph was stopped and the sphygmomanometer deflated rapidly.

Exposure to the Cold Pressor Test

Participants were allowed 30 minutes rest in the laboratory in the sitting position before commencement of tests. Basal blood pressure (mmHg) was determined before exposure to the CPT. With the sphygmomanometer left in place in readiness for determining blood pressure, the subject's foot was immersed for 1 minute up to the ankle, in ice slurry composed of equal parts water and crushed ice and maintained at 4°C.[19-21] Participants were asked to be still, breathe normally and to avoid muscle contractions as well as all forms of Valsalva manoeuvre [20,22] as all these will modify their response to the cold pressor test. At the end of 1 minute and with the foot still immersed up to the ankle, peak blood pressure (mm Hg) was determined as the highest of three 15 sec serial blood pressure readings taken after 1 minute with the foot still immersed.[19,23] The foot was used in this study in order to obtain maximal haemodynamic and sympathetic responses to the CPT.[20]

Salt-Loading

After control parameters of blood pressure and forearm blood flow had been determined, participants were given a salt-load at a dose of 200mmol/day Na⁺ (HK Pharma Ltd.) for 5 days.[24] The salt was administered in two divided doses after participants had eaten and given with 500ml of

orange squash to mask the salt taste. participants

The tests for forearm vascular resistance (FVR) were repeated before and after the participants had been salt-loaded with 200 mmol/day Na^+ daily for 5 days as described above. Participants were also exposed to the cold pressor test by immersing their right foot for 1 minute in ice slurry composed of equal parts of water and crushed ice maintained at 4°C, before and after salt-loading.

Data Analysis

Body mass Index (kg/m^2) was calculated from $\text{weight}/(\text{height})^2$. Mean arterial blood pressure (mm Hg) was obtained from $\text{DBP} + 1/3 \text{ PP}$ of the BP reading. The rate of forearm blood flow (ml/s) was calculated from the initial rate of rise of the slope of the blood flow tracing obtained from the plethysmograph. FVR was calculated in mmHg/ml/s, from mean arterial blood pressure (mmHg) divided by the forearm blood flow (ml/s). [25] Percentage difference (%Δ) in forearm vascular resistance (FVR) was calculated as the percentage difference of the values before and after CPT (after CPT minus before CPT/before CPT $\times 100$). It signifies the actual effect of the CPT on FVR.

Statistical analyses

Data were analysed using the GraphPad Statistical software, Prism 5 for Windows (GraphPad Software, San Diego, California, USA). Data were expressed as $\bar{X} \pm \text{SEM}$. Differences were determined using students t-test and one-way analysis of variance (ANOVA) followed by Newman-Keuls post-hoc test as required. Statistical significance was accepted at 95% confidence interval.

RESULTS

The anthropometric data of the participants are shown in Table 1. There was no significant difference in the age and heights between the normotensive (NT) and hypertensive (HT) participants, but the Body Mass Index (BMI) was significantly higher ($p < 0.05$) in the HT group.

Forearm Vascular Resistance in Normotensive and Hypertensive Participants

The mean forearm vascular resistance (FVR) in the normotensive participants (NT) before intervention was 115.5 ± 14.00 mmHg/ml/s. This was significantly lower ($p < 0.05$) than the 149.30 ± 13.4 mmHg/ml/s recorded among the hypertensive participants (HT) (Figure 1). Following salt-loading, FVR increased slightly ($p > 0.05$) among NT to 117.70 ± 10.0 mmHg/ml/s. On the other hand, there was a slight reduction ($p > 0.05$) in FVR among HT following salt-

loading to 136.30 ± 9.1 mmHg/ml/s. Results of this study also show that in NT, salt+amiloride caused a significant fall ($p < 0.01$) in FVR from 117.70 ± 10.0 mmHg/ml/s recorded after salt-loading alone to 92.22 ± 8.0 mmHg/ml/s which was only slightly less ($p > 0.05$) than the baseline value of 115.5 ± 14.00 mmHg/ml/s (Figure 1). Among the HT on the other hand, FVR increased slightly ($p > 0.05$) to 138.9 ± 12.8 mmHg/ml/s which was only slightly less ($p > 0.05$) than the baseline value of 149.6 ± 13.4 mmHg/ml/s

Response of Forearm Vascular Resistance to the Cold Pressor Test

Before salt-loading, exposure to the cold pressor test (CPT) resulted in significant increase ($p < 0.001$) in forearm vascular resistance (FVR) among the normotensive participants (NT) from 115.5 ± 14.00 mmHg/ml/s to 176.20 ± 18.0 mmHg/ml/s (Figure 2a). Also, FVR increased significantly ($p < 0.001$) in the hypertensive participants (HT) from 149.30 ± 13.4 mmHg/ml/s at baseline to 264.20 ± 29.0 mmHg/ml/s on exposure to the CPT (Figure 2b). Following salt-loading, exposure to the CPT led to a significant increase ($p < 0.001$) in FVR among the NT from 118.00 ± 9.75 mmHg/ml/s to 202.90 ± 26.97 mmHg/ml/s and among HT from 142.40 ± 10.82 mmHg/ml/s to 227.70 ± 18.75 mmHg/ml/s (Figure 2). After salt+amiloride ingestion, there was a significant increase ($p < 0.001$) in FVR among the NT from 89.92 ± 7.91 mmHg/ml/s to 167.90 ± 19.46 mmHg/ml/s on exposure to the CPT. After salt+amiloride-loading, the HT also responded with a significant increase ($p < 0.001$) in FVR from 144.60 ± 13.70 mmHg/ml/s to 270.20 ± 32.10 mmHg/ml/s on exposure to the CPT.

Effect of Cold Pressor Test on Forearm Vascular Resistance Before salt-loading, after salt-loading and after salt-loading plus amiloride

The actual effect of the cold pressor test was determined as the percentage difference (increase or decrease) following exposure to the cold pressor test (CPT). This was necessary because FVR is a derived measure.

There was a percentage increase of $91.78 \pm 18.4\%$ in FVR among NT after salt+amil which was significantly higher ($p < 0.05$) than that recorded after salt-loading alone but marginally different ($p > 0.05$) from that recorded before salt-loading in these normotensive participants (Table 2)

The percentage increase of $79.1 \pm 10.3\%$ recorded in FVR among HT after salt+amil, was not significantly different ($p > 0.05$) from those recorded before and after salt-loading in these participants (Table 2).

Table 1: Baseline characteristics of normotensive and hypertensive participants

	NT (n = 39)	HT (n = 52)	p
Age (y)	41.2 ± 1.4	$44.4 \pm 1.$	30.12
Weight (Kg)	66.53 ± 1.8	78.31 ± 1.68	<0.00
Height (m)	1.68 ± 0.02	1.66 ± 0.02	0.37
BMI (Kg/m^2)	23.66 ± 0.68	28.69 ± 0.70	<0.0001
SBP (mmHg)	117 ± 1	145 ± 3	<0.0001
DBP (mmHg)	80 ± 1	96 ± 2	<0.0001

Modified with permission from [9]

Key: NT = normotensive; HT = hypertensive

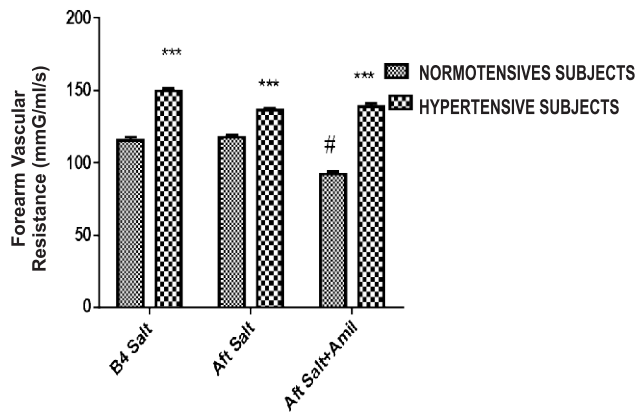


Figure 1: Effect of salt and combined salt and amiloride on forearm vascular resistance in normotensive (NT) and hypertensive (HT) participants

Normotensive (NT) n = 39; Hypertensive (HT) n = 52
KEY: *** p < 0.001 NT versus HT all groups; # p < 0.05 before salt versus after salt+amiloride NT; B4 = before; Aft = after

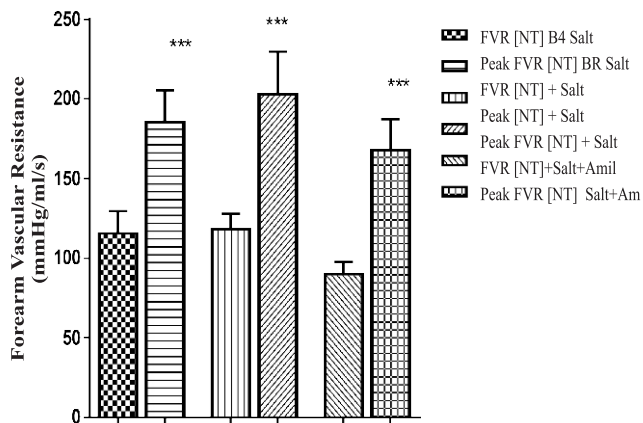


Figure 2a: Forearm vascular resistance response to the cold pressor test (CPT) in normotensive participants before salt-loading, after salt-loading and after salt-loading plus Amiloride (n = 39)

KEY: *** p < 0.001 = significant effect of CPT before salt, after salt-loading and after salt+amiloride; FVR = forearm

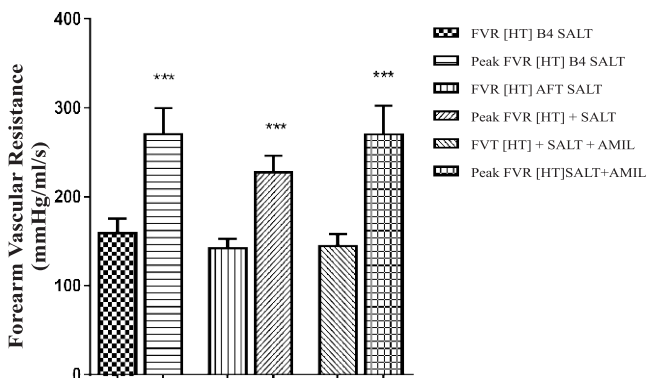


Figure 2b: Forearm vascular resistance response to the cold pressor test (CPT) in hypertensive participants before salt-loading, after salt-loading and after salt-loading plus amiloride (n = 52)

KEY: *** p < 0.001 = significant effect of CPT before salt, after salt-loading and after salt plus amiloride-loading; FVR = forearm vascular resistance; B4 = before; Amil = amiloride; Peak = after CPT

Table 2: Effect of Cold Pressor Test on Forearm Vascular Resistance Before Salt-Loading, After Salt-Loading and After Salt-Loading Plus Amiloride

	Normotensive	Hypertensive	p
Before Salt %Δ	74.9 ± 11.3 %	78.3 ± 13.6 %	0.85
After Salt %Δ	61.9 ± 8.6 %	76.5 ± 16.6 %	0.50
After Salt+ Amiloride %Δ	91.8 ± 18.4 %*	79.1 ± 10.3 %	0.52

Key: %Δ = Percentage Difference; * p < 0.05 vs After salt, Normotensive

DISCUSSION

The cold pressor test (CPT) is a provocative test designed by Hines to predict future hypertension.[23] The test was used as a sympathetic autonomic stress in this study. Usually the non-dominant hand is inserted up to the wrist in cold water and the blood pressure recording taken from the other hand.[26] In this study, the foot was immersed in the cold slurry in order to obtain maximal haemodynamic and sympathetic responses to the CPT[20]; participants' blood pressure and heart rate were determined simultaneously with the foot still immersed. This method differed from that used in an earlier work [27] in which the hand was inserted into ice water for 2 min and blood pressure measured after the hand had been removed from the water. It was however similar to the method of Roy-Gagnon *et al.*, [28] in which blood pressure was measured with the participants' hand still immersed. The difference in methodology did not however affect the result of the studies.

The regulation of peripheral vascular resistance is important in the control of regional blood flow. Also, since the CPT causes increased sympathetic activity, this results in vasoconstriction leading to an increase in peripheral vascular resistance.[29] At baseline, FVR was lower in the normotensive participants (NT) compared with the hypertensive participants (HT) in this study. Enhanced haemodynamic changes to physiological and psychological stressors have been recorded among Blacks. These are thought to be mediated largely through an increase in peripheral vascular resistance.[19] Blacks have been shown to experience a greater increase in vascular tone in response to stress probably due to increased sympathetic activity or altered vascular sensitivity to sympathetic stimulation. This may lead to increased peripheral vascular resistance which in addition to the high environmental stress to which blacks are exposed, predisposes them to sustained hypertension.[19]

Following salt-loading in the present study, whereas FVR increased slightly among the NT, there was a slight reduction among the hypertensive participants. These results differ from that of Schmidlin *et al.*, [30] in which a decrease in systemic vascular resistance was recorded in normotensive participants over the first three days following a salt load. Such a fall in vascular resistance in their study does not tally with the nephrogenic explanation of salt-induced hypertension. By this formulation and as mentioned above, it is expected that during the initial days following a salt load, an increase in blood pressure is basically as a result of a change in cardiac output while vascular resistance remains normal. However, FVR was not measured on a daily basis in this present study, being measured only at the end of the 5-day

salt-loading period and the 5-day salt+amiloride period. It is therefore possible that what was observed in the NT in this study was at the point when autoregulation had occurred in response to the increased cardiac output that accompanies a salt load. On the other hand, the slight increase in FVR in the HT may be due to the fact that although peripheral vascular resistance is said to remain normal following a salt-load, this does not mean that it remains totally unchanged. On the contrary, this suggests a systemic vascular dysfunction that is expressed as impaired vasodilatory response to the increased salt intake.[30]

In this study, stimulation of the sympathetic nervous system by means of the cold pressor test resulted in significant increases in FVR among both the NT and HT. The actual effect of the CPT (determined by the percentage difference) on FVR was not significantly different between NT and HT before and after the ingestion of a salt load. However, after addition of amiloride concomitantly with the salt load, the increase in Forearm Vascular Resistance (FVR) was much higher in NT than in HT ($91.8 \pm 18.4\%$ vs $79.1 \pm 10.3\%$). This suggests that the drug amiloride did not affect the FVR response to CPT in HT participants suggesting a reduction in sympathetically-mediated vascular resistance change as possible additional action of amiloride in our HT. However in another study in which salt ingestion elevated blood pressure and 70% of Maximum Voluntary Handgrip Contraction was used to excite sympathetic nervous system, and the effect of Losartan, an angiotensin receptor blocker (ARB) was tested, losartan attenuated the vascular response to MVC.[31] Thus, it appears that Amiloride, unlike the ARB losartan, may not be mediating its response by majorly sympathetic system inhibition.

Peripheral vascular resistance and its regulation play a central role in the control of arterial blood pressure. Response of resistant arteries to cold may be as a result of a balance between adrenergic vasoconstriction and vasodilation; the latter is mediated by endothelial function. Endothelial function of resistant arteries is a vascular function that has been identified as a primary target of injury from hypertension.[32] Increased arterial stiffness and reduced arterial compliance may both be associated with endothelial dysfunction which is again present in cardiovascular disease.[20] Activation of the sympathetic nervous system by the cold pressor test increases vascular tone in resistant arteries. This simulates a natural occurrence in winter during which period it has been observed that blood pressure is higher compared to the other seasons.[33,34]

CONCLUSION

Forearm vascular resistance increased significantly in response to sympathetic stimulation in normotensive participants after salt-loading; this post salt-loading level in NT approached the baseline FVR level among the hypertensive participants. This indicates that significant adrenergic nervous potentiation is important in the development of salt sensitive hypertension. Amiloride generally brought the FVR back towards the baseline value, indicating a role for the epithelial sodium channel in the vascular response to sympathetic autonomic stress. In addition, sympathetically-mediated vascular response was absent or reduced in HT, suggesting that lack of, or inhibition of sympathetic drive may be additional mechanism of the

blood pressure-lowering action of amiloride.

REFERENCES

1. World Health Organisation (2019). Key facts on hypertension. <https://www.who.int/news-room/fact-sheets/detail/hypertension> Last accessed 6th Aug 2020
2. Olamoyegun MA, Oluyombo R, Iwuala SO and Asaolu SO. Epidemiology and patterns of hypertension in semi-urban communities, south-western Nigeria. *Cardiovasc J Afr* 2016;27:356-360
3. Kaplan NM (2006). *Clinical Hypertension*, 9th edn. Philadelphia, USA: Lippincott Williams and Wilkins
4. Lawes CMM, Hoorn SV and Rodgers A for The International Society of Hypertension. Global burden of blood-pressure-related disease, 2001. *Lancet* 2008; 371: 1513-1518
5. Rothwell PM, Howard SC, Dolan E, O'Brien E, Dobson JE, Dahlof B, Sever PS and Poulter NR. Prognostic significance of visit-to-visit variability, maximum systolic blood pressure and episodic hypertension. *Lancet* 2010;375:895-905
6. Coats P. The effect of peripheral vascular disease on structure and function of resistance arteries isolated from human skeletal muscle. *Clin Physiol Funct Imaging* 2010;30:192-197
7. Edwards DG and Farquhar WB. Vascular effects of Dietary Salt. *Curr Opin Nephrol Hypertens* 2015;24(1):8-13
8. Chen J, Gu D, Jaquish CE, Chen C-S, Liu D, Hixson JE, Hamm LL, Gu CC, Whelton PK and He J for the GenSalt Collaborative Research Group. Association between blood pressure responses to the cold pressor test and dietary sodium intervention in a Chinese population. *Arch Intern Med* 2008;168:1740-1746
9. Elias SO, Sofola OA, Jaja SI. Vascular Reactivity and Salt Sensitivity in Normotensive and Hypertensive Adult Nigerians. *J. Afr. Ass. Physiol Sci.* 2014;2(2): 95-103
10. Malpas SC. Sympathetic nervous system overactivity and its role in the development of cardiovascular disease. *Physiological reviews.* 2010;90:513-557.
11. Kelsey RM, Alpert BS, Patterson SM, Barnard M. Racial differences in haemodynamic responses to environmental thermal stress among adolescents. *Circulation* 2000;101:2284-2289
12. Virdis A, Savoia C, Grassi G, Lembo G, Vecchione C, Seravalle G, Taddei S, Volpe M, Rosei EA, Rizzoni D. Evaluation of microvascular structure in humans: a 'state-of-the-art' document of the Working Group on Macrovascular and Microvascular Alterations of the Italian Society of Arterial Hypertension. *J Hypertens* 2014;32:2120-2129; discussion 2129.
13. Savoia C and Grassi G. The key role of microvascular structure assessment in treated hypertensive patients. *J Hypertens* 2012;30:688-690
14. Sofola OA, Knill A, Hainsworth R and Drinkhill MJ. Change in endothelial function in mesenteric arteries of Sprague Dawley rats fed a high salt diet. *J. Physiol.* 2002;543:255-260
15. Baker EH, Duggal A, Dong Y, Ireson NJ, Wood M, Markandu ND and McGregor GA. Amiloride, a specific drug for hypertension in black people with

- T594M variant? Hypertension 2002;40:13-17
16. Eng J. Sample size estimation: How many individuals should be studied? Radiology 2003;227:309-313
17. Pratt JH, Ambrosius WT, Agarwal R, Eckert GJ and Newman S. Racial difference in the activity of the amiloride-sensitive epithelial sodium channel. Hypertension 2002;40:903-908
18. Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure, 7th Report (2003). National Institute of Health, United States
19. Stein CM, Lang CC, Singh I, He HB and Wood, AJJ. Increased vascular vasoconstriction and decreased vasodilation in blacks. Additive mechanisms leading to enhanced vascular reactivity. Hypertension; 2000;36:945-951
20. Kawano H, Tanimoto M, Yamamoto K, Sanada K, Gando Y, Tabata I, Higuchi M and Miyachi M. Resistance training in men is associated with increased arterial stiffness and blood pressure but does not adversely affect endothelial function as measured by arterial reactivity to the cold pressor test. Exp Physiol 2007;93:296-302
21. Flaa A, Eide IK, Kjeldsen SE and Rostrup M. Sympathoadrenal stress reactivity is a predictor of future blood pressure. An 18-year follow-up study. Hypertension 2008;52:336-341
22. Elias SO, Ajayi RE. Effect of Sympathetic Autonomic Stress from the Cold Pressor Test on Left Ventricular Function in Young Healthy Adults. Physiol Rep, 2019;7(2), e13985, <https://doi.org/10.14814/phy2.13985>
23. Wood DL, Sheps SG and Elveback LR. Cold pressor test as a predictor of hypertension. Hypertension 1984;6:301-306
24. Tzemos N, Lim PO, Wong S, Struthers AD and MacDonald TM. Adverse cardiovascular effects of acute salt loading in young normotensive individuals. Hypertension 2008;51:1525–1530
25. Arosio E, De Marchi S, Rigoni A, Prior M and Lechi A. Effects of smoking on cardiopulmonary baroreceptor activation and peripheral vascular resistance. Eur J Clin Invest 2006;36:320-325
26. Moriyama K and Ifuku H. Increased cardiovascular reactivity to the cold pressor test is not associated with increased reactivity to isometric handgrip exercise. Eur J Appl Physiol 2010;108:837-843
27. Mei H, Gu D, Rice TK, Hixson JE, Chen J, Jaquish GE, Zhao Q, Chen C-S, Chen J-C, Gu CC, Kelly TN and He J. Heritability of blood pressure responses to cold pressor test in a Chinese population. Am J Hypertens 2009;22:1096-1100
28. Roy-Gagnon MH, Weir MR, Sorkin JD, Ryan KA, Sack PA, Hines S, Bielak LF, Peyser PA, Post W, Mitchell BD, Shuldeiner AR and Douglas JA. Genetic influences on blood pressure response to the cold pressor test: results from the Heredity and Phenotype Intervention Heart Study. J Hypertens 2008;26:729-736
29. Kelsey RM, Patterson SM, Barnard M and Alpert BS. Consistency of haemodynamic responses to cold stress in adolescents. Hypertension 2000;36:1013-1017
30. Schmidlin O, Forman A, Sebastian A and Morris RC Jr. What initiates the pressor effect of salt in salt-sensitive humans? Hypertension 2007;49:1032-1039
31. Agbaraolorunpo FM, Sofola OA, Anigbogu CN, Azinge EC. Angiotensin receptor blockade with losartan attenuates pressor response to hand grip contraction and enhances natriuresis in salt loaded hypertensive subjects: a quasi-experimental study among Nigerian adults. The Pan African Medical Journal 2019;34:article 188 doi:10.11604/pamj/09/12/2019
32. Moyna NM and Thompson PD. The effect of physical activity on endothelial function in man. Acta Physiol Scand 2004;180:113-123
33. Modesti PA, Morabito M, Bertolozzi I, Massenti L, Panci G, Lumachi C, Giglio A, Bilo G, Caldara G, Lonati L, Orlandini S, Mancia G, Gensini GF and Prati G. Weather related changes in 24-h blood pressure profile: effect of age and implication for hypertension management. Hypertension 2006;47:155-161
34. Murakami S, Otsuka K, Kono T, Soyama A, Umeda T, Yamamoto N, Morita H, Yamanaka G and Kitauro Y. Impact of outdoor temperature on prewaking morning surge and nocturnal decline in blood pressure in a Japanese population. Hypertens Res 2011;34:70-73