SALT INTAKE, SALT SENSITIVITY AND HYPERTENSION IN NIGERIANS: AN OVERVIEW.

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ABSTRACT

It is widely recognized that a high dietary intake of salt can result in hypertension and various studies have confirmed this link. Epidemiological studies have shown that communities that consume large amounts of salt in their diet have a high incidence of hypertension. Studies in experimental animals show that giving high salt in the diet can result in high blood pressure. Some of the mechanisms responsible for this observation include enhanced constriction response as well as reduced relaxation of resistance vessels. There also appears to be sexual dimorphism in the responses. In humans, our experiments have reported elevated blood pressure in response to oral salt loading and this elevation or Salt Sensitivity may be related to increased salt retention. Some suggestions include genetic defect in the renal tubules in the handling of sodium ions, due to mutation in the Epithelial Sodium Channel (ENaC), which is common in blacks. Our studies with the drug, amiloride that blocks ENaC tend to confirm this, as well as experiments that test sympathetic nervous system mechanisms by the Cold Pressor Test (CPT) which confirm vascular hyperreactivity. Subsequently we hope to conclude genetic studies on ENaC polymorphism and mutations in our subjects. The eventual goal is the development of screening mechanisms to identify individuals that are salt sensitive and so advise on dietary salt restriction in order to reduce the incidence of hypertension.

Key words and phrases: Salt, Hypertension, Salt Sensitivity, Epithelial Sodium Channel (**ENaC**).

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1. INTRODUCTION

Salt or sodium chloride has been used over the years as food condiment as well as preservative. Over the millennia it has been available from sources including sea water, rock salt and recently, manufactured industrially. The universal use of salt has also resulted in behavioural trends in which its consumption level varies in many different communities. Sodium in salt is the major cation in the body fluids and blood. Its osmotic property allows it to attract water along with it especially at the nephrons such that excessive salt intake can result in expansion of blood volume which can result in high blood pressure or hypertension.

Thus, there is extreme interest in the relationship between the amount of salt consumption in food and the level of arterial blood pressure. The recommended average daily intake is about 5g (or 86 mmol) per day, equivalent to about 1 teaspoon but actual consumption ranges between 1 g per day to as high as over 10 - 12g in some populations (Meyer, 2010). Epidemiological studies have shown a correlation between the level of salt intake and the incidence of hypertension. For example, communities that consume very little or no salt in their diet such as Eskimos, Kalahari tribesmen of Southern Africa and Yanomamo Indians in Brazil among others have incidence of hypertension which is virtually zero whereas those that consume a high salt in their diet such as the inhabitants of Northern Japan do have incidence of hypertension that is as high as 40% of the population (Oliver, Cohen, & Neel, 1975). Indeed the most well-known community with regard to low salt intake are the Yanomamo Indians on the border of Venezuela and Brazil who have been reported to ingest as little as 0.46g/day (20mmol/day) of sodium (Food and Nutrition Board, 2004) and at the age of 50 years, the average blood pressure in this community is only 100/64 mmHg (Meneton, Jeunemaitre, De Wardener et al, 2005).

A large-scale study that involved 52 centres in 32 countries and encompassing over 10,500 subjects, the INTERSALT study (INTERSALT 1988), found a correlation between the quantity of salt consumption and the level of blood pressure. Salt intake was estimated from the quantity of sodium excreted in urine in 24 hours which is termed 24 hours Urinary Na excretion. By contrast, another study that looked into the effect of salt restriction in the diet, the Dietary Approach to Stop Hypertension (DASH) showed that reduction of salt intake in the diet resulted in the lowering of blood pressure in both normotensive and hypertensive subjects (Sacks, Svetkey, Volman et al, 2001). The DASH diet which has been made popular in the United States involves the reduction in consumption of high salt-containing processed food and replacements with fruits and germ cereals. The results show significant beneficial effects on not only blood pressure but also on cardiovascular well-being. A recent meta-analysis of studies on salt and blood pressure has estimated that reduction in salt intake of 5*gm* (1 teaspoon) could result in 25% fewer strokes and other cardiovascular events (Meyer, 2010).

The afore-mentioned observations became an impetus to embark on studies to find out the mechanisms by which high salt in the diet can result in elevation of blood pressure. Studies were then carried out on experimental animals in order to test the effects of high salt diet on blood pressure. Previously, Dahl (Dahl, Heine & Tassinari, 1962) had developed genetically-selected rats that developed hypertension when subjected to a high salt diet, the so-called Dahl Salt Sensitive (DahlSS) rats, as well as the subset of salt resistant strains or Dahl-SR rats. However, in our studies we have used normal or non-genetically selected Sprague - Dawley (SD) rats. If weanling SD rats of 4 - 6 weeks of age are fed a high salt diet containing 8% sodium chloride, for about 6 - 8 weeks, they often developed high blood pressure (Miyajima & Bunnang, 1985; Obiefuna, Ebeigbe, Sofola et al, 1991). Other studies involving chimpanzees (Denton, Weisinger, Mundy et al., 1995). or dogs (Hainsworth, Sofola, Knill & Drinkhill, 2003), fed with high salt diet have also reported elevated blood pressure in these experimental animals.

2. STUDIES ON EXPERIMENTAL ANIMALS

In our rat studies, high dietary salt intake resulted in high blood pressure which can be attributed to vascular mechanisms. We therefore set out to investigate the responses of isolated blood vessels of rats and dogs that have been fed a diet that has a high salt content. In arterial vessels, which we studied by using isolated aortic ring segments (Figs 1, 2), or the pressurized mesenteric artery preparation (Fig 3), a high salt diet resulted in enhanced constriction tone (Obiefuna, Ebeigbe, Sofola et al, 1991; Sofola, Knill, Hainsworth et al., 2002) as well as reduction in relaxation responses to agonists (Sofola, Knill, Myers et al, 2004), both factors that will increase vascular resistance and hence blood pressure. In addition, the veins which act as conduit vessels, that return blood to the heart, also show enhanced constriction tone after a high salt diet in the dog (Hainsworth, Sofola, Knill et al, 2003). The effect of this is that venous return to the heart will be increased leading to high cardiac output and hence high blood pressure.

The reduced relaxation response that we reported in arterial resistance vessels was shown to be mediated by changes in the signaling mechanism in the vessel where the usual vasodilator agent - the Endothelium Derived Relaxing Factor (EDRF) is replaced by another vasodilator agent, the Endothelium Derived Hyperpolarizing Factor (EDHF) (Sofola, Knill, Hainsworth et al., 2002). In these experiments, perfused and pressurized mesenteric vessels exhibited identical relaxation responses to acetylcholine but this response was reduced by the inhibitor of Nitric Oxide Synthase (NOS), N-methyl L-arginine methyl ester (L-NAME) in rats fed a high salt diet, but abolished by blocking EDHF with apamin and charybdotoxin in both normal salt and high salt rats (Sofola, Knill, Hainsworth et al, 2002). We have also reported reduction in cyclic- AMP mediated relaxation responses in isolated aortic ring preparation (Sofola, Momoh, Igbo et al, 2003). Equally, the concurrent administration of potassium ions (K+) or the drug spironolactone, a potassium-sparing agent, has been shown to reverse or inhibit elevated blood pressure as well as the constriction responses of blood vessels to agonists following a high salt diet (Sofola & Adegunloye, 1998). This observation is important in that it has been well reported that intake of potassium e.g. in fruits as well as calcium as in skimmed milk, can reduce the tendency to develop high blood pressure, as shown as part of the recipe of the DASH diet (Sacks, Svetkey, Volman et al., 2001). In addition, we have reported that the male hormone, testosterone, when administerd exogenously, may actually reduce the tendency for blood vessels to contract and so prevent development of hypertension in contrast to the belief that this hormone contributes to the higher incidence of hypertension in males (Oloyo, Sofola, Nair et al, 2011). However, following orchidectomy, the elevation in blood pressure in response to a high salt diet is reduced suggesting that endogenous testosterone production contributes to blood pressure elevation via genomic mechanisms (Oloyo, Sofola & Anigbogu, 2011), in contrast to the vasorelaxant action of exogenously administered testosterone, acting via non-genomic mechanisms (Oloyo, Sofola, Nair et al, 2011).

3. SALT LOADING AND BLOOD PRESSURE IN HUMAN SUBJECTS

Following several reports and experiments in laboratory animals, we have now shifted our focus to studies in human subjects. As mentioned earlier, dietary salt intake can be evaluated from the 24-hour urinary excretion of sodium ion. Experiments were carried out in which oral salt of 200 - 400 mmol (about 11 - 22g of salt) was given to subjects over a period of three days. Blood pressure and other parameters such as serum levels of sodium and potassium as well as urinary excretion of these ions were then monitored (Azinge, Mabayoje, Sofola et al, 1999). The background to these series of studies is based on reports that blacks e.g. African Americans or native Africans do have a higher incidence of hypertension when compared with Caucasians. Furthermore, it has been shown conclusively that blacks tended to respond

with a high blood pressure in response to salt than their Caucasian counterparts. The incidence of salt sensitive hypertension in normotensive and hypertensive Caucasians in the United States is about 29% and 56% respectively compared with 43% and 72% respectively in normotensive and hypertensive African Americans (Weinberger, 1996). In Nigeria, the incidence of hypertension has recently been reported to be about 32.8% (Ulasi, Ijoma & Onodugu, 2010). In a study in Britain, the incidence of hypertension in blacks living in South London has been reported to be as high as 50% (Cappucio, Cook, Atkinson et al). There thus appears to be a higher association between salt intake and hypertension in blacks. This may have some relationship to salt sensitivity.

Salt sensitivity in blacks has historical and scientific backgrounds. The historical background on bio-history of slavery suggests that surviving "slaves" after the transatlantic shipment did survive because of their possessing inherent sodium conserving mechanisms during heat exposure and this trait was then transferred to later generations (Wilson & Grim, 1991). However, lately it has been shown that some aspects of salt sensitivity in blacks is possibly related to defective Epithelial Sodium Channel (ENaC), which is the membrane channel mechanism that regulates the final adjustments for sodium reabsorbed at the distal tubules of the kidney thus regulating the final amount of sodium ions that are retained in the body. A defective channel that results in excessive sodium reabsorption will lead to its accumulation in the body and hence cause the tendency for blood pressure to increase. This defect in ENaC occurs in about 5% of hypertensive blacks in the United States compared with less than 1% of Caucasians (Baker, Duggal, Dong et al, 2002). The defect has been linked to a transmutation of T594M gene, where Threonine is exchanged for Methionine, in the β subunit of the genes. The ENaC is regulated by Aldosterone and is Amiloride-sensitive. Thus, the drug Amiloride can be useful in cases of hypertension with defective ENaC (Baker, Duggal, Dong et al, 2002).

Our current studies are thus looking at:

- Salt Taste Threshold variability in individuals
- Salt sensitivity of blood pressure in normotensive and hypertensive Nigerians
- Blood pressure responses to neurally-mediated sympathetic challenges using the Cold Pressure Test and
- The role of ENaC in salt sensitivity in normotensive and hypertensive Nigerian subjects

Salt Taste threshold (STT) examines individual perception of salt taste at different salt concentrations. A high salt taste threshold will suggest tendency to a high salt consumption because of the reduced taste perception for salt which can then lead to an increase in its consumption and hence a rise in blood pressure. Some studies in our environment have reported correlation between a high salt taste threshold and hypertension (Obasohan, Ukoh, Onyia et al. (1992).

Salt sensitivity describes the propensity of individuals to show meaningful changes, increases or decreases, in mean arterial blood pressure (MABP)in response to sodium repletion or restriction respectively (Sanders, 2008). Hypertensive subjects are generally more salt sensitive than normotensive subjects (Franco, Oparil, 2006; He, Gu, Chen et al, 2009). Salt sensitivity is useful in predicting target organ damage such as left ventricular hypertrophy, renal dysfunction, and increased mortality (Titze & Machnik, 2010). Salt sensitivity is determined when the Mean Arterial Blood Pressure (MABP) increases by ≥ 5mmHg following a salt load (Cooper & Hainsworth, 2002; Schmidlin, Forman, Sebastian et al, 2007). Preliminary

results among our Nigerian subjects, following salt loading with 200mmol sodium daily for 5 days, have shown that salt sensitivity occurred in 52% of normotensive subjects and in 60.7% of age-matched hypertensive group of subjects respectively (Elias, Azinge, Umoren et al, 2011).

4. SALT SENSITIVITY AND SALT REACTIVITY

The Cold Pressor Test (CPT), determined from blood pressure response to cold immersion of the foot in ice slurry at $4^{\circ}C$, was carried out in our normotensive and hypertensive subjects before and after salt loading with 200mmol of sodium daily for 5 days. The CPT allows categorization of vascular reactivity in subjects. Hyperreactivity occurs when the elevated blood pressure response to the CPT is $\geq$ 15mmHg, systolic or diastolic (Kasagi, Akahoshi, Shimaoka, 1995; Chen et al, 2008). Our recent preliminary results have shown that 71% of normotensive subjects and 68.2% of hypertensive subjects are hyperreactive and these figures increased and reduced respectively following salt-loading. More subjects are being recruited to study this in more detail.

Results from our laboratory also show that salt sensitivity among normotensive and hypertensive subjects is positively correlated with systolic reactivity. Being a predictor of the tendency to develop hypertension, this Cold Pressor Test is being developed as a screening test for pre-hypertensive subjects among our populace.

Our current on-going work seeks to eventually develop scientifically based criteria for determining salt sensitivity in an individual and therefore the prediction of hypertension in Nigerians. If this is established, we can therefore carry out dietary counseling to susceptible individuals so as to reduce the tendency towards developing high blood pressure. Our experiments on the assessment of ENaC will also allow us eventually to develop a possible genetic marker for identifying those with defective ENaC gene. The ultimate is to be able to carry out real genetic studies in order to determine actual genetic profiling, identify genetic determinants and hopefully determine the genomic mechanism for salt sensitivity and hence of salt induced hypertension.

In conclusion, the large number of studies on experimental animals and man has linked a high intake of salt with the possibility of developing hypertension. However, not everyone will develop hypertension from the intake of salt but a large proportion of people that are salt sensitive will do so. Therefore a reduction in the quantity of salt in cooked food for both normotensives and hypertensives, avoidance of high consumption of processed food, development of the habit of NOT adding extra salt to table food, identification of salt sensitive individuals and their dietary counseling will go a long way in reducing significantly, the incidence of hypertension as a result of salt intake in Nigerians.

References

Azinge E, Mabayoye OM, Sofola OA, Oshibogun A (1999). 24-hour urinary sodium, potassium, and creatinine and their relationship to blood pressure in adult Nigerians, *Nig Qt. J. Hosp. Med.* 9: 17-20.

Baker EH, Duggal A, Dong Y, Ireson NJ, Wood M, Markandu ND et al (2002). Amiloride, a specific drug for hypertension in black people with T594M variant? *Hypertension* 40: 13-17.

Cappucio F, Cook DG, Atkinson RW et al. (1997). Prevalence, detection and management of cardiovascular risk factors in different ethnic groups in South London. *Heart* 78: 555-562.

Chen J, Gu D, Jaquish CE, ChenC-S, Liu D, Hixson JE et al (2008). Association between blood pressure responses to the cold pressor test and dietary sodium intervention in a Chinese population, *Arch Intern Med* 168: 1740-1746.

Cooper VL & Hainsworth R (2002). Effects of dietary salt on orthostatic tolerance, blood pressure and baroreceptor sensitivity in patients with syncope, *Clin Auton Res.* 2002; 12: 236-241.

Dahl LK, Heine M & Tassinari L. (1962). Effects of chronic salt ingestion; evidence that genetic factors play an important role in susceptibility to experimental hypertension, *J. Exp Med.* 115: 1173-1180.

Denton D, Weisinger R, Mundy MI et al. (1995). The effect of increased salt intake on blood pressure of chimpanzees. Nature Medicine, 1: 1009-1016.

Elias SO, Azinge EC, Umoren GA, Jaja SI, Sofola OA (2011). Salt sensitivity in normotensive and hypertensive Nigerian subjects, *Nig Qtly J Hosp Med 21: 85 - 91*.

Food and Nutrition Board, Institute of Medicine: Sodium and Chloride. In "Dietary Reference intakes, Water, Potassium, Sodium, Chloride, and Sulfate", *Washington DC: Food and Nutrition Board, Institute of Medicine,* 2004.

Franco V, Oparil S (2006). Salt sensitivity, a determinant of blood pressure, cardiovascular disease and survival, *J Am College of Nutrition* 25: 247S-255S.

Hainsworth R, Sofola OA, Knill JP, Drinkhill MJ (2003). Influence of dietary salt intake on the response of isolated perfused mesenteric veins of the dog to vasoactive agents, *Am J Hypertens* 16: 6-10.

He J, Gu D, Chen J et al (2009). Gender difference in blood pressure responses to dietary sodium intervention in the GenSalt study, *J Hypertens* 27:48-54.

INTERSALT (1988). An international study of electrolyte excretion and blood pressure. Results of 24hr urinary sodium and potassium excretion, *BMJ* 297: 311-328.

Kasagi F, Akahoshi M, Shimaoka K. (1995). Relation between cold pressor test and development of hypertension based on 28-year follow-up, *Hypertension* 25(1):71-76.

Meneton P, Jeunemaitre X, De Wardener HE, MacGregor G (2005). Links between dietary salt intake, renal salt handling, blood pressure and cardiovascular diseases, *Physiol Rev* 85: 679-715.

Meyer BJ. Could reducing salt consumption prevent cardiovascular events? *Journal Watch Cardiology*, 2010 [Link to: http://cardiology.jwatch.org].

Miyajima E, Bunnang RD (1985). Dietary salt loading produces baroreflex impairment and mild hypertension in rats, *Am J. Physiol.* 24: H27-H284.

Obasohan AO, Ukoh VA, Onyia KA et al. (1992). Salt taste threshold in normotensive and hypertensive Nigerians, *Tropical Cardiol.* 18: 183-187.

Obiefuna PCM, Ebeigbe AB, Sofola OA, Aloamaka P (1991). Altered responses of aortic smooth muscle from Sprague Dawley rats with salt-induced hypertension, *Clin. Exp. Pharmacol. Physiol.* 18: 813-818.

Oliver, WJ, Cohen EL, & Neel JV. (1975). Blood pressure, sodium intake and sodium-related hormones in Yamomono Indians, a "no salt" culture, *Circulation*, 52: 146-151.

Oloyo AK, Sofola OA, Nair RR, Harikrishnan VS, Fernandez AC (2011). Testosterone Relaxes Abdominal Aorta in Male Sprague-Dawley Rats By Opening Potassium (K+) Channel and Blockade of Calcium (Ca2+) Channel, *Pathophysiology* 18: 247 - 53.

Oloyo AK, Sofola OA, Anigbogu CN (2011). Orchidectomy attenuates impaired endothelial effects of a high salt diet in Sprague Dawley rats, *Can J Physiol Pharmacol* 89: 295 - 304 doi:10. 1139/Y11-023.

Sacks FM, Svetkey LP, Volman WM et al., (2001). Effects on blood pressure of reduced dietary sodium and dietary approaches to stop hypertension (DASH) diet, *N Engl. J. Med.*, 344: 3-10.

Sanders PW (2008). Salt sensitivity; It is not always in the genes, *Hypertension* 51: 823-824.

Schmidlin O, Forman A, Sebastian A, Morris RC Jr. (2007). What initiates the pressor effect of salt in salt-sensitive humans? *Hypertension* 49: 1032-1039.

Sofola OA and Adegunloye, B.J. Effects of potassium supplementation and potassium-sparing agent-Spironolactone on blood pressure and vascular responses of salt-loaded rats, *Nig. Qtl. J. Hosp. Med.* 8: 298-300, 1998.

Sofola OA, Knill A, Hainsworth R. Drinkhill MJ (2002). Change in endothelial function in mesenteric arteries of Sprague Dawley rats fed a high salt diet, *J. Physiol.* 543: 255-260.

Sofola OA, Knill A, Myers D, Hainsworth R, Drinkhill MJ (2004). High salt diet and responses of pressurised mesenteric artery to noradrenaline and acetylcholine, *Clin Exp Pharmacol Physiol* 31: 696-699.

Sofola OA, Momoh Y, Igbo I, Newa ZM, Oyekan A (2003). High salt diet modulates c-AMP and nitric oxide-mediated relaxation responses to isoproterenol in the rat aorta, *Eur J Pharmacol* 474: 241-247.

Titze J, Machnik A (2010). Sodium sensing in the interstitium and relationship to hypertension, *Curr Opin Nephrol Hypertens* 19: 385-392.

Ulasi II, Ijoma CK and Onudugo (2010). A community-based study of hypertension and cardio-metabolic sundrome in a semi-urban and rural communities in Nigeria, *BMC Health Serv Res*, 10: 71

Weinberger MH (1996). Salt-sensitivity of blood pressure in humans, *Hypertension* 18: 483-493.

Wilson TW & Grim CE (1991). Biohistory of slavery and blood pressure differences in blacks today, *Hypertension* 17: I-122 - I-128.

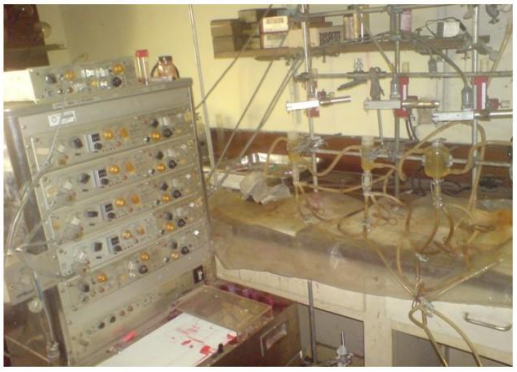

Figure 1. Grass Polygraph Model 7D Recorder and Organ Baths. The aortic rings are suspended in an array of organ baths which can record from 3 to 4 rings simultaneously

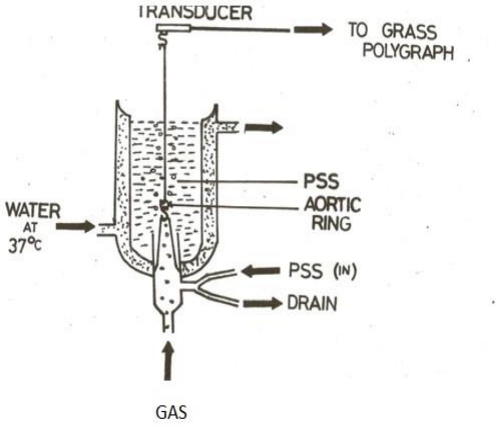

Figure 2. Schematic diagram of an Organ Bath. The aortic ring is suspended between a hook at the bottom of the bath and a Force transducer above and perfused with Physiological Salt Solution (PSS) at $37^{\circ}C$ bubbled with $95\%O_2$-$5\%CO_2$ gas mixture. The Force transducer is connected to the Grass polygraph to record tension developed by the ring during experimentation

Figure 3. Pressurized vessel set up. The mesenteric artery is viewed with an inverted microscope (on the left), connected via a video camera, to a monitor which records the luminal diameter of the vessel digitally (displayed below the screen). Constriction of the vessel results in decreased diameter which can be measured.

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