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Research article

Testosterone Enhances the Early Onset and Promotes the Increase in Magnitude of Salt-Induced Hypertension in Male Sprague-Dawley Rats.

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ABSTRACT: Blood pressure has been reported to be consistently higher in males compared with females from puberty onwards and men show an increased risk for hypertension compared to women, a risk that interacts with genes and with diet. Experiments were designed to assess the effect of testosterone deficiency on blood pressure in male Sprague-Dawley rats on normal or high salt diet. Weanling male rats were randomly divided into 8 groups (n = 6 each) that were either orchidectomised or sham-operated (under ketamine and xylazine anaesthesia), with or without testosterone replacement (10mg/kg sustanon 250® i.m once in 3 weeks), and were placed on normal (0.3%) or high (8%) NaCl diet for 6 weeks. Arterial blood pressure was determined before and weekly throughout the experimental period using non-invasive tail cuff method. There was a significant increase ($P < 0.001$) in the mean arterial blood pressure of rats placed on high salt diet when compared with control or orchidectomised rats. Orchidectomy elicited a reduction in MABP while testosterone replacement normalized MABP to values observed in intact rats placed on high salt diet. Endogenous testosterone promotes blood pressure-elevating effect of a high salt diet.

Keywords: Orchidectomy, Testosterone Supplementation, Salt-induced Hypertension, Blood Pressure

INTRODUCTION

Blood pressure has been reported to be consistently higher from puberty onwards, in males when compared with females and there is also a greater incidence of hypertension, which is one of the most common cardiovascular diseases, in men and post-menopausal women than in the pre-menopausal women (Orshal and Khalil, 2004). There is an increasing interest in the influence of high dietary salt intake on the incidence of high blood pressure. High salt diet has been implicated in the pathogenesis of human hypertension, particularly in salt sensitive individuals, as high salt in the diet was correlated with the level of blood pressure (Hollenberg, 2006; Sanders, 2009). Experimental studies have also shown that high salt diet results in elevated blood pressure in animals such as Dahl salt sensitive rats (Zheng *et al.*, 2008), Spontaneously hypertensive rats (SHR) (Matavelli *et al.*, 2007), Sabra rats (Khalid *et al.*, 2002) Sprague-Dawley rats (Sofola *et al.*, 2002) and dogs (Hainsworth *et al.*, 2003). Sex differences in Salt sensitivity in humans have been the subject of many studies, but at best it has been inconsistent. Some studies have reported that girls are more likely than boys to show BP reduction in response to low sodium diet (Wilson *et al.*, 1999), while boys showed more increase in BP compared with girls in response to high sodium diet (Wilson *et al.*, 1996), a finding that is consistent with that of the Dietary Approach to Stop Hypertension DASH – Sodium trial (Vollmer *et al.*, 2001). On the contrary, a more recent study by the

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Genetic Epidemiology Network of Salt Sensitivity (GenSalt) group reported that BP response to dietary sodium intake was greater in females when compared with males suggesting that females have higher sensitivity to salt diet (He *et al.,* 2008). Sexual dimorphism has also been reported in salt sensitivity in experimental animals. Several studies have suggested that male rats are more sensitive to dietary NaCl exposure than female rats (Chappel *et al.,* 2008). When male and female Dahl salt sensitive rats are fed high salt diet, males become more hypertensive (Hinojosa – Larbode *et al.,* 2004).

Gender disparity in salt-sensitive hypertension has been interpreted primarily as reflecting estrogen-mediated protection of females to vascular effect of a high salt diet. Whereas previous studies have demonstrated acute relaxant responses to testosterone in various vascular beds, this observation is not consistent with the greater predisposition of males to hypertension. Therefore this study was designed to evaluate the effect of orchidectomy and subsequent testosterone replacement the onset and magnitude of salt-induced hypertension in male Sprague-Dawley rats.

MATERIALS AND METHODS

Weanling male rats (8 weeks old) with weight range of 90-110g were used. They were housed in steel cages and maintained at 12 hours light and 12 hours dark period. Food and water was provided *ad libitum*. The rats were divided into 8 groups of 8 rats each. Groups 1 and II were intact rats, groups III and IV were orchidectomised rats, groups V and VI were rats that were given Sustanon® injection as testosterone replacement following orchidectomy and groups VII and VIII were sham orchidectomised rats. For orchidectomy, rats were anesthetized with ketamine and xylazine (90mg and 10mg/Kg/body weight i.m) (Gonzales *et al.,* 2004), respectively, for bilateral removal of the testes under aseptic surgical conditions while in groups VII and VIII rats, the scrotal sacs were opened and sutured back as a model of sham orchidectomy. All operated rats received an injection of penicillin 300,000 i.u/Kg body weight at the time of surgery to prevent infections and were allowed a 3-day recovery period before the beginning of the experiments (Zhu *et al.,* 2005). After recovery from anesthesia, all animals were returned to their cages. Rats in groups I, III, V, and VII were fed with rat diet containing normal salt concentration (0.3% NaCl) and tap water for 6 weeks. Rats in groups II, IV, VI and VIII were fed with a high salt diet (8% NaCl), and tap water *ad libitum*, for 6 weeks (Adegunloye and Sofola, 1997). Group V and VI rats received 10mg/Kg body weight of testosterone suspension (Sustanon 250® i.m) once in 3 weeks during the study, for testosterone replacement. Sustanon 250® is a trade name for an oil-based injectable blend of four esterized testosterone compounds viz: 30mg Testosterone Propionate, 60mg Testosterone Phenylpropionate, 60mg Testosterone Isocaproate, and 100mg Testosterone Decanoate. The composition is a blend of fast, short acting and slow but long acting testosterone esters.

Determination of body weight

The animals were weighed before and weekly throughout the period of the experiment, using a Duet top loading scale (Salter, England). After the experimental period, the percentage weight gain was calculated by subtracting the initial weight from the final weight and dividing the difference by the final weight and then multiplied by 100. The percentage weight gains of the animals were compared across the groups.

Blood Pressure measurement

The conscious rats were placed in a restrainer on a heated pad and allowed to adapt / rest inside for 15 minutes before blood pressure was measured weekly. The procedure was performed in a room with an ambient temperature of 25^0C. The rat tail was placed inside 9mm or 11mm tail cuff, and the cuff was inflated and released several times to allow the animal become conditioned to the procedure. Five consecutive blood pressure and heart rates measurements were obtained using the non – invasive blood pressure monitor MP35 (BIOPAC System Inc USA), which was connected to a computer. Blood pressure tracings were obtained through preinstalled software for BSL Pro.3.7.

Determination of Heart and Kidney weights

After sacrifice the, heart and the kidneys were removed, carefully cleared of connective tissues, dried between filter paper and weighed. The weight index of each organ was taken as the division of the weight of such organ by the total body weight multiplied by 100. The weight index of each organ was compared across the groups.

Serum Testosterone Assay

Serum testosterone levels were measured by enzyme-linked immunoassay (EIA) (Marcus and Durnford, 1986) using a commercial kit from Biotech Laboratories (Suffolk, UK) according to the protocol of the manufacturer. The kit uses the principle of competitive microplate enzyme immunoassay, whereby testosterone present in the sample competes with

enzyme-testosterone conjugate for binding with anti-testosterone coated microplate to form an antigen-antibody complex.

Statistical Analysis

The collected data were expressed as means ± S.E.M. The data were analyzed using one way analysis of variance (ANOVA). Student-Newman-keuls post Hoc test was used to identify differences between individual means. Confidence interval was placed at 95%, so that in all cases a value of $P < 0.05$ was considered significant.

RESULTS

Body and organs weights

Table 1 shows the percentage weight gain and the mean heart and kidneys indices in each group. In all groups, there was an increase in the body weight after six weeks. However the percentage increase in weight in the high salt group was significantly lower ($P < 0.001$) when compared with that of the intact plus normal salt group (control). The percentage weight gain in the testosterone replacement plus high salt diet group and sham orchidectomised plus high salt diet group was also significantly lower ($P < 0.001$) when compared with their corresponding normal diet (control) groups. However the percentage weight gain of the orchidectomy plus high salt diet group was significantly higher ($P < 0.01$) when compared with that of intact and high salt diet group. There were no significant differences in the percentage weight gain of the sham-orchidectomised groups when compared with that of their corresponding intact groups. There was a significant increase ($p < 0.05$) in the mean heart index of the intact plus high salt diet group when compared with the intact plus normal salt diet group (control). However there was a significant decrease ($P < 0.05$) in the heart index of orchidectomy plus high salt group when compared with intact plus high salt diet group, while there was no significant difference between the orchidectomised plus high salt group when compared with the intact plus normal salt group (control). There was a significant increase ($p < 0.001$) in the kidney index of high salt diet group when compared with their corresponding control groups, except in the orchidectomised groups where there was no significant difference between the kidney index of the normal diet group and high salt group. There was a significant decrease ($P < 0.01$) in the kidney index of orchidectomy plus high salt group when compared with intact plus high salt diet group.

Blood pressure

Table 2 shows the initial, final and percentage increase in mean arterial blood pressure (MABP) of the rats in all the groups. Figure 1 shows the weekly mean MABP of the rats. Mean arterial blood pressure (MABP) of 115mmHg was used as the cut-off point for hypertension in this study. This was achieved by adding 10% increase to 105mmHg, the mean value of MABP in non-hypertensive rats (Rapp and Dene, 1985).

Table. 1:
Percentage body weight gain, heart and the kidneys weight indices of the rats across the groups.

Groups (n =10)	% Weight Gain	Heart Index	Kidney Index
INT + NS	58.73 ± 0.33	0.29 ± 0.006	0.57 ± 0.03
INT + HS	46.72 ± 1.04***	0.31 ± 0.007$^{\#}$	0.65 ± 0.04$^{\#\#}$
ORCH + NS	57.71 ± 0.80	0.26 ± 0.012	0.55 ± 0.01
ORCH + HS	51.10 ± 0.49††	0.28 ± 0.009$^{\wedge}$	0.57 ± 0.02$^{\wedge\wedge}$
ORCH + TES + NS	58.79 ± 0.55	0.28 ± 0.009	0.55 ± 0.02
ORCH + TES + HS	50.57 ± 0.47***	0.28 ± 0.008	0.60 ± 0.01$^{\#\#}$
SHAM + NS	58.19 ± 0.50	0.28 ± 0.002	0.55 ± 0.01
SHAM + HS	47.24 ± 0.72***	0.30 ± 0.004$^{\#}$	0.67 ± 0.02$^{\#\#\#}$

Keys: ***INT*** *= intact,* ***ORCH*** *= orchidectomy,* ***NS*** *= normal salt,* ***HS*** *= high salt,* ***TES*** *= testosterone,* ***SHAM*** *= sham orchidectomy. Data are presented as Means ± S.E.M (n = 10).* ****Significantly less ($P < 0.001$) when compared with corresponding control groups,* ††*Significantly high ($P < 0.01$) when compared with intact plus high salt diet group. Significantly high ($P < 0.05^{\#}$; $P < 0.01^{\#\#}$; $P < 0.001^{\#\#\#}$) when compared with control. Significantly less ($P < 0.05^{\wedge}$; $P < 0.01^{\wedge\wedge}$) when compared with intact plus high salt group.*

Table. 2:
Initial, final, and percentage difference of the mean arterial blood pressure of the rats across the groups.

Groups (n = 6)	Initial MABP (mmHg)	Final MABP (mmHg)	% Difference
INT + NS	99.5 ± 2.43	112.2 ± 3.71	15.23 ± 0.78
INT + HS	100.3 ± 2.33	134.3 ± 4.28***	28.57 ± 1.23***
ORCH + NS	100.0 ± 1.68	106.0 ± 3.02$^{\#\#}$	7.55 ± 0.65$^{\#\#}$
ORCH + HS	100.3 ± 1.95	††124.2 ± 2.96***	††19.08 ± 1.01***
ORCH + TES + NS	100.5 ± 1.56	112.5 ± 2.85	10.67 ± 0.74
ORCH + TES + HS	101.0 ± 1.68	132.7 ± 3.52***	23.71 ± 1.65***
SHAM + NS	99.67 ± 1.84	116.1 ± 3.01	14.23 ± 0.81
SHAM + HS	101.7 ± 2.49	134.7± 3.52***	28.3 ± 1.62***

***Keys: INT** = intact, **ORCH** = orchidectomy, **NS** = normal salt, **HS** = high salt, **TES** = testosterone, **SHAM** = sham orchidectomy. Data are presented as means ± S.E.M (n = 6). ***significant increase (P < 0.05) when compared with corresponding control, ††significant decrease (P < 0.01), when compared with intact plus high salt group. $^{\#\#\#}$significant decrease (P < 0.001) when compared with control.*

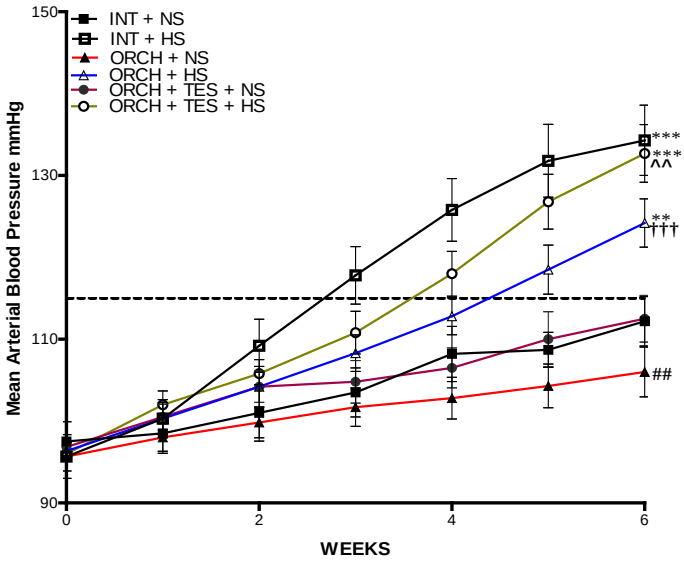

Fig. 1:
Mean arterial blood pressure of intact and orchidectomised male Sprague Dawley rats fed a normal or high salt diet with or without testosterone supplementation ORCH = orchidectomy, NS = normal salt, HS = high salt, TES = testosterone.
Data are presented as means ± S.E.M (n = 6). Significantly high (**P < 0.01, ***P <0.001) when compared with Control. $^{\wedge\wedge}$Significantly high (P < 0.01) when compared with orchidectomy plus high salt diet group. $^{\#\#}$significantly less (P < 0.01) when compared with control plus testosterone replacement plus normal salt diet group. †††significantly less (P < 0.001) when compared with intact plus high salt group.

Non-invasive blood pressure monitoring via tail plethysmography showed that the blood pressure elevating effect of a high salt diet set in around the second week of salt loading, and by the third week the intact and high salt group had developed hypertension. Orchidectomy delayed the onset of hypertension following a high salt diet till about the fifth week of salt loading. In rats that were given testosterone replacement following orchidectomy and concurrently fed a high salt diet, blood pressure elevation also set in at about the second week, and reached the hypertensive level about the third week. After the six week experimental period, there was an increase in the mean arterial blood pressure of rats in all the groups. However, the increase in MABP in the high salt diet groups was significantly higher (P < 0.001)

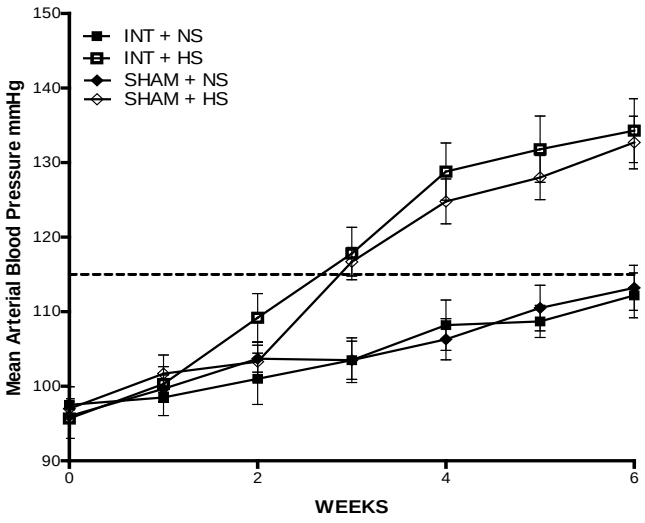

Fig. 2:
Mean arterial blood pressure of intact and sham-orchidectomised Sprague-Dawley rats fed a normal or high salt diet. ORCH = orchidectomy, NS = normal salt, HS = high salt, TES = testosterone. Data are presented as means ± S.E.M (n = 6).

from the third week upward to the sixth and the final week of the experiment, when compared with their corresponding control (normal salt diet) groups.

Whereas the elevation in the MABP in the orchidectomy plus high salt group was significantly less ($P < 0.05$) when compared with the intact plus high salt diet group (Figure 1). On the other hand the increase in the MABP of rats in the testosterone replacement groups was significantly higher ($P < 0.01$) when compared with their corresponding orchidectomised groups without testosterone replacement. The increase in MABP of the testosterone replacement groups is almost comparable to that of the intact groups. Also elevation of MABP after the six week experimental period in the orchidectomy plus normal salt group was significantly less ($P < 0.01$) when compared with the intact plus normal salt diet group. Sham-orchidectomy has no significant effect ($P > 0.05$) on the MABP both in the normal diet plus high salt diet groups (Figure 1). There was no significant difference in the MABP of the sham-orchidectomised groups when compared with that of the intact groups (Figure 2). The percentage difference between the final and initial MABP of the rats follows the same trend as described above, i.e. after the six week experimental period, there were increase in the final MABP when compared with the initial MABP across all the groups.

However the percentage increase in the MABP of all the high salt fed groups were significantly higher ($P < 0.001$) when compared with the groups fed a normal salt diet, but the percentage increase recorded in the orchidectomy plus high salt group was significantly less ($P < 0.01$) when compared with intact plus high salt diet group. Also the percentage increase in the MABP of the orchidectomy plus normal diet group was significantly less ($P < 0.01$) when compared with the intact plus normal diet group (Table 2). Conversely, the increase in the percentage difference in the testosterone replacement groups was significantly higher ($P < 0.01$) when compared with their corresponding orchidectomised groups i.e. groups without testosterone replacement (Table 2).

Testosterone Assay

Figure 3 shows the serum level of testosterone across the groups. There was a significant decrease ($P < 0.001$) in the serum level of testosterone in the orchidectomised groups when compared with the intact groups. Serum level of testosterone was also significantly reduced ($P < 0.001$) in the orchidectomised groups when compred with that of the groups that recieved testosterone replacement following orchidectomy and the sham-orchidectomised groups.

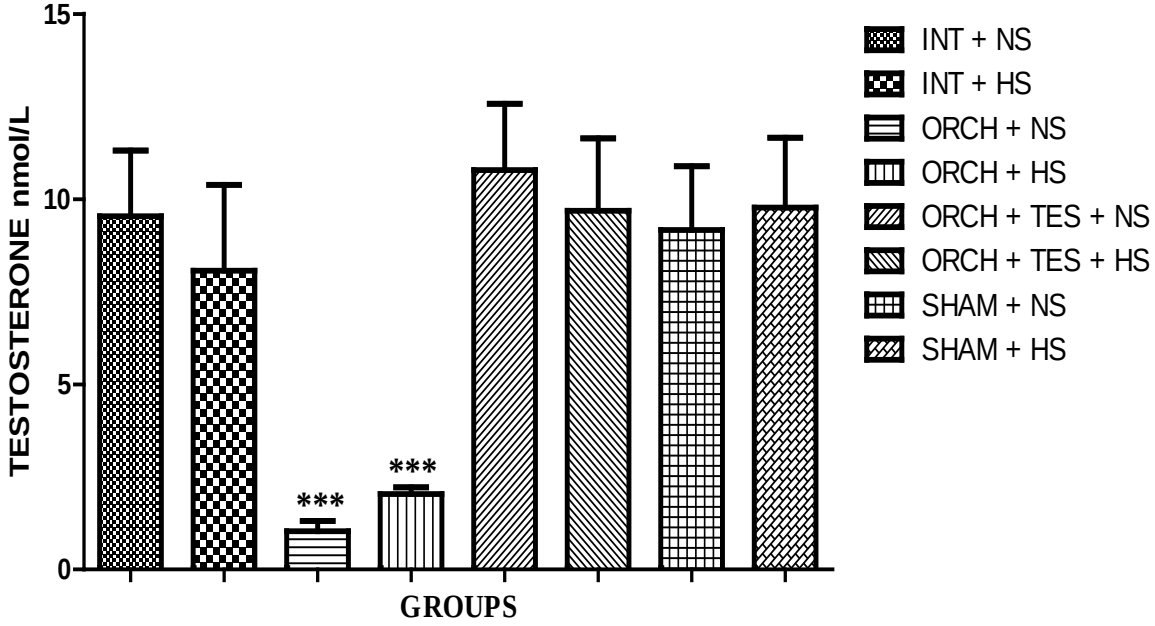

Fig 3:

Serum level of testosterone across the groups. INT = intact, ORCH = orchidectomy, NS = normal salt, HS = high salt, TES = testosterone supplementation, SHAM = sham orchidectomy. Data are presented as means ± S.E.M, (n = 5). Significant decrease ($^{***}P < 0.001$) when compared with the intact, testosterone replacement and sham-orchidectomised groups.

Testosterone replacement restored the serum testosterone level to that observed in the intact groups as there was no significant difference ($P > 0.05$) between the testosterone replacement group and the intact group. Likewise there was no significant difference ($P > 0.05$) in the serum testosterone level of the sham-orchidectomised groups when compared with that of the intact groups. High salt diet did not affect the serum level of testosterone, because there was no significant change ($P > 0.05$) in the serum level of testosterone in the groups fed a normal salt diet when compared with that of the high salt fed groups.

DISCUSSION

Results from this study show that a high salt diet resulted in reduction in the bodyweight of the rats. The higher magnitude in the increase in the final mean body weight of rats in the orchidectomy plus high salt group when compared with the intact plus high salt group indicates that orchidectomy attenuated body weight reducing effect of a high salt diet. This observation of reduced gain in body weight is similar to the report of Coelho *et al.,* (2006), and this may be related to an increase in body water loss through excessive urination in the intact high salt diet group which was observed during the experiments. High salt diet increased the heart and kidney weight indices in the rats. More recently, it has been recognized that excess salt is strongly associated with cardiac hypertrophy, a structural pattern observed in both hypertensive men and rats independently of the level of blood pressure (Ahn *et al.,* 2004; Matavelli *et al.,* 2007). A temporal link between increased NaCl intake and aortic hypertrophy has also been noted (Limas *et al.,* 1980; Partovian *et al.,* 1998) in spontaneously hypertensive rats (SHR) in the absence of a significant change in blood pressure. The finding that the heart and kidney indices of the orchidectomy plus high salt group were comparable to those of the control group, unlike those of the intact plus high salt group that was higher than the control implicates testosterone in the increase in the weight indices of the heart and the kidney of rats fed a high salt diet in this present study.

Normal non-genetically modified Sprague-Dawley rats develop high blood pressure when fed a high salt diet from weaning onwards (Adegunloye and Sofola, 1997; Ebuehi *et al.,* 2003). In this study, non-genetically modified Sprague-Dawley rats were used as a model of salt-sensitivity in normotensive males. This enabled us to ask how normotensive males develop high blood pressure in response to a high salt diet and what the role of androgens in this process is.

Feeding the rats with a high salt diet for six weeks elevated the mean arterial blood pressure. In this study, the blood pressure elevating effect of a high salt diet set in around the second week of salt-loading. By the third week, the intact as well as the orchidectomised rats that were given testosterone replacement and fed a high salt diet had developed hypertension. But the onset of hypertension was delayed to about the fifth week of salt-loading in the orchidectomised rats fed a high salt diet. Absence of any significant differences between the sham orchidectomy groups and the intact groups shows that the observed differences in the orchidectomy and testosterone supplemented groups when compared with the intact groups is definitely not due to the surgical trauma the rats underwent during the surgical procedure. These findings implicate testosterone in the modulation of mechanisms that are responsible for regulation of blood pressure during salt-stress and suggest that orchidectomy delayed while testosterone promoted the development of salt-induced hypertension in male Sprague-Dawley rats. Although blood pressure was elevated in the rats that were fed a high salt diet, the higher magnitude of blood pressure elevation in the groups of intact, and orchidectomised rats that were given testosterone replacement showed that testosterone is involved in the blood pressure elevating and consequently hypertension inducing effect of a high salt diet. The reduction in the magnitude of blood pressure elevation in orchidectomised rats fed a high salt diet shows that orchidectomy countered or prevented the blood pressure elevating effect of a high salt diet, therefore showing that testosterone plays a role in or complements the blood pressure elevating effect of a high salt diet and consequently salt-induced hypertension in male Sprague-Dawley rats. Our result is similar to the findings of other, using different strains of rats, Reckelhoff *et al.* 1999; Reckelhoff, 2001, reported that castration of young male SHR and DSS attenuates the development of hypertension when they were fed a high-salt diet. The study by Reckelhoff and Colleagues implicated renin angiotensin system in the gender difference in the development of hypertension in SHR, as disparity in blood pressure between male and female was abolished by chronic treatment with angiotensin-converting enzyme inhibitor. In addition, in their study, androgen failed to increase blood pressure in the presence of RAS blockade (Reckelhoff *et al.,* 2001), suggesting that testosterone elicit blood pressure elevation in these rats by activation of the RAS. Khalid *et al.* (2002) also reported that in male Sabra rats (SBH), a genetically-modified salt-sensitive strain, gonadectomy prevented the full development of

hypertension, however they suggested the effect of testosterone on the role of α_{2A}-adrenoceptor as the basis for the blood pressure reducing effect of orchidectomy in this rat strain. Conversely, Yagil and Yagil, (2000) reported that both in male and female newly selected Sabra rats (SBH_y) strain, gonadectomy had no effect on salt-induced hypertension. Discrepancy in their findings could be due to the different strains used, but this also shows that differences occur in physiological functions of laboratory animals from species to species and even within the same species. Findings from this present study shows that even in the absence of genetic risk factor for onset of hypertension such as in SHR, DSS and SBH (as exemplified in Sprague-Dawley rats), high salt diet could induce high blood pressure and that development of high blood pressure is delayed and attenuated by orchidectomy, but promoted by testosterone in Sprague-Dawley rats.

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REFERENCES

Adegunloye, B.J., and Sofola, O.A. (1997): Effect of dietary salt loading and high-calcium diet on vascular smooth muscle response and endothelium functions in rats. *Clin Exp Pharmac Physiol.* **24:** 814-818.

Ahn, J., Varagic, J., Slama, M., Susic, D. and Frohlich, E.D. (2004): Cardiac structural and functional responses to salt loading in SHR. *Am J Physiol Heart Circ Physiol.* **287**: H767– H772.

Chappell, M.C., Westwood, B.M., and Yamaleyeva, L.M. (2008): Differential Effects of Sex Steroids in Young and Aged Female mRen2.Lewis Rats: A Model of Estrogen and Salt-Sensitive Hypertension. *Gend Med.* **5**(Suppl A): S65–S75.

Coelho, M.S., Passadore, M.D., Gasparetti, A.L., Bibancos, T., Prada, P.O., Furukawa, L.L., Furukawa, L.N.S., Fukui, R.T., Casarini. D.E., Saad, M.JA., Luz, J., Chiavegatto, S., Dolnikoff, M.S., and Heimann, J.C. (2006): High- or low-salt diet from weaning to adulthood: effect on body weight, food intake and energy balance in rats. *Nutr Metab Cardiovasc Dis.* **16**:148e55.

Ebuehi, O.A.T., Elekolusi, O.A., Edegunloye, B.J., and Mojiminiyi, F.B.O. (2003): Effect of dietary salt-loading on blood pressure and erythrocyte Na^+-K^+-ATPase in rats. *Afr J.Medicine and Pharmaceut Sci.* **7**: 42-50.

Gonzales, R.J., Krause, D.N., and Duckles, S.P. (2004): Testosterone suppresses endothelium- dependent dilation of rat middle cerebral arteries. *Am J Physiol Heart Circ Physiol.* **286**: H552 – H560.

Hainsworth, R., Sofola, O.A., Knill, A.J.P., and Drinkhill, M.J. (2002): 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., Jaquish, C.E., Rao, D.C., Hixson, J.E., Chen, J., Duan, X., Huang, J., Chen, C., Kelly, T.N., Bazzano, L.A., and Whelton, P.K. (2008): Gender difference in blood pressure responses to dietary sodium intervention in the GenSalt study. *J Hypertens.* **27**:48–54.

Hinojosa-Laborde, C., Craig, T., Zheng, W., Ji, H., Haywood, J.R., and Sandberg, K. (2004): Ovariectomy augments hypertension in aging female Dahl salt sensitive rats. *Hypertension.* **44**: 405–409.

Hollenberg, N.K. (2006): The influence of dietary sodium on blood pressure. *JACN.* **25**: (3) 240S-246S.

Khalid, M., Ilhami, N., Giudicelli, Y., and Dausse, J.P. (2002): Testosterone dependence of salt-induced hypertension in Sabra rats and role of renal $\alpha_{(2)}$-adrenoceptor subtypes. *J Pharmacol Exp Ther.* **300**:43–49.

Khalil, R.A. (2006): Dietary salt and hypertension: new molecular targets add more spice. *Am J Regulatory Comp Physiol.* **290**: 509-513.

Limas, C., Westrum, B., Limas, C. J. and J. N. Cohn. (1980): Effect of salt on the vascular lesions of spontaneously hypertensive rats. *Hypertension.* **2**: 477–489.

Marcus, G.J., and Durnford, R. (1986): A simple enzyme-linked immunosorbent assay for testosterone. *Steroids.* **46**: 975–986.

Matavelli, L.C., Zhou, X., Varagic, J., Susic, D., and Frohlich, E.D. (2007): Salt loading produces severe renal hemodynamic dysfunction independent of arterial pressure in spontaneously hypertensive rats. *Am J Physiol Heart Circ Physiol.* **292**: H814–H819.

Orshal, J.M., and Khalil, R.A. (2004): Gender, sex hormones, and vascular tone. *Am J Physiol Regul Integr Comp Physiol.* **286**: R233–R249.

Partovian, C., Benetos, A., Pommie`s, J-P., Mischler, W. and Safar, M.E. (1998): Effects of a chronic high-salt diet on large artery structure: role of endogenous bradykinin. *Am. J. Physiol. Heart Circ. Physiol.* **43**: H1423–H1428.

Rapp, J.P. and Dene, H. (1985): Development and characteristics of inbred strains of Dahl salt-sensitive and salt-resistant rats. *Hypertension.* **7**: 340-349.

Reckelhoff, J.F. (2001): Gender differences in the regulation of blood pressure. *Hypertension* 37:1199–1208.

Reckelhoff, J.F., Zhang, H., Srivastava, K., and Granger, J.P. (1999): Gender differences in hypertension in spontaneously hypertensive rats: role of androgens and androgen receptor. *Hypertension.* **34**:920–923.

Sader, M., and Celermajer, D.S. (2002): Endothelial functions, vascular reactivity and gender differences in the cardiovascular system. *Cardiovasc Res.* **53**: 597 – 607.

Sanders, P.W. (2009): Vascular consequences of dietary salt intake. *Am J Physiol Renal Physiol.* **297**: F237–F243.

Schmidlin, O., Forman, A., Sebastian, A., and Morris, Jr. **R.C. (2007):** What initiates pressor effect of salt in salt sensitive Humans? Observation in normotensive blacks. *Hypertension.* **49:** 1032 – 1039.

Sofola, O.A., Knill, A.J.P, Hainsworth, R., and Drinkhill, M.J. (2002): Changes in endothelial functions in mesenteric arteries of Sprague Dawley rats fed a high salt diet. *J. Physiol.* **543:** 255-60.

Vollmer, W.M., Sacks, F.M., Ard, J., Appel, L.J., Bray, G.A., Simons-Morton, D.G., *et al.* (2001): For the DASH-Sodium Trial Collaborative Research Group. Effects of diet and sodium intake on blood pressure: subgroup analysis of the DASH-Sodium trial. *Ann Intern Med.* **135**:1019–1028.

Wilson, D.K., Bayer, L., and Sica, D.A. (1996): Variability in salt sensitivity classifications in black male versus female adolescents. *Hypertension.* **28**:250–255.

Wilson, D.K., Sica, D.A., and Miller, S.B. (1999): Ambulatory blood pressure non-dipping status in salt-sensitive versus salt-resistant black adolescents. *Am J Hypertens.* **12:**159–165.

Yagil Y., and Yagil C. (2000): The lack of a modulating effect of non-genetic factors (age, gonads and maternal environment) on the phenotypic expression of the salt susceptibility genes in the Sabra rat model of hypertension. *J Hypertens* **18:**1393–1399.

Zheng, W., Ji, H., Maric, C., Wu, X., and Sandberg, K. (2008): Effect of dietary sodium on estrogen regulation of blood pressure in Dahl salt-sensitive rats. *Am J Physiol Heart Circ Physiol* **294**: H1508–H1513.

Zhu, J., Yu, M., Friesema, J., Huang, T., Roman, R.J., and Lomband, J.H. (2005): Salt –induced ANG II suppression impairs the response of cerebral artery smooth muscle cells to prostacyclin. *Am J. physiol Heart Circ Physiol.* **288**: H 908-H913.