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Plasma Renin, Angiotensin II, Aldosterone and Atrial Natriuretic Peptide Concentrations on Acute Salt-Loading in Normotensive and Hypertensive Nigerians

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ABSTRACT

Blacks have a delayed ability to excrete salt when compared with whites despite comparable levels of renal function. The basis is not fully understood. Salt retention is a major cause of hypertension**.** This study aimed to assess and compare renin, angiotensin II, aldosterone and atrial natriuretic peptide concentrations on acute salt-loading in normotensive and hypertensive Nigerians**.** Forty-three (43) apparently healthy normotensive and thirty-seven (37) newly diagnosed age-matched hypertensive Nigerian volunteers ingested 11.6g of salt each per day for 5 days. Plasma concentrations of renin, angiotensin II, aldosterone and atria natriuretic peptide were determined before and after salt salting in the participants. The results showed basal plasma renin concentrations to be similar and salt loading did not raise its levels in the study population. Besides, basal angiotensin II hormone concentrations were not different but salt loading increased its levels significantly ($p < 0.001$) in normotensive and hypertensive participants. However, the mean change in the angiotensin levels after salt loading was significantly higher ($p < 0.05$) in hypertensive than normotensive volunteers. Basal aldosterone concentrations were higher ($p < 0.05$) in normotensive participants but its levels were more suppressed in the normotensive ($p < 0.001$) than hypertensive ($p < 0.01$) participants. Basal atria natriuretic peptide concentrations were also similar but salt loading significantly elevated the hormone levels ($p < 0.001$) in normotensive participants only and its mean change after salt loading, was also significantly higher ($p < 0.05$) in the normotensive participants. In conclusion, acute salt loading did not suppress plasma renin, angiotensin II; and ANP levels were not significantly raised in the hypertensive Nigerians. These findings suggest the basis for the racial difference in renal salt handling.

Keywords: Renin-angiotensin-aldosterone system, salt loading, hypertension, atrial natriuretic peptide

INTRODUCTION

Sustaining a normal arterial blood pressure is crucial to healthy living but this appears to be difficult to achieve in some individuals.[1]Hypertension remains the major cause of morbidity and mortality, worldwide;[2,3]affecting no fewer than one billion people globally[4,5]and its prevalence is said to be highest in Africa.[3,6]

Blacks are reported to have a delayed ability to excrete salt and are risk for a more serious form of hypertension and its complications when compared with whites despite comparable levels of renal function.[7,8]The underlying factor responsible for these racial differences is not fully known. Although, the etiology of essential hypertension is quite complex and largely unknown, failure of the body to adequately regulate salt and body fluid balance, has been implicated in its pathogenesis.[9,10]

The renin-angiotensin-aldosterone system (RAAS) is a major control mechanism of salt and water homeostasis, as well as blood pressure in humans.[11,12] However, the activity of this system is reported to be raised in hypertension,[13] the basis for the hyperactivity in a hypertensive state, is not fully known. The effect of high salt intake on RAAS is still controversial. High sodium consumption is reported to be associated with decreased levels of plasma-renin activity (PRA), plasma angiotensin II, and aldosterone concentrations in normotensive individuals[14,15]while some researchers have attributed salt-induced hypertension to inadequate suppression of aldosterone.[16] The underlying factor responsible for this weak suppression of the

aldosterone in a hypertensive condition is not fully known.

Atrial natriuretic peptide (ANP) is a cardiac hormone that plays an important role in regulating salt and water balance.[17]It is secreted primarily from the cardiac atria in response to myocardial stretch, as a result of increased extracellular volume.[18,19]It exerts its physiological effects by binding to its receptors (natriuretic peptide- A receptors) in the kidneys and blood vessels, thereby promoting natriuresis, diuresis and vasodilation.[17,19] The hormone is also inhibitory to RAAS.[20] The activity of RAAS which is said to be increased in a hypertensive condition, might be due to abnormal plasma ANP concentrations or inadequate suppression of RAAS by the ANP hormone. There is paucity of data regarding relationship between plasma ANP and renin, angiotensin II and aldosterone concentrations on salt loading, particularly in a Nigerian population.

Hence, this study was designed to assess and compare renin, angiotensin II, aldosterone and atrial natriuretic peptide concentrations on acute salt-loading in normotensive and hypertensive Nigerians in order to shed light on this regard.

MATERIALS AND METHODS

The study was carried-out in forty-three (43)apparently healthy normotensive and thirty-seven (37) newly diagnosed age-matched hypertensive participants. Approval to conduct the study was obtained from the Health Research Ethic Committee, College of Medicine of the University of Lagos. The volunteers were briefed about the study and duly signed informed consent forms were obtained.

Inclusion Criteria: The normotensive Nigerian volunteers that were included in the study had their blood pressure less than 140/90 mmHg while the hypertensive volunteers had sustained systolic blood pressure $\geq$ 140 mmHg or diastolic blood pressure $\geq$ 90 mmHg or both.[21]They were not diabetic, not suffering from any cardiovascular, renovascular or cerebrovascular disorder.

Determination of Blood Pressure: Systolic and diastolic blood pressure were determined by auscultatory method, using Accoson mercury sphygmomanometer as per the described instructions of American Heart Association.[22] Participants were briefed and allowed to rest for 10 minutes in sitting position before the commencement of measurements. Appropriate cuff sizes were used based on their mid-arm circumferences. This was done so as to prevent under-cuffing or over- cuffing, as this could adversely affect the readings. The cuff was wrapped on the right arm of each of the volunteers with the midline of bladder over the brachial arterial pulsation.
Cuff was inflated rapidly while palpating radial pulse. Reading at which pulse disappeared was noted, pressure was further elevated by 20-30 mmHg above this value. Then the bladder was slowly deflated while listening for appearance of Korotkoff's sounds using a stethoscope placed on the brachial arterial pulsation. Systolic blood pressure and diastolic blood pressure were recorded to the nearest 2mmHg, as the first appearance and disappearance of Korotkoff's sounds, respectively. The blood pressure was measured thrice in each of the participants and the average value was determined and recorded.

Protocol for Venous Blood Sample Collection: The participants were briefed about the collection of their venous blood samples. They fasted overnight and reported at 9 a.m in the laboratory for the venous blood collection. The volunteers were allowed to sit comfortably. Tourniquet was applied in each of participants' arms in order to identify antecubital veins. Skin surface was properly cleaned with methylated spirit and cotton swab with hands gloved so as to achieve aseptic condition. Venous blood was withdrawn from the antecubital vein and emptied into appropriately labeled blood sample bottles. Lithium heparin bottles were used for estimations of plasma renin, angiotensin II and aldosterone while chilled EDTA bottles were used for ANP estimation. The blood samples were spin immediately at 3,000 rpm at 4^0C C for 10 minutes. The supernatants were stored at - 40^0C until analyses were carried out.

Hormonal Assays: Plasma concentrations of renin, angiotensin II, aldosterone and ANP were determined using their respective human Elisa kits (Sunlong Biotech, China) and analyzed as described by their manufacturer's instructions.

Acute Salt Loading in the Study Participants: Having determined plasma renin, angiotensin II, aldosterone and ANP concentrations in the normotensive and hypertensive volunteers, they were given a salt load of 11.6 g of salt each, to ingest per day in two divided doses for 5 days.[23,24] Compliance with the salt ingestion was assessed by determining their 24-hour urinary sodium excretions before and after salt loading in the volunteers. They reported in the laboratory in the morning on the 6th day for measurements of these hormone concentrations, following the 5-day period of salt loading.

Data Analysis: Data analysis was performed using GraphPad Statistical software, Version 5 for Windows (GraphPad Software, SanDiego, California, USA). Data was expressed as mean ± standard error of the mean. Paired student's t-test was used to analyze data within the group andunpaired t testfordatabetweenthestudygroups.Statisticalsignificancewasacceptedatp<0.05level.

RESULTS

Plasma Renin Concentrations on Acute Salt Loading in the Study Population: The renin concentrations before and after acute salt loading are shown in Figure 1. There was no significant difference between basal renin levels measured in normotensive and hypertensive Nigerian volunteers and salt did not significantly raise the hormone concentrations in the study population. Besides, there was no significant difference in the mean changes of their renin concentrations after salt loading.

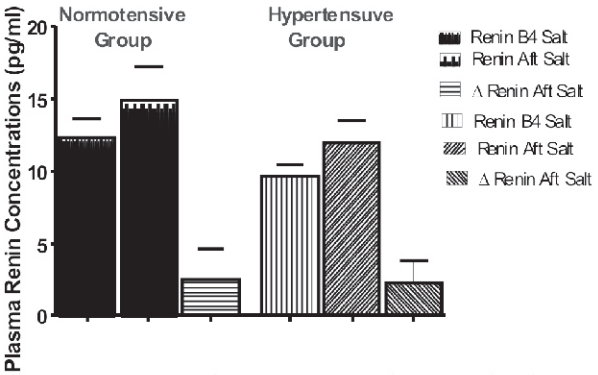

Figure 1: The Plasma Renin Concentrations before and after Salt Loading in the Study Groups

KEY:

B4 = before salt loading
Aft = after salt loading
Δ = change

Plasma Angiotensin II Concentrations on Acute Salt Loading in the Population

Plasma concentrations of angiotensin II hormone before and after salt loading in the study normotensive and hypertensive participants are shown in Figure 2. Basal angiotensin II levels were similar in the study population. Salt loading significantly increased the angiotensin II concentrations ($p < 0.001$) in both normotensive and hypertensive participants but the mean change in angiotensin II level observed in the hypertensive volunteers was significantly higher ($p < 0.05$) than what was seen in the normotensive participants (Figure 2).

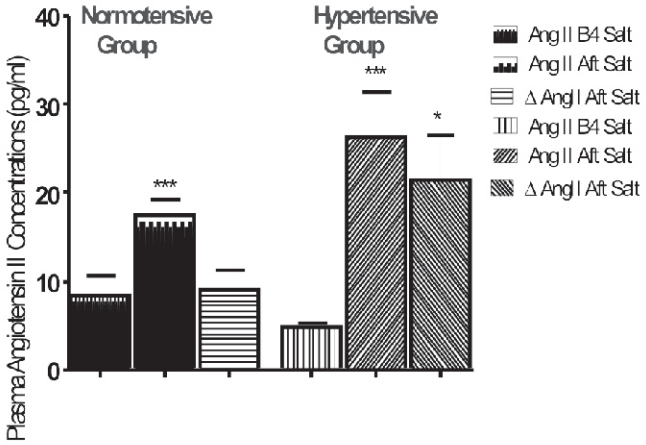

Figure 2: The Plasma Angiotensin II Concentrations before and after Salt Loading in the Study Groups

***$p < 0.001$ between angiotensin II levels before and after salt loading in both normotensive and hypertensive participants

*$p < 0.05$ between mean changes in angiotensin II hormone in normotensive and hypertensive participants after salt loading

KEY:

Ang II = angiotensin II hormone
B4 = before salt loading
Aft = after salt loading
Δ = change

Plasma Aldosterone Concentrations on Acute Salt Loading in Study Participants: Plasma Aldosterone levels before and after salt loading in the normotensive and hypertensive Nigerian volunteers are shown in Figure 3. The basal plasma concentrations of aldosterone hormone were observed to be significantly higher ($p < 0.05$) in normotensive participants than hypertensive counterparts. However, after salt loading, the levels of aldosterone hormone fell more greatly in the normotensive ($p < 0.001$) than hypertensive ($p < 0.01$) participants (Figure 3).

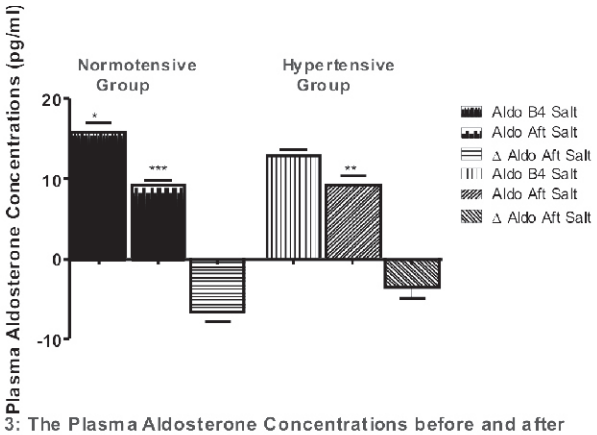

Figure 3: The Plasma Aldosterone Concentrations before and after salt Loading in the Study Groups.

*$p < 0.05$ between basal aldosterone between normotensive and hypertensive participants

**$p < 0.001$ between aldosterone levels before and after salt loading in normotensive volunteers
***$p < 0.01$ between aldosterone levels before and after salt loading in hypertensive volunteers

KEY:
Aldo = aldosterone
B4 = before salt loading
Aft Salt = after salt loading
Δ = change

Plasma ANP Concentrations before and after salt loading in the Study Groups: Figure 4 shows the concentrations of ANP hormone in the study normotensive and hypertensive groups. Basal ANP levels were similar in the studygroups. However, after salt loading; the concentrations of the hormone rose significantly ($p < 0.001$) in the normotensive group but not significantly in the hypertensive group. Besides, the mean change in the ANP concentration was significantly greater ($p < 0.05$) in the normotensive participants (Figure 4).

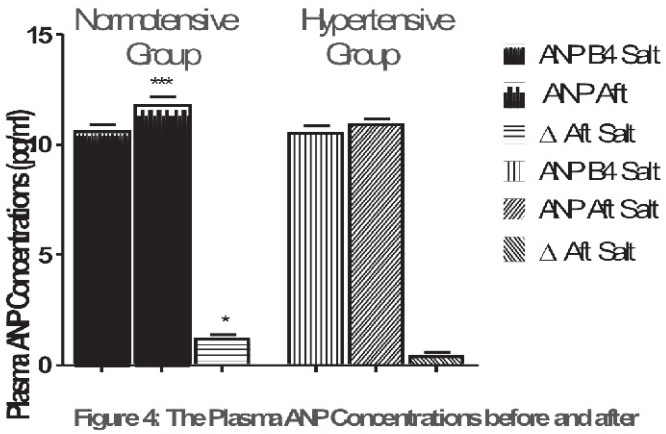

Figure 4: The Plasma ANP Concentrations before and after Salt Loading in the Study Groups

KEY:
B4 = before salt loading
Aft = after salt loading
Δ = Change

$p < 0.001$ between the mean plasma ANP levels before and after the salt loading in the normotensive groups
*$p < 0.05$ between the mean changes in Plasma ANP levels observed in normottensive and hypertensive Groups

DISCUSSION

The selection of the participants in the study was mainly based on their health status. They were age-matched and not suffering from any end organ damage such as left ventricular hypertrophy, ischemic heart disease, congestive heart failure, chronic kidney disease or stroke. These disease conditions have been associated with abnormally raised plasma ANP concentrations.[25,26]Besides, none of the participants in the study groups was diabetic, as abnormally low ANP concentrations have also been documented to be seen in diabetes mellitus patients.[27]

Basal ANP levels in the normotensive and hypertensive Nigerians were similar. Salt loading significantly increased the hormone concentrations in the normotensive participants but the hypertensive Nigerians demonstrated a blunted response.

Basal renin concentrations in the normotensive and hypertensive Nigerians were similar and salt loading did not raise the levels of this hormone significantly in both study groups. In addition, salt loading did not inhibit renin release in these participants. This finding is inconsistent with what has been previously documented among whites that high salt diet suppresses renin release.[28]Although, this might be due to the fact that blacks are reported to have low plasma renin levels,[29] it could also be due low concentrations of ANP observed in the study population. It has been reported that blacks have lower ANP levels when compared with whites.[30]

On the plasma levels of angiotensin II hormone observed in the study population, the basal angiotensin II concentrations of the participants in the study groups were not significantly different. This finding is consistent with a previous report that normotensive and hypertensive persons have similar angiotensin II concentrations.[31] Salt significantly raised the levels of the hormone in the study population, the magnitude of these increases was observed to be greater in normotensive than normotensive participants.

The greater increase might be due to the blunted ANP response to salt loading that was observed in the hypertensive group. Angiotensin II is a potent vasoconstrictor that increases peripheral resistance and promotes sodium reabsorption in the proximal convoluted tubule of the kidney, leading to increased blood volume and elevated blood pressure[32]but these effects are normally counteracted by the ANP hormone which inhibits and antagonizes angiotensin II hormone.[33]

Pertaining to plasma aldosterone concentrations in the study population, the basal aldosterone levels in this study population were observed to be higher in the normotensive than hypertensive participants. This finding agrees with what has been previously documented.[34]However, after salt loading, the hormone levels significantly fell in both study groups but the magnitude of these decreases was found to be higher in normotensive participants than hypertensive counterparts. The significant fall in the aldosterone levels as observed in this study to be more pronounced in the normotensive than hypertensive subjects, might be due to increased ANP concentrations, caused by increased blood volume as a result of salt loading.

ANP suppresses aldosterone secretion.[20] Since, increased plasma aldosterone levels enhance sodium reabsorption at the distal convoluted tubule and collecting duct of the kidney, leading to increased blood volume and pressure.[35] ANP opposes these actions of aldosterone hormone at these sites, thereby preventing the sodium reabsorption so as to reduce blood volume and pressure.[36,37]

CONCLUSION

In this study population, acute salt loading did not suppress plasma renin, angiotensin II concentrations; and ANP levels were not significantly increased in the hypertensive Nigerians. These findings might be the basis for the racial difference in renal salt handling between black and white populace.

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