EFFECTS OF PRENATAL EXPOSURE TO DIFFERENT SALT CONCENTRATIONS ON THE THIRD MONTH'S WEIGHT AND BLOOD PRESSURE IN WISTAR RAT

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Background: *In utero* alterations in fluid and electrolyte endocrine systems may result in permanent effects on offspring. A low sodium intake during prenatal life jeopardizes growth in young rats, prenatal high-salt diet in Sprague-Dawley rats caused an increase in MAP at postnatal day 30. The objective of this study was to determine the effect of prenatal exposure to different salt concentrations on the third month's weight and blood pressure in Wistar rat. **Methods:** This study was performed at the Department of Physiology, Isfahan University of Medical Science, Isfahan, Iran, over a period from 1998 to 2003. Six groups of rat, 1 male and 5 female in each group were exposed to 0.5, 1, 1.4, 1.6, 1.8 and 2 percent of salt concentrations during pre-pregnancy, pregnancy and lactation period, another test group consumed distilled water and control group used Isfahan tap water, other living conditions for all groups were similar. Exposure to different salt concentrations on the third month's weight and blood pressure was evaluated. **Results:** Prenatal exposure to 0.5 and 1% salt concentrations gives birth to more alive and healthy infants, and third month's weight increased significantly, but blood pressure was not influenced significantly. Salt concentrations higher than 1% increased the maternal and infant mortality rate and blood pressure significantly, but some concentrations decreased third month's weight significantly. **Conclusion:** Level of dietary salt during intrauterine development can influence on the number of alive and healthy infants, birth weight, third month' weight and blood pressure significantly. There is no need to introduce a salt restricted diet in prenatal care, a balanced diet in sodium during pregnancy is recommended, high salt diet creates harmful effect.

Keywords: Blood Pressure, Pregnancy, Prenatal, Rat, Salt

INTRODUCTION

The development of chronic non-communicable diseases (CNCD) in epidemic proportions characterizes populations undergoing the nutrition and epidemiologic transitions (Forrester T, 2004). A recent World Health Organization report states that elevated blood pressure alone causes 50% of cardiovascular disease worldwide (Ezzati *et al*, 2002). Like obesity and diabetes, essential hypertension is one of the 'diseases of civilization' that results from the collision of a modern lifestyle with Paleolithic genes. Regardless of its genetic substrate, hypertension is clearly an ecogenic diseases, that is, environmental factors interact with genes to result in high blood pressure (Hollenberg NK, and Braunmwald E, 1998). Environmental factors also appear to make an important contribution to the development of hypertension in the SHR. Such factors include a variety of dietary and psychosocial stimuli (Di Nicolantonio *et al*, 2005). As far as the role of various electrolytes in blood pressure modulation is concerned, prohypertensive effects of dietary Na^+ and antihypertensive effects of dietary Ca^{2+} are enhanced in immature animals, whereas vascular protective and antihypertensive effects of dietary K^+ are almost independent of age (Zicha J, Kune J, 1999). The importance of dietary sodium chloride in the regulation of blood pressure has received much attention over the past few years (Jones DW, 2004). Prenatal salt loading or restriction exerts considerable effects on BP development in normotensive and genetically hypertensive rats. With the exception of two studies, high salt intake during pregnancy and lactation in normotensive, Dahl salt-sensitive, borderline hypertensive, or spontaneously hypertensive mother is always associated with elevated BP of their progeny later in life. On the other hand, severe Na^+ restriction (17 mmol Na^+/Kg diet) during intrauterine life and suckling period impairs body growth of SHR and lowers their BP in adulthood, but less severe Na^+ restriction (20–22 mmol Na^+/Kg diet) fails to modify body weight and BP in the offspring of normotensive Sprague-Dawley or SHR mothers. It is still not clear how the changes in Na^+ intake of the rat mother can affect the foetus or the pup (Magalhães *et al*, 2005). *In utero* hypertonicity and perhaps maternal nutrient stress may program offspring osmoregulation and systemic arterial hypertension (Ross *et al*, 2005). Association of adult SBP and diastolic BP (DBP) with determinants of foetal growth, size at birth, and growth in infancy was reported (Järvelin *et al*, 2004). Recent human studies have provided evidence that the *in utero* environment has an impact on foetal development and may alter homeostatic regulatory mechanisms, resulting in chronic diseases of adulthood [i.e., the Barker hypothesis] (Ross *et al*, 2005). In few studies the effect of different salt

concentrations consumption during prenatal period on the next generation's weight and Blood Pressure in rat has been noticed. The objective of this study was to determine the effect of prenatal exposure to different salt concentrations on the third month's weight and Blood Pressure in Wistar rat.

MATERIAL AND METHODS

This study was conducted at the Department of Physiology, Faculty of Medicine, Isfahan University of Medical Science, Isfahan, Iran. Prior to the initiation of experiment, study protocols were reviewed and approved by the animal research committee of Isfahan University of Medical Science. All works involving experimental animals were performed in full compliance with NIH Guidelines for the Care and Use of Laboratory Animals. This study was performed at the Department of Physiology, Isfahan University of Medical Science, Isfahan, Iran, over a period from 1998 to 2003. The animals were purchased from the Iranian Razi Institute, one of the certified centres of laboratory animals breeding in Iran. Male and female Wistar Rats were received at 12 weeks of age and were quarantined for one week prior to the administration of test or control articles. Before administration of salt concentrations, healthy animals were selected following physical examinations and 48 rats were allocated randomly to eight groups. Each group consisting of 5 non-pregnant female rats and one male rat, weighing 200±20 g were chosen to perform the experiment., animals were housed at 24 °C on a 12 h–12 h light–dark cycle with free access to Isfahan tap water and standard pellet chow containing 0.5% salt, Isfahan city potable water was delivered via a manual bottle watering system. During salt administration periods, different test groups consumed distilled water and special salt concentration as drinking water and control group consumed Isfahan potable water. In contrast to water, all groups were fed with same consistency of diet, containing approximately 0.5% salt, and other living conditions for all groups were alike. Chemical drugs including Ketamine hydrochloride of Alfasan Co. (Woerden, Holland) and NaCl of Merck Co. (Darmstadt, Germany) Brands were purchased. 0.5, 1, 1.4, 1.6, 1.8 and 2 percent in distilled water were prepared for inducing hypernatremia (Six concentrations of NaCl). 5, 10, 14, 16, 18 and 20 g of salt was dissolved in one litre of distilled water for preparing 0.5, 1, 1.4, 1.6, 1.8 and 2 percent salt concentrations. These salt concentrations contained about 75, 150, 210, 240, 270 and 300 mmol/L Na and Cl respectively and their osmolarity was about 150, 300, 420, 480, 540 and 600 mmol/L respectively. Test groups were exposed to 0.5, 1, 1.4, 1.6, 1.8 and 2 percent of salt concentrations during pre-pregnancy, pregnancy and lactation period, one test group consumed distilled water and control group used Isfahan tap water, other living conditions for all groups were alike. In this study, the effect of exposure to different salt concentrations in pregnant rat on the infant's third month weight and Blood Pressure were evaluated. At the end of breeding period (in 90 days), 21 alive and healthy infants were required for this experiment. As the maternal mortality rates in groups consuming concentrated salt concentrations were high and the number of healthy infants was low, so it was decided to perform breeding task in more cages. The number of healthy and dead infants was recorded. Consuming salt concentrations was continued by infant's mother during lactation period. After lactation period, each group's infants were kept in special cages. During breeding period, all groups consumed Isfahan potable water as the only source of water intake, with similar living conditions up to 90 days (End of breeding period). At the 90^{th} day, BP was measured directly under anaesthesia condition. On the basis of previous studies, ketamine hydrochloride was selected as anaesthetic drug at an anaesthetic dosage of 125 mg/Kg with intraperitoneally (Heydarpour F, 2008). Rat's weight was exactly measured and recorded, the ketamine dosage for each rat was calculated suspiciously as 125 mg/Kg and the drug was administrated via IP. After anaesthesia induction and skin and muscle dissection, with the help of an intra artery canula, blood pressure was measured directly via carotid or femoral arteries, using a Harvard model Physiograph. The data were presented as Mean±SD. Statistical analysis of collected data (Third month's weight and BP) was performed by different groups ANOVA test using SPSS version of 11.5. A minimum significance level of $p<0.05$ was used for all comparisons and $p<0.05$ was considered as significant changes.

RESULTS

The 0.5 and 1% salt concentrations and distilled water were tolerated by pregnant rat without significant problems, pregnant rat consumed 0.5 and 1% salt concentrations as the only source of potable water gives birth to more alive and healthy infants. Their birth weight in comparison with other groups was higher and these groups maintained this difference in weight with other groups during breeding period. The Mean±SD of third month's weight of groups were exposed to 0.5 and 1% salt concentrations were 261.9±67.8 g and 225.2±54.9 g respectively. The Mean SBP±SD and Mean DBP±SD of groups were exposed to 0.5 and 1% salt concentrations were 100.7±18.6, 64.76±19.90 and 96.7±25.5, 60.24±18.81 mmHg respectively in 90 days. Prenatal exposure to 0.5 and 1% salt concentrations and distilled water did not influence SBP and DBP significantly. Salt concentrations higher than 1% were not tolerated by pregnant rat, consuming these

concentrations associated with some problems in pregnant rats, higher salt concentrations increased the maternal and infant mortality rates. Pregnant rat consumed salt concentrations higher than 1% as the only source of potable water gives birth to lower alive and healthy infants and lower infant's birth weight. The rate of alive and healthy infants in groups were exposed to 1.8 and 2% salt concentrations in comparison with groups exposed to 0.5 and 1% salt concentrations was 1/6. After lactation period, when similar living conditions were provided for all groups, no significant changes in the animal's weight and growth curve were observed and the animal's weight remain low. The Mean±SD of third month's weight of groups were exposed to 1.8 and 2% salt concentrations were 168.1±45.8 g and 164.05±33.49 g respectively. Salt concentrations higher than 1% decreased third month's weight, these decreases in some concentrations were significant. The Mean SBP±SD and mean DBP±SD of groups were exposed to 1.8 and 2% salt concentrations were 112.4±17.8, 75.24±26.48 and 112.4±15.6, 77.62±17.07 mmHg respectively in 90 days. Prenatal exposure salt concentrations higher than 1% increased infant's SBP and DBP, these increases were significant in some concentrations. Table-1 shows Mean±SD of third month's weight and Table-2 shows mean SBP±SD and mean DBP±SD in different groups**.**

Table-1: Third month's weight in different groups (Mean±SD)

Groups	Third month's weight (g)	*p*-Value
Control Group (Tap Water)	230.7±55.8	---
Test Group-1 (Distilled Water)	202.7±55.7	0.1661
Group-2 (0.5% salt)	261.9±67.8	0.1516
Group-3 (1% salt)	225.2±54.9	0.88
Group-4 (1.4% salt)	180.5±56.7	0.0048
Group-5 (1.6% salt)	174±31.6	0.0004
Group-6 (1.8% salt)	168.1±45.8	0.0004
Group-7 (2% salt)	164.05±33.49	0.0001

Table-2: Systolic and Diastolic BP in different groups (Mean±SD)

Groups	SBP (mmHg)	*p*-Value	DBP (mmHg)	*p*-Value
Control Group (Tap Water)	100.7±17.4	---	67.86±17.51	---
Group-1 (Distilled Water)	104.3±19.4	0.4565	68.57±19.18	1
Group-2 (0.5% salt)	100.7±18.6	0.8701	64.76±19.90	0.7153
Group-3 (1% salt)	96.7±25.5	0.7059	60.24±18.81	0.1995
Group-4 (1.4% salt)	109.3±16.8	0.0543	74.29±16.15	0.1866
Group-5 (1.6% salt)	110±11.6	0.0305	74.76±8.29	0.0969
Group-6 (1.8% salt)	112.4±17.8	0.1627	75.24±26.48	0.5212
Group-7 (2% salt)	112.4±15.6	0.0142	77.62±17.07	0.0872

DISCUSSION

This study presented several key findings in relation to the effect of prenatal exposure to different salt concentrations. The findings are as follow: First, 0.5 and 1% salt concentrations led to an increase in the number of alive and healthy infants, birth's weight and third month's weight. Sodium necessity increases during pregnancy, Nourishing with sufficient sodium during pregnancy gives birth to more healthy animals. Higher salt necessity during pregnancy was shown in most species of animals. Second, infant's SBP and DBP were not affected significantly by 0.5 and 1% salt concentrations. Although, a low sodium diet prevents hypertension in non-pregnant individuals, but there is no need to introduce a salt restricted diet in prenatal care, a balanced diet in sodium during pregnancy is recommended. Third, salt concentrations higher than 1 percent decreased the number of alive and healthy infants, birth's weight and third month's weight significantly, these decreases in some concentrations were significant. Dietary salt overload present an increased oxidative stress and prenatal stress and malnutrition associated with low birth weights and high blood pressure. Fourth, salt concentrations higher than 1% increased SBP and DBP, these increases in some concentrations were significant. Higher Salt concentrations decreased birth weight and third month's weight significantly. Many studies have shown low birth weight association with higher blood pressure during offspring in rat and human. Fifth, there was a reverse relationship between BP and third month's weight, rat groups with lower third month's weight had higher SBP and DBP and on the contrary rat groups with higher third month's weight had lower SBP and DBP. Many studies have demonstrated a link between the intrauterine environment and diseases in adulthood (Leandro *et al*, 2008). The intrauterine (prenatal) period varies from 21 to 23 days and is terminated by birth, when the foetal circulation, nutrition, and other physiological functions are profoundly rearranged. In the rat, postnatal life begins with a 14-day period of milk consumption as the only source of water and nutrients, i.e., the suckling period (Zicha J, Kune J, 1999). Salt overload during pregnancy and/or lactation has long-term effects on offspring's body weight and blood pressure. In addition, high salt diet during the perinatal period induced renin-angiotensin system functional disturbances in the offspring (Da Silva *et al*, 2003). Prenatal high-salt diet in Sprague-Dawley rats caused an increase in MAP at postnatal day 30 (Di Nicolantonio *et al*, 1990). Therefore, *in utero* alterations in fluid and electrolyte endocrine systems may result in permanent effects on offspring.[8] Contreras et al. have shown that perinatal high salt can also produce a lasting hypertension in Sprague-Dawley rats. This model involved giving a high-salt (HS) diet before and during pregnancy, during lactation, and to weaned offspring for 10 days before switching to a normal-salt (NS) chow (Porter *et al*, 2007). Several studies suggest that prenatal exposure to plasma osmolality alterations may

permanently alter (imprint) osmoregulatory pathways. Dehydration of pregnant rats increased the salt appetite and blood pressure of adult offspring. In humans, prenatal and/or neonatal exposure to conditions resulting in plasma hyperosmolality enhances the salt preference of offspring at later life (Wang *et al*, 2003). It has also been shown that salt restriction during pregnancy is associated with low birth weight in male and female rat offspring (Lopes *et al*, 2006). Similarly, low-sodium diets during pregnancy produce long-lasting changes in adult offspring, including greater water intake by the offspring and increased adrenal gland weight. High-salt intake by the dam during pregnancy also results in permanent physiological and behavioural alterations in offspring, including increased salt intake, salt preference, and blood pressure (McBride *et al*, 2006). A low sodium intake during perinatal life jeopardizes growth in young rats (Magalhães *et al*, 2005). The obtained results in this study coincide with similar findings released by other researchers. In agreement with some earlier studies our study revealed that early life experience with low-and high-sodium diets, during the prenatal or early postnatal period, is a stress that may result in permanent effects on offspring and produces diseases in adulthood. While treating high blood pressure in middle age is beneficial in terms of reducing the occurrence of cardiovascular disease, treated and well controlled hypertensive adults still have a substantial excess mortality and reduced survival compared with normotensives. Therefore, identification of the means of preventing hypertension in earlier life is an important objective. There is increasing evidence that adult blood pressure is determined by a range of characteristics from the intrauterine period, through infancy and childhood (Lawlor DA and Smith GD, 2005). Hence, prescribing a sodium-restricted diet or a diet rich in sodium during pregnancy is not effective. Therefore there is no need to introduce a salt restricted diet in prenatal care, although increasing evidence shows that a low sodium diet prevents hypertension in non-pregnant individuals (Knuist *et al*, 1998). By the 1940's a low salt diet was standard during pregnancy, particularly for women with pre-eclampsia. In the late 1950's and early 1960's this practice began to be questioned, and it was even suggested that high salt intake might prevent or treat pre-eclampsia (UK 1958, UK 1961). Subsequently, interest in salt consumption during pregnancy has largely faded away. In most parts of the world women are no longer advised by clinicians to alter their salt intake during pregnancy. A notable exception is in the Netherlands where this practice has, until recently, remained widespread. Nevertheless, some lay literature aimed at pregnant women continues to advocate salt restriction during pregnancy (Duley L and Henderson-Smart DJ, 2009).

CONCLUSION

There is no need to introduce a salt restricted diet in prenatal care, a balanced diet in sodium during pregnancy is recommended, high-salt diet creates harmful effect.

Prospective: Prenatal salt loading or restriction exerts considerable effects on BP development, identification of the means of preventing hypertension in earlier life is an important objective in future studies.

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REFERENCES

1. Forrester T. Historic and early life origins of hypertension in Africans, The American Society for Nutritional Sciences. J Nutr 2004;134:211–6.
2. Ezzati M, Lopez AD, Rodgers A, Vander Hoorn S, Murray CJL. and the Comparative Risk Assessment Collaborating Group. Selected major risk factors and global and regional burden of disease. Lancet 2002;360: 1347–60.
3. Hollenberg NK, Braunmwald E. A Atlas of Heart Diseases, Hypertension: mechanism and therapy. Philadelphia, US. Current Medicine Inc 1998: pp:1–5.
4. Di Nicolantonio R, Westcott KT, Koutsis K, Wlodek ME. Lack of evidence for a role for either the in utero or suckling periods in the exaggerated salt preference of the spontaneously hypertensive rat. Physiology & Behavior 2005;86:500–7.
5. Zicha J, Kune J. Ontogenetic Aspects of Hypertension Development: Analysis in the Rat. Physiol Rev 1999;79:1227–82.
6. Jones DW. Dietary Sodium and Blood Pressure. Hypertension 2004;43:932–5.
7. Magalhães JCG, da Silveira AB, Mota DL, Paixão ADO. Renal function in juvenile rats subjected to prenatal malnutrition and chronic salt overload. Exp Physiol 2005;91(3):611–9.
8. Ross MG, Desai M, Guerra C, Wang S. Prenatal programming of hypernatremia and hypertension in neonatal lambs. Am J Physiol Regul Integr Comp Physiol 2005;288:R97–R103.
9. Järvelin MR, Sovio U, King V, Lauren L, Xu B, McCarthy MI, *et al.* Early Life Factors and Blood Pressure at Age 31 Years in the 1966 Northern Finland Birth Cohort. Hypertension 2004;44:838–46.
10. Heydarpour F. The effect of hypernatraemia on ketamine anaesthesia in male rats: Iranian J Veterinary Research 2008;9(2):162–7.
11. Leandro SM, Furukawa LNS, Shimizu MHM, Casarini DE, Seguro AC, Patriarca G, *et al.* Low birth weight in response to salt restriction during pregnancy is not due to alterations in uterine-placental blood flow or the placental and peripheral rennin-angiotensin system. Physiology & Behavior 2008;95:45–151.
12. Da Silva AA, de Noronha IL, de Oliveira IB, Malheiros DM, Heimann JC. Renin-angiotensin system function and blood pressure in adult rats after perinatal salt overload. Nutr Metab Cardiovasc Dis 2003;13:133–9.
13. Di Nicolantonio R, Hoy K, Spargo S, Morgan TO. Perinatal salt intake alters blood pressure and salt balance in hypertensive rats. Hypertension 1990;15:177–82.
14. Porter JP, King SH, Honeycutt AD. Prenatal high-salt diet in the Sprague-Dawley rat programs blood pressure and heart rate hyperresponsiveness to stress in adult female offspring. Am J Physiol Regul Integr Comp Physiol 2007;293:R334–R342.

15. Wang Sh, Chen J, Kallichanda N, Azim A, Calvario G , Ross MG. Prolonged prenatal hypernatremia alters neuroendocrine and electrolyte homeostasis in neonatal sheep. Exp Biol Med 2003;228:41–5.
16. Lopes KL, Furukawa LNS, de Oliveira IB, Dolnikoff MS, Heimann JC. Perinatal salt restriction: A new pathway to programming adiposity indices in adult female Wistar rats. Life Sciences 2008;82:728–32.
17. McBride SM, Culver B, Flynn FW. Prenatal and early postnatal dietary sodium restriction sensitizes the adult rat to amphetamines. Am J Physiol Regul Integr Comp Physiol 2006;291:R1192–R1199.
18. Lawlor DA, Smith GD. Early life determinants of adult blood pressure. Curr Opin Nephrol Hypertens 2005;14:259–64.
19. Knuist M, Bonsel GJ, Zondervan HA, Treffers PE. Low sodium diet and pregnancy-induced hypertension: a multi-centre randomised controlled trial. Br J Obstet Gynaecol 1998;105:430–4.
20. Duley L, David J Henderson-Smart DJ. Reduced salt intake compared to normal dietary salt, or high intake, in pregnancy, Cochrane Database of Systematic Reviews 2009;1:1–15.

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