



Research Article

Effect of salt loading and gender influences on plasma atrial natriuretic peptide levels and cardiovascular parameters in normotensive and hypertensive Nigerians

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Keywords:

Atrial natriuretic peptide, blood pressure, salt loading, gender, hypertension.

ABSTRACT

Background: Prevalence of hypertension is higher among Sub-Saharan Africans than whites and there is a sex difference in the prevalence. Though, salt retention has been implicated in pathogenesis of hypertension, the basis is not completely known. Atrial natriuretic peptide (ANP) might play a role. **Method:** 43 apparently healthy normotensive male and female Nigerians and 37 aged-matched hypertensive counterparts were orally administered 11.6g of dietary salt each per day for 5 days. Their plasma ANP levels, heart rate (HR), systolic (SBP), diastolic (DBP) and mean arterial blood pressure (MABP) were determined before and after salt loading. **Results:** Normotensive and hypertensive male and female subjects had similar basal ANP levels. However, salt loading significantly raised the ANP concentrations ($p = 0.0001$) in normotensive subjects but not significantly in hypertensive counterparts. ANP levels rose significantly in the normotensive males ($p = 0.0024$) and females ($p = 0.0002$) but not significantly in the hypertensive counterparts. Besides, salt significantly decreased HR in normotensive ($p = 0.0095$) and hypertensive ($p = 0.0397$) subjects but increased SBP ($p < 0.01$) and MABP ($p < 0.01$) in both study groups and DBP ($p = 0.0014$) in hypertensive group only. SBP, DBP and MABP were all significantly elevated ($p < 0.05$) in hypertensive males and females but not significantly in normotensive females. **Conclusion:** Although, basal ANP levels were similar in the study normotensive and hypertensive subjects, other findings in this study suggest that ANP as well as female gender could ameliorate increased blood pressure response to salt loading especially in a normotensive state

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INTRODUCTION

Prevalence of hypertension in Africa is estimated at 46% in adult individuals aged 25 years and above with higher prevalence in males than females (WHO, 2013a; Akinlua *et al.*, 2015). Hypertension affects more than one billion people, globally (Mills *et al.*, 2016) and is responsible for about 9 million deaths, annually, worldwide (WHO, 2013b). It is the single most important risk factor for stroke and coronary artery disease (Garg, 2014). The current levels (9 – 12 g/day) of salt consumption in most countries, have exceeded

daily recommended levels (<5 g/day) (Samogan *et al.*, 2014; Dötsch-Klerk *et al.*, 2015). Salt retention has been implicated in the pathogenesis of hypertension (He and MacGregor, 2007; Hauck *et al.*, 2012). Although, most people exhibit an increase in blood pressure on salt loading (Weinberger, 1996), the quantity of salt required to induce a hypertensive state, varies (Richardson *et al.*, 2013). The basis for this variation is still a subject of intense research.

The mechanisms by which salt raises blood pressure are complex, multi-factorial and incompletely understood (Hauck *et al.*, 2012). Some of the mechanisms include a genetic defect in salt transport, increased sympathetic activity, pathological condition of the kidney, adrenal cortex or pituitary gland (Strazzulo *et al.*, 2001; Brooks *et al.*, 2005; Blaustein *et al.*, 2010). Other mechanisms such as reduced production of vasodilators like nitric oxide, dopamine and kallikrein have also been

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implicated in the pathogenesis of hypertension (Fujwara *et al.*, 2000). The role of atrial natriuretic peptide in the development of salt-induced hypertension has not been fully investigated. There is paucity of data regarding the effect of salt loading on plasma atrial natriuretic peptide concentrations in normotensive and hypertensive Nigerians. Previous measurements of plasma atrial natriuretic peptide levels, assessed among individuals with essential hypertension in European and North American population, have provided conflicting results. Some investigators have reported low ANP concentrations in hypertension (Macheret *et al.*, 2012) while others have documented high amounts (Irzanski *et al.*, 2007).

Atrial natriuretic peptide (ANP) is an endocrine hormone that regulates salt and water balance as well as blood pressure by promoting renal loss of sodium and water (Song *et al.*, 2015). It is produced mainly in the cardiac atria and released into the circulation in response to volume expansion and increased atrial distension (Klar *et al.*, 2007; Wang *et al.*, 2012). It has been suggested that Sub-Saharan Africans have lower plasma atrial natriuretic levels than their white counterparts (Guptal *et al.*, 2015). The higher prevalence of hypertension reported in Sub-Saharan Africans might be due to abnormal ANP concentrations. In mice, ANP deficiency caused salt-sensitive hypertension and cardiac hypertrophy (Song *et al.*, 2015). It is not clear if sex difference exists on plasma ANP levels among Africans particularly in a hypertensive condition when high salt diet is consumed; hence, the study was designed to assess plasma ANP levels in a Nigerian population as well as to determine the effects of salt loading and gender influences on plasma ANP levels and blood pressure in normotensive and hypertensive Nigerian volunteers.

METHODS

Forty-three (43) apparently healthy normotensive and thirty-seven (37) age-matched newly diagnosed hypertensive subjects who were yet to be on antihypertensive medications, participated in the study. Ethical approval with Ref No: CM/HREC/10/16/101; dated February 16, 2017 was obtained from Health Research Ethic Committee of College of Medicine, University of Lagos. The volunteers were briefed about the study and duly signed informed consent forms were obtained.

Inclusion Criteria:

The normotensive subjects had their blood pressure below 140/90 mmHg. They were not on any antihypertensive medication, not suffering from chronic

kidney, cardiovascular or cerebrovascular disease. They did not have any abnormal ECG findings such as left ventricular hypertrophy, ischaemic heart disease, myocardial infarction, atrial fibrillation and they were not diabetic.

The hypertensive volunteers had sustained systolic blood ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg or both (Franklin, 2004). The subjects were not on any antihypertensive drug, not diabetic or on any hypoglycaemic medication and they were not suffering from any complication arising from their hypertensive condition.

Exclusion Criteria:

The normotensive volunteers who were diabetic, smokers or had history of chronic consumption of alcohol were excluded from the study. Hypertensive volunteers that had severe hypertension (BP $\geq 180/110$ mmHg) or history of cardiovascular disease or chronic kidney disease as evident by plasma $K^+ \geq 5.5$ mmol/l and or creatinine ≥ 150 μ mol/l, were also excluded from the study. Pregnant women were not allowed to participate in the study for medical and ethical reasons.

Experimental procedures

Anthropometric data of the subjects were determined. Age was recorded in years and body weight was measured with the aid of weighing scale and recorded in kilogrammes. Height in metres was measured using stadiometer and the body mass index (BMI) was calculated by dividing the body weight (kg) by square of the height (m) (Monterio *et al.*, 2012).

Measurements of Cardiovascular Parameters of the Subjects

The cardiovascular parameters were determined before and after salt loading in the study groups of subjects

Determination of Heart Rate

Heart rate (beats/minute) was determined using an electrocardiograph machine. The procedure was briefly explained. Subjects were allowed to rest for 10 minutes in sitting position. The placement of electrodes was done in conformity with the American Heart Association recommendations (Kligfield *et al.*, 2007). The heart rate was determined from the R-R intervals in Lead II of the electrocardiogram, using formula: $25/R - R \times 60$ (beats/min) (Kligfield *et al.*, 2007).

Determination of Blood Pressure

Blood pressure was determined by auscultatory method using Accoson mercury sphygmomanometer (Accoson, United Kingdom, 2014), as per the described instructions of American Heart Association (Beavers *et*

al., 2001). Subjects were allowed to rest for 10 minutes in sitting position. Appropriate cuff size was wrapped on the right arm with the midline of cuff over the brachial arterial pulsation and inflated rapidly while palpating radial pulse. Reading at which pulse disappeared was noted and pressure was further elevated 20 – 30mmHg above this value. Then the cuff was slowly deflated while listening to the Korotkoff's sounds using a stethoscope placed on brachial arterial pulsation. Systolic blood pressure and diastolic blood pressure were recorded to the nearest 2mmHg as the first appearance and disappearance of the Korotkoff's sounds, respectively. The blood pressure was taken thrice and the average determined and recorded.

Determination of Mean Arterial Blood Pressure

Mean arterial blood pressure was determined from the sum of diastolic blood pressure and one-third of pulse pressure (Zheng *et al.*, 2008). The pulse pressure is the difference between systolic and diastolic blood pressure.

Measurements of Laboratory Parameters

The laboratory parameters measured in the subjects were fasting blood sugar, plasma creatinine, sodium, potassium and ANP levels as well as the urine sodium and potassium concentrations and urine volumes.

Protocol for Venous Blood Collection

The subjects fasted overnight and reported at 9a.m in the laboratory for their blood collection. They were briefed about the procedure. Venous blood was withdrawn from the antecubital vein under aseptic condition and emptied into appropriately labeled blood sample bottles. Lithium heparin bottles were used for blood creatinine, sodium and potassium estimations while chilled EDTA bottles were used for ANP estimation. The withdrawn blood samples were spinned immediately at 2000 x g at 4°C for 10 minutes. The supernatants were stored at – 40°C until analyses were carried out.

Fasting blood sugar was measured using Accu-check glucometer (Roche Diabetes Care Inc; USA, 2008). Plasma creatinine was determined using COBAS C 111 machine (Roche Diagnostics, USA, 2012). Plasma and urinary sodium and potassium concentrations were measured before and after salt loading, using ion selective electrode (ISE 6000) machine (SFRI, France, 2011). The 24- hour urine volumes were also measured before and after salt loading, using a measuring cylinder. Plasma ANP concentrations were determined before and after salt loading, using Human Atrial Natriuretic Peptide Eliza Kits (Sunlong Biotech, China, 2016). The

ANP levels were analyzed as described by the manufacturer's instructions.

Acute Salt Loading in the Study Subjects

Having measured and recorded the baseline cardiovascular and laboratory parameters, each of the subjects in the study group ingested 200mmol of sodium (11.6 g of dietary salt) per day in two divided doses for 5 days (Tzemos *et al.*, 2008; Elias *et al.*, 2014). The subjects were followed up during the course of salt administration and compliance with the salt ingestion was assessed by determining their 24-hour urine sodium excretions before and after the salt loading. They reported in the laboratory on the 6th day for measurements of their cardiovascular and laboratory parameters, following the 5-day period of salt ingestion.

Data Analysis

Data analysis was carried out with the help of GraphPad Statistical software, Version 5 for Windows (GraphPad Software, San Diego, California, USA). Data was expressed as mean ± standard deviation. Paired student's t- test was employed to analyze data within the group and unpaired t- test for data between the study groups. Statistical significance was accepted at p < 0.05 level.

RESULTS

Baseline Characteristics of the Subjects in the Study Groups

The baseline characteristics of the participants in the study groups are shown in Table 1.

Although, the mean body weight and BMI of subjects in the hypertensive group were significantly higher than those of normotensive counterparts, there was no significant difference in their mean ages. The fasting blood sugar and creatinine levels were also observed to be similar in the study groups (Table 1).

Table 1: The Baseline Characteristics of the Study Groups of Subjects

Parameters	Normotensive Group (n = 43)	Hypertensive Group (n = 37)	p Value
Age (yr)	43.09 ± 8.14	46.43 ± 6.73	0.0513
Body weight (kg)	67.60 ± 10.62	77.00 ± 14.53	0.0012
Height (m)	1.64 ± 0.07	1.65 ± 0.07	0.9019
BMI (kg/m ²)	25.10 ± 3.92	28.48 ± 4.60	0.0007
FBS (mg/dl)	81.86 ± 10.12	83.78 ± 9.40	0.3838
Creatinine(μmol/l)	69.00 ± 18.54	71.65 ± 17.94	0.5208

Values are means ± SD as analyzed by student's t-test. n = number of subjects; SD = standard deviation; BMI = body mass index; FBS = fasting blood sugar NS=not significant

Effect of Salt Loading on the Plasma ANP Levels in the Study Groups

The basal plasma ANP levels observed in the normotensive and hypertensive subjects were similar (Table 2). However, salt loading significantly increased

plasma ANP levels ($p = 0.0001$) in the normotensive group but had no significant effect on ANP concentrations in the hypertensive group. In addition, when comparing the mean changes in the plasma ANP concentrations observed in the study groups after salt loading, the mean change in the normotensive group was significantly higher ($p = 0.0137$) than that of the hypertensive group (Table 2).

Influence of Gender on Plasma ANP Levels

The plasma ANP levels before and after salt loading in male and female normotensive and hypertensive volunteers are also shown in Table 2. In the normotensive group, basal ANP concentrations between males and females were similar. Also, in the hypertensive group, the basal ANP levels observed in males and females were not significantly different. The basal ANP concentrations seen in the normotensive males and hypertensive females or normotensive females and hypertensive males were not significantly different (Table 2). However, after salt loading in these subjects, plasma ANP levels rose significantly in both normotensive males ($p = 0.0024$) and females ($p < 0.0002$) but there were no significant increases in the hypertensive male and female counterparts (Table 2).

The mean ANP concentration after salt loading in the normotensive females was observed to be significantly higher ($p = 0.0263$) than that of the hypertensive female counterparts but those of the normotensive and hypertensive males were not significantly different. In addition, no significant difference was seen in the mean changes in the normotensive males and females. The mean ANP changes in the hypertensive male and female subjects were also similar (Table 2).

Effect of Salt Loading on Cardiovascular Parameters Measured in the Normotensive and Hypertensive Subjects

The mean values of heart rates, systolic and diastolic blood pressure as well as the mean arterial blood pressure measured before and after salt loading in the study groups are shown in Tables 3.

Heart Rates:

There was no significant difference between the mean values of the heart rates observed in the normotensive and hypertensive subjects before salt loading (Tables 3). However, after salt loading, the heart rates fell significantly in both normotensive ($p = 0.0095$) and hypertensive ($p = 0.0397$) groups (Table 3). There was no significant difference in the mean heart rate changes, observed in these study groups.

Systolic Blood Pressure:

Hypertensive subjects had significantly higher basal

SBP ($p = 0.0001$) than normotensive subjects. Salt loading significantly elevated SBP in the normotensive ($p = 0.0023$) and hypertensive (0.0037) volunteers (Tables 3). When comparing the mean changes in SBP observed in the study groups after the salt ingestion, they were not significantly different (Table 3).

Diastolic blood pressure:

Hypertensive volunteers also had significantly higher basal DBP ($p = 0.0001$) than normotensive counterparts (Tables 3). Salt loading increased DBP significantly ($p = 0.0014$) in hypertensive subjects but not significantly in the normotensive counterparts. The mean changes in DBP in the study groups though, higher in the hypertensive subjects, were not significantly different.

Mean arterial blood pressure (MABP):

Basal MABP was significantly higher ($p = 0.0001$) in the hypertensive subjects than normotensive volunteers. Salt loading significantly elevated mean MABP in both normotensive ($p = 0.0025$) and hypertensive ($p = 0.0014$) subjects and the mean changes in MABP after salt loading, were not significantly different.

Table 2: Plasma ANP Levels Measured before and after Salt Loading in Normotensive and Hypertensive Groups of Subjects

Subjects m (f)	Normotensive 20 (23) ANP (pg/ml)	Hypertensive 16 (21) ANP (pg/ml)	p Value
Before Salt loading			
Males	10.16 ± 1.17 (n=22)	10.75 ± 2.13 (n = 16)	0.2932
Females	10.99 ± 2.70 (n = 21)	10.33 ± 1.61 (n = 21)	0.3383
Combined (M & F)	10.60 ± 2.15 (n = 43)	10.52 ± 1.84 (n = 37)	0.8440
After Salt Loading			
Males	11.24 ± 1.76**	11.23 ± 0.38	0.9783
Females	12.26 ± 2.76***	10.68 ± 1.70	0.0286
Combined (M & F)	11.78 ± 2.38***	10.92 ± 1.63	0.0649
Δ (Changes after Salt)			
Males	1.05 ± 1.42	0.48 ± 1.37	0.2348
Females	1.26 ± 1.43	0.35 ± 1.17	0.0263
Combined (M & F)	1.16 ± 1.42	0.40 ± 1.25	0.0137

Values are expressed in mean ± SD as analyzed by student's t-test.: n = number of subjects; SD = standard deviation M & F = combined male and female subjects in the group; Δ = changes in ANP levels after salt loading m (f) = males (females). ** $p = 0.0024$ in ANP levels observed in the normotensive males before and after salt loading; *** $p = 0.0002$ in ANP levels observed in normotensive females before and after salt loading; *** $p = 0.0001$ in ANP levels observed before and after salt loading in normotensive group (M&F combined)

Influences of Gender on the Cardiovascular Parameters Measured in the Normotensive and Hypertensive Subjects

Influences of gender on the cardiovascular parameters measured in the study normotensive and hypertensive subjects are shown in Table 4. The basal heart rates in

the normotensive male and female subjects were not significantly different but hypertensive females had a significantly higher basal heart rate ($p = 0.0161$) than their counterpart males. After salt loading, heart rates significantly fell in normotensive ($p = 0.0236$) and hypertensive ($p = 0.0171$) females but not significantly in normotensive and hypertensive males (Table 4).

In addition, normotensive male and female subjects had similar basal systolic blood pressure values and there was no significant difference in the basal systolic blood pressure observed in the hypertensive male and female counterparts (Table 4). However, after salt loading, systolic blood pressure was significantly elevated ($p = 0.0008$) in the normotensive males but not significantly in the normotensive females. In the hypertensive group, on the other hand, the systolic blood pressure was significantly increased in both males ($p = 0.0490$) and females ($p = 0.0404$).

Furthermore, normotensive males had a significantly higher basal diastolic blood pressure ($p = 0.0375$) than normotensive females. In the same vein, hypertensive males had a significantly higher basal diastolic blood pressure ($p = 0.0113$) than their females. However, salt loading significantly increased diastolic blood pressure in both hypertensive males ($p = 0.0310$) and females (0.0208) but not significantly in the normotensive male and female counterparts (Table 4).

The basal mean arterial blood pressure values observed in normotensive males and females were not significantly different. Also, in the hypertensive male and female subjects, the basal MABP values were similar. However, after salt loading, the mean arterial blood pressure values were significantly raised in the hypertensive males ($p = 0.0147$) and females ($p = 0.0353$) but not significantly in the normotensive male and female counterparts (Table 4)

DISCUSSION

The subjects in the study groups were aged- matched. This was ensured so as to eliminate age factor that has been reported to be associated with high blood pressure (Rockwood and Howlett, 2011). The participants were selected primarily based on their health status. They were not suffering from any end organ damage such as left ventricular hypertrophy, ischaemic heart disease, congestive heart failure, chronic kidney disease or cerebrovascular disease; as any of these disease conditions, has been reported to be associated with abnormally high plasma ANP concentrations (Minamino and Nishikimi, 2013; Volpe *et al.*, 2016; Ogawa *et al.*, 2015). In addition, none of the subjects was diabetic, as abnormally low ANP concentrations

have been documented to be observed in diabetes mellitus patients (Wang *et al.*, 2007; Magnusson *et al.*, 2012).

The basal plasma ANP levels in the study population were similar. This finding disagrees with earlier studies carried out among Caucasians that hypertensive individuals have higher basal ANP levels than normotensive counterparts (Irmanski *et al.*, 2007; Hu *et al.*, 2015). However, when compared the basal ANP levels observed in the study population with those reported in western population (Castro and Gombein, 1994), the levels observed in the Nigerian population were found to be lower. Abnormally low plasma ANP concentrations have been implicated in the pathogenesis of salt induced hypertension (Song *et al.*, 2015). This finding agrees with what Gupta and his co-workers (2015) reported that African Americans have lower plasma ANP concentrations when compared with Caucasians.

Table 3: Cardiovascular Parameters Measured before and after Salt Loading in Normotensive and hypertensive Groups of Subjects

Cardiovascular Parameters Measured	Normotensive Group (n = 43)			Hypertensive Group (n = 37)		
	Before Salt Loading	After Salt Loading	p-Value	Before Salt Loading	After Salt Loading	p Value
HR(beats/min)	73.00 ± 7.29	69.77 ± 8.54	0.0095	76.11 ± 8.67	73.51 ± 10.66	0.0397
SBP(mmHg)	116.60 ± 10.09	122.00 ± 12.43	0.0023	136.4*** ± 9.00	142.80 ± 13.10	0.0037
DBP (mmHg)	80.21 ± 7.24	82.81 ± 10.01	0.0642	97.00*** ± 5.91	101.9 ± 9.44	0.0014
MABP(mmHg)	92.35 ± 7.50	96.40 ± 10.02	0.0025	110.50*** ± 5.55	115.50 ± 8.37	0.0014

Values are expressed in mean \pm SD, as analyzed by student's t-test. MABP = mean arterial blood pressure; n = number of subjects in the group; SBP = systolic blood pressure; DBP = diastolic blood pressure SD = standard deviation; HR = heart rate, ***P = 0.0001 in SBP values between normotensive and hypertensive groups of subjects before salt loading. ***P = 0.0001 in DBP values between normotensive and hypertensive groups of subjects before salt loading. ***P = 0.0001 in MABP values between normotensive and hypertensive groups of subjects before salt loading.

Salt loading increased plasma ANP concentrations significantly in the normotensive subjects but the hypertensive counterparts demonstrated a blunted ANP response to the salt challenge. Furthermore, the normotensive volunteers had higher mean change in the plasma ANP levels than hypertensive counterparts. The slight increase in the plasma ANP levels, seen in the hypertensive subjects, might be due to impaired response of stretch receptors to increased blood volume, caused by salt loading in these individuals. Stretch-

induced ANP release is said to be suppressed by an endogenous angiotensin II (Oh *et al.*, 2011) which has since been documented to be raised in hypertension (Catt *et al.*, 1971).

Table 4: Cardiovascular Parameters Measured before and after Salt Loading in the Normotensive and Hypertensive Male and Female Subjects

Cardiovascular Parameters Measured	Normotensive Group					
	Male Subjects (n = 20)			Female Subjects (n = 23)		
	Before Salt	After Salt	p Value	Before Salt	After Salt	p Value
HR (mmHg)	71.85 ± 6.49	69.70 ± 8.53	0.2052	74.00 ± 7.92	69.83 ± 8.73	0.0236
SBP (mmHg)	117.20 ± 8.52	125.00 ± 9.83	0.0008	116.10 ± 11.46	119.40 ± 14.01	0.2117
DBP (mmHg)	82.65* ± 6.04	86.95 ± 7.98	0.5047	78.09 ± 7.64	79.22 ± 10.35	0.5324
MABP (mmHg)	94.17 ± 6.15	99.63 ± 7.81	0.0086	90.77 ± 8.31	93.58 ± 11.01	0.1112
Cardiovascular Parameters Measured	Hypertensive Group					
	Male Subjects (n = 16)			Female Subjects (n = 21)		
	Before Salt	After Salt	p Value	Before Salt	After Salt	p Value
HR (beats/min)	71.81 ± 5.49	71.69 ± 6.72	0.9355	79.38* ± 10.94	74.90 ± 12.88	0.0171
SBP (mmHg)	134.80 ± 7.48	141.90 ± 13.18	0.0490	137.70 ± 9.99	143.50 ± 13.33	0.0404
DBP (mmHg)	99.75* ± 6.73	104.20 ± 8.70	0.0310	94.90 ± 4.27	100.20 ± 9.82	0.0208
MABP (mmHg)	111.40 ± 6.01	116.70 ± 9.29	0.0147	109.70 ± 5.20	114.60 ± 9.55	0.0353

Values are expressed in mean ± SD as analyzed by student's t-test; MABP = mean arterial blood pressure, n = number of subjects; SBP = systolic blood pressure; DBP = diastolic blood pressure HR = heart rate; SD = standard deviation; before salt = before salt loading; after salt = after salt loading * p = 0.0161 in basal HR values observed values between hypertensive males and females, * p = 0.0113 in basal DBP values observed values between hypertensive males and females. * p = 0.0375 in basal DBP values between normotensive males and females

Regarding influences of gender on plasma ANP concentrations in the normotensive and hypertensive subjects in this study, the basal ANP levels were similar in males and female subjects of the study groups. However, after salt ingestion what was observed in the study subjects was quite interesting as males and females in the normotensive group demonstrated significant increases in their ANP levels but salt loading did not significantly elevate ANP levels in their hypertensive male and female counterparts. The blunted ANP response demonstrated by these hypertensive individuals, might be an underlying cause for poor salt

handling that has been reported to be seen in a hypertensive state (O'Shaughnessy and Karet; 2004). The higher ANP response observed in the normotensive females than males might be due to oestrogen effect as it has been documented that oestrogen induces ANP release from the heart via oestrogen receptor (Vishwarkama *et al.*, 2016).

On the cardiovascular parameters measured in the study population, basal heart rates were similar in the study groups. However, basal systolic, diastolic and mean arterial blood pressure were observed to be significantly higher in the hypertensive subjects than normotensive counterparts. These were not quite surprising as hypertension is a chronic disease condition, characterized with abnormal elevations in systolic and diastolic blood (Michael *et al.*, 2007).

In this study, salt loading significantly increased systolic and mean arterial blood pressure as well as causing significant decreases in the heart rates in both normotensive and hypertensive groups of subjects. The magnitude of these heart rate decreases though found to be higher in the normotensive than hypertensive subjects, the decreases were necessitated by a reflex response to increased blood pressure demonstrated by the subjects (McNeely *et al.*, 2008; Messerli, 2013).

The expansion in blood volumes that led to increase in blood pressure caused stretch receptors (baroreceptors) in the carotid sinus and aortic arch to be stimulated, leading to increased vagal discharge to the heart from nucleus ambiguus and dorsal motor nucleus of the vagus in the medulla oblongata (Wang *et al.*, 2001; Messerli, 2013). Hence, the observed decreases in heart rates in the normotensive and hypertensive subjects.

Salt loading significantly elevated systolic and mean arterial blood pressure in both study groups but diastolic blood pressure was not significantly raised in the normotensive group. Although, impaired baroreflex function has been documented in hypertension (Heusser *et al.*, 2005), the differential response in diastolic blood pressure that was seen in the normotensive subjects following salt loading, might also be due to a significant increase in their ANP concentrations. ANP is a vasodilator that reduces peripheral resistance leading to a decrease in blood pressure (Chopra *et al.*, 2013; Song *et al.*, 2015; Ogawa *et al.*, 2015). It is also inhibitory to sympathetic nervous activity (Nakagawa *et al.*, 2015).

On the effect of gender on the measured cardiovascular parameters on salt loading, female gender played a protective role on cardiovascular function especially in a normotensive condition as evident by insignificant increases observed in heart rate, systolic, diastolic and mean arterial blood pressure seen in the normotensive females after salt ingestion but these were not the case in

the hypertensive female counterparts is as all these parameters (SBP, DBP and MABP) were significantly elevated in them. This implies that oestrogen though is reported to play a protective role in cardiovascular function in premenopausal women (Lorga *et al.*, 2017), this role seems to be impaired in a hypertensive state.

CONCLUSION

In the study subjects, basal ANP levels were similar in normotensive and uncomplicated hypertensive states. However, salt loading significantly elevated ANP concentrations in normotensive subjects with greater ANP response in females than males but not significantly increased in hypertensive volunteers. Findings in this study suggest that ANP as well as female gender could ameliorate increased blood pressure response to salt loading primarily in normotensive individuals.

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