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Long Term Consumption Of High Fructose And High Salt Diet Did Not Induce Blood Pressure Elevation In Female Mice. Is Estrogen Protective Against Dietary-induced High Blood Pressure?

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INTRODUCTION

High fructose and high salt (HFHS) consumption are linked to hypertension, which is the leading cause of death worldwide. Dai et al., 1994 reported that the effect of HFHS diet on blood pressure (BP) is dependent on amount consumed and the duration of consumption. The kidney plays a major role in BP regulation. Sex differences in renal regulation of BP have been reported and are partially attributed to the effects of sex steroids on key renal sodium transporters especially NCC and NKCC.

OBJECTIVES /HYPOTHESIS

The aim of this study is to ascertain if female mice will be protected from dietary-induced BP elevation over time (3 months) and also, to determine the effect of the HFHS diet on the expression of NCC and NKCC renal sodium transporters.

Based on findings from our previous study (6 weeks), we hypothesized that HFHS diet would increase BP in both sexes and females will have higher expression of renal NCC and NKCC.

METHODS

Animals: Four-week old intact CD-1 mice from Envigo (Indianapolis, IN) were used. The study lasted for three months and one week.

Protocol: Mice (n= 6/group) were placed in metabolic cages and consumed standard chow and water for seven days, followed by 3months of 4% sodium chloride (NaCl) diet and a drinking solution of 1% NaCl and 20% fructose. Separate mice on the same diet in the bins were sacrificed and kidneys extracted at the end of the first week, first and second months, and used for molecular studies.

Blood Pressure: BP (SBP, mmHg) measurement was done via tail cuff technique using CODA instrument from Kent Scientific. Measurements were taken on each mouse in the metabolic cages 5days/week during the baseline period and subsequently 5days/week during the last week of every month.

Physiological Measurements; Body weight, food intake, fluid intake, and urine volume were measured from mice in metabolic cages . Sodium intake (Nai) was calculated from the food and fluid. Urinary sodium excretion (Nae) was measured. Sodium balance was determined from the difference between Nai and Nae.

RESULTS

Fig. 1: Body Weight

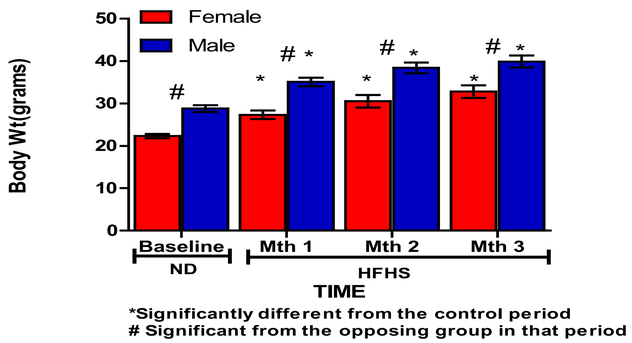

Fig. 1 shows the average body weight during the 3 months of the study. Male and female mice progressively increased in weight throughout the study; however, males had higher body weight in each of the periods.

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Fig. 2: Systolic Blood Pressure

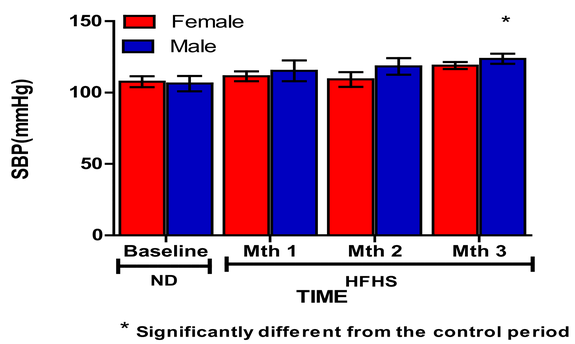

Fig. 2 shows no sex difference in BP from baseline to the third month. Males showed an increase in BP in the third month compared to baseline (123.6 ± 3.6mmHg and 106.3 ± 5.4mmHg respectively, P< 0.05).

Fig. 3: Sodium Retention

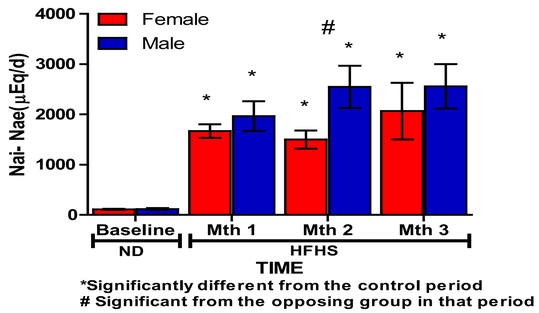

Fig. 3 shows higher sodium retention during HFHS diet (Month 1- 3) compared to baseline (ND). No sex difference in sodium retention throughout the study except in month 2 when males had higher retention.

Fig. 4: mRNA expression of NCC and NKCC transporters (Female to Male)

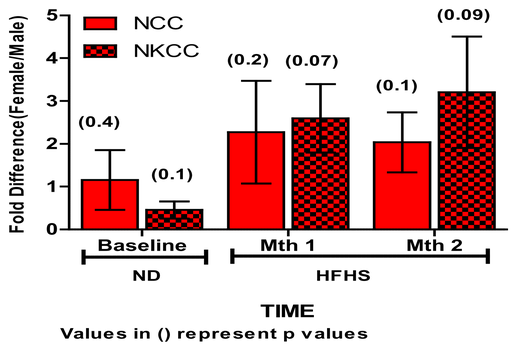

Fig. 4 shows the relative expression(fold Difference) of NCC and NKCC transporters in female versus male mice. Female kidneys tended to have higher expression of NCC compared to males in all the periods. NKCC also tended to be higher in females during the first and second month of the study.

mRNA Measurements: Quantitative real-time PCR (QT-PCR) was used to measure relative expression of renal NCC and NKCC transporters. Total RNA was extracted from renal cortical tissue and cDNA was synthesized. Real-time PCR was conducted using specific primers for NCC and NKCC Na^+ transporters. QT-PCR was performed via Sybr green technology on an Opticon 2 (MJ Research) thermocycler. Relative expression data were calculated via the ΔΔCt method with GapDH as the reference gene

Statistics:

Data are shown as Mean ± SEM. Repeated measures 2-way ANOVA was used to analyze SBP. QT-PCR data (relative gene expression with reference to GapDH) were compared using 2-way ANOVA (GraphPad Prism6, Ca), Fisher LSD was used for multiple comparisons. Differences of $P < 0.05$ were considered statistically significant

SUMMARY /CONCLUSION

No significant sex difference in BP from baseline to the third month, though, males tended to be higher. A significant increase in blood pressure was observed in male mice in third month compared to the baseline. There was no significant sex difference in sodium retention, though males had higher sodium retention than females in the second month only. Both sexes showed increased body weight from week one to third month. Most expression levels were 2 fold or higher in female kidneys.

We conclude that females may have protection against HFHS induced elevation in blood pressure which may be due to the effect of female sex hormones (estrogen). A longer duration (6 months) study is being carried out for further investigation on the. possible sex differences under HFHS diet.

ACKNOWLEDGEMENTS

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