The Effects of High Salt Diet on Blood Pressure and the Renal Handling of Sodium

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Abstract

Introduction: The association between elevated dietary salt consumption and high blood pressure is well known. Hypertension carries elevated risk for stroke, cardiovascular disease, liver disease, and nervous disorders. Interestingly, sex differences in many areas of pathophysiology. Pre-menopausal women have shown to be protected against hypertension and renal diseases compared to age-matched men. It is reasonable to expect that how the kidney handles sodium in presence of high-salt consumption plays a key role in sex differences. The purpose of this study was to determine sex differences in the renal handling of sodium in mice consuming a high-salt diet. We also investigated the effects of high-salt consumption on blood pressure in these mice.

Methods: Intact male and female mice (n=6/group) consumed a high-salt (4%, Harlan Teklad) diet for 30 days. Mice were placed individually in metabolic cages where urine could be collected for volume and measurement of Na^+ concentration. Urinary Na^+ excretion (NAE, mg/day) was determined from daily measurements of urine sodium concentration and urine volume. Sodium intake (Nai, mg/day) was determined from daily food intake of 4% salt diet *ad libitum*. Blood pressure was measured daily via the tail-cuff method. Expression of key sodium transport proteins in the kidney was measured via real-time quantitative PCR.

Results: From the data accumulated during the 30-day period of high salt consumption, female mice showed a significantly lower average of the output-to-input Na+ ratio (NAE/Nai) compared to male mice (53.3 ± 2.7 vs 68.1 ± 1.8, respectively, p<0.0001). Female mice showed lower mean blood pressure (MBP, mmHg) compared to male mice over the 30-day period (78.4 ± 1.0 vs 84.9 ± 1.2 respectively, <0.0005). Molecular expression of the key sodium transporter Na^+-$2Cl^-$-K^+ (NKCC) in the thick ascending limb was over 5-fold higher in the female kidney.

Conclusion: Interestingly, results from this study demonstrated that female mice retained more ingested sodium compared to male mice while on a high-salt diet. Moreover, female mice had lower MBP compared to male mice while on a high-salt diet. We suspect that sex steroids are playing important roles in the renal handling of sodium and in the control of blood pressure. This study suggests that females are protected from deleterious effects of high-salt consumption.

Introduction

Hypertension increases the risk for numerous pathologies particularly stroke and cardiovascular disease. Numerous causes of hypertension exist, genetic, environmental, life-style, etc. High salt consumption is prevalent globally and is one of the well-known causes of hypertension. The American Heart Association, American College of Cardiology, physicians, and general health-care professionals recommend reducing dietary salt consumption although this is difficult in our society. The renal system is responsible for excreting excess salt from the body and preventing salt-induced hypertension. Sex differences in cardiovascular and kidney disease are well documented with females being protected compared to their age-matched males. Thus, it is important to study sex differences related to the control of blood pressure and in renal function on how the kidney excretes sodium under high salt consumption.

The **purpose** of this study was to investigate sex differences in the renal handling of sodium and blood pressure in mice during a 30-day period of high salt consumption. Based on previous data we **hypothesized** that female mice would demonstrated lower blood pressure and excrete less sodium than males during this this 30-day period of high salt consumption.

Methods

Animals: Female and male mice at 12 weeks of age were purchased from Envigo Laboratories (Indianapolis, IN) and placed in metabolic cages (n=6/group) with access to food and water a*d libitum*.

Daily measurements included body weight, food & water intake, urine flow rate, and urine Na^+ concentration (UNa). An EasyLyte electrolyte analyzer was used to measure the latter.

Blood pressure of each mouse was measured periodically via the tail-cuff method (Kent Scientific). This method uses a restrainer and a warming platform to maintain normal body temperature. After a three-day training period, mice became accustomed to the restrainer ensuring that blood pressure was recorded under minimal stress.

Quantitative real-time PCR (QT-PCR) was employed to measure relative expression of key sodium transporters in the kidney. Kidney tissue was harvested from separate mice placed on the same dietary protocol as those in metabolic cages. (Data presented in Table 1 are from samples harvested on days 5, 8 12, 19, 23, and 26.) Total RNA was extracted from renal cortical tissue using the MELT Nucleotide kit (Ambion) and cDNA was synthesized using the RT^2 first-strand kit (SA Biosciences). Real-time PCR was conducted using PCR arrays designed with specific primers for mouse Na^+ transporters. The PCR arrays were made by Qiagen SA Biosciences and QT-PCR was performed via SBYR green technology on an Opticon 2 (MJ Research) thermocycler.

Statistics: ANOVA with repeated measures and Student T-test were used to determine differences between groups with Graphpad Prism 9.0. Statistical significance was a p value < 0.05.

Results

Figure #1. Systolic Blood Pressure

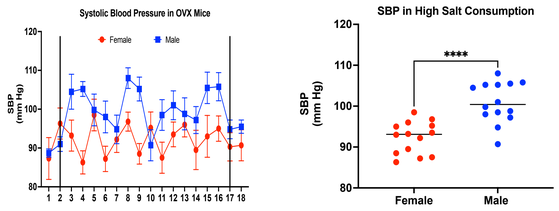

Figure shows periodic blood pressure measurements, i.e., every-other-day, of male and female mice. Vertical lines indicate normal diet – high salt diet – normal diet periods. SBP was measured via the tail-cuff measurement every-other-day. Male mice had higher SBP (p<0.0001).

Figure #2. Sodium Balance

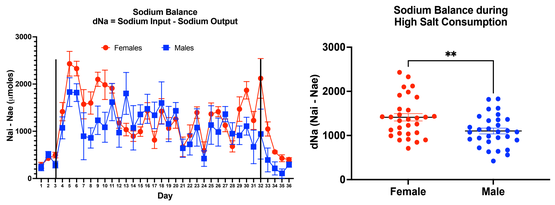

Figure shows daily sodium balance (input – output) and the comparison between female and male mice. Mice showed higher sodium retention during the high salt period (p<0.005).

Table 1. Molecular Expression of Renal Transporters

Gene	Name	Fold Difference (Female/Male)
Scnn1a	ENaCa	2.1 : p < 0.001
Scnn1b	ENaCb	2.2 : p < 0.01
Scnn1g	ENaCg	2.5 : p < 0.01
Slc12a1	NKCC	6.1 : p < 0.06
Slc12a3	NCC	2.8 : p < 0.0005
Slc9a3	NHE3	1.27 : p < 0.2
Slc9a2	NHE2	3.0 : p< 0.005
Slc34a1	Na-Pi	-1.1 : p < 0.5
Slc5a12	SGLT	-1.1 : p < 0.7
Atp1a1	Na/K ATPase	1.3 : p < 0.5
Agtr1a	Angiotensin Receptor Type 1	1.2 : p < 0.3
Agtr2	Angiotensin Receptor Type 2	No Expression

Conclusions

The physiological regulation of renal sodium excretion plays a significant role in determining blood pressure. This study was conducted to determine the effects of high salt consumption on blood pressure and the renal sodium handling in male and female mice. The major focus was to determine if sex differences would be observed in blood pressure and in how the kidney excretes sodium in mice consuming a high salt diet.

Results showed that female mice had lower blood pressure during the high salt consumption period. Interestingly, female mice excreted less of the ingested sodium and thus retained more sodium than the male mice. Moreover, female mice kidneys had higher expression of key sodium transporters suggesting an explanation for the higher sodium retention during the high salt consumption.

Results of this study support the protective nature of the female sex during high salt consumption. Despite the apparent higher sodium retention, female mice had lower blood pressure than male mice.

Future studies should be designed to determine sex differences in how the body stores sodium especially during elevated dietary salt. Recent evidence indicates that sodium can be stored in various parts of the body especially the skin. Understanding these mechanisms of how male and female kidneys excrete sodium and how the body stores sodium could lead to more effective therapeutic measures to control salt-induced hypertension.

It is well known that premenopausal women have lower blood pressure and fewer renal and cardiovascular diseases compared to age-matched males. The underlying mechanisms for these sex differences are not well understood. Clinically, men and women receive basically the same treatment for hypertension, kidney disease, and cardiovascular pathologies. A better understanding of the sex differences in the overall regulation of blood pressure will lead to more effective and focused treatment of these diseases.

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