Sex Differences in Protein Excretion in Mice Consuming High Protein Diet

CENTER FOR HEALTH SCIENCES

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Abstract

Background: Normally, the renal excretion of protein (or proteinuria) is absent or very small. Ingesting high-protein diets can elevate proteinuria and in the long term, increase the work on the kidney by increasing glomerular filtration and higher energy requirement to handle the protein. Sex differences in renal function are well known and thus, differences in proteinuria may exist. The purpose of this study was to determine if sex differences exist in proteinuria in mice consuming high protein diet and investigate the potential roles of the sex steroids 17beta-estrogen (E2) and testosterone.

Methods: Healthy 3-4-week-old male and female intact and gonadectomized mice were used. Mice were placed in individual metabolic cages where the urine of each mouse could be collected and measured for protein concentration. Mice consumed a 40% casein protein diet for 25 days (normal protein = 20% protein). Some gonadectomized female mice received exogenous E2 and gonadectomized male mice received exogenous testosterone. Proteinuria was measured via dipstick measurement and protein excretion (mg/day) i.e., urine flow rate (ml/day) x urine protein concentration (mg/day).

Results: Intact male mice had significantly higher proteinuria compared to intact female mice (5-10 mg/day vs 25-30 mg/day, p<0.001). Gonadectomized male and female mice had very low proteinuria (3-5 mg/day). Gonadectomized testosterone-treated male mice had high proteinuria not different from the intact male mice. Gonadectomized E2-treated female mice had similar proteinuria compared to intact female mice and slightly though not significantly higher than gonadectomized placebo-treated female mice.

Conclusion: The results of this study suggest that the male sex steroid induces high proteinuria in mice consuming high protein levels. The female sex steroid plays no role or only a minor role in proteinuria under these experimental conditions. Our results suggest that androgens may account for the higher incidence of kidney disease in males compared to age-matched pre-menopausal females.

Introduction

The sex steroids estrogen and testosterone profoundly influence many physiological mechanisms in addition to the development of sex characteristics and differentiation. Hormone replacement therapy in both women and men continues to be a promising yet controversial method to treat pathologies particularly those related to cardiovascular disease. It has been noted that these sex steroids affect the renal system that apparently result in important sex differences. Men have a higher incidence of renal disease than age-matched premenopausal women, in addition to diseased kidneys worsening progressively faster in men. Interestingly, postmenopausal women appear to lose their renoprotection. The underlying mechanisms responsible for these sex differences are not well understood. An important function of the kidney centers around protein and sex differences related to the renal handling of protein have been proposed.

The **purpose** of this study was to determine sex differences in urinary protein excretion (PE) and the influence of estrogen and testosterone on PE in mice. We hypothesize opposing rather than similar effects of estrogen and testosterone.

Methods

Animals. Male and female mice (Hsd:ICR (CD-1®)) at 20-25 day old and 17-20 g body weight were purchased from Envigo, Inc. (Indianapolis, IN). Envigo technicians performed the surgical procedures (ovariectomy or castration) 10-14 days prior to the studies. Upon arrival to the institution's animal facilities, mice were placed in plastic bins for 4-7 days with standard rodent chow (Harlan Teklad 18% protein) and water before being placed in metabolic cages. The OSU-CHS Institutional Animal Care and Use Committee approved all experimental procedures with animals.

Metabolic Cages & Physiological Measurements. Experiments were conducted with mice in metabolic cages. Daily measurements included body weight, food and water intake, urine volume (UV), urine osmolarity (Uosm), urine pH, solute excretion (SE), and urinary pH via urinalysis reagent strips. Total protein concentration in the urine was measured at the OSU-CHS Medical Center via a colorimetric method using an Olympus AU400™ analyzer. PE (mg/day) was determined by measuring total protein concentration (mg/ml) in the urine and multiplying by the UV (ml/day). Upon entering metabolic cages, all mice consumed a high-protein diet of 40% casein (Teklad, Harlan) with water *ad libitum* throughout the experimental protocols.

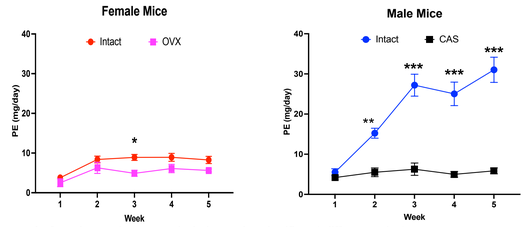
Experimental Protocols. Two major studies, each with two protocols (one in males and the other in females) were conducted. All protocols lasted for a 25-day period and PE was measured on days 2, 7, 14, 21, and 25. The first study included one protocol with intact and castrated (CAS) male mice and the other protocol with intact and ovariectomized (OVX) female mice (n=6 in each group). The second study included two protocols each with three groups (n-4 in each group). The first protocol included intact male mice, CAS mice treated with placebo, and CAS mice treated with testosterone. The second protocol included intact female mice, OVX mice treated with placebo, and OVX mice treated with estrogen (E2). Treatments involved specialized pellets (placebo, 1.5 mg E2, or 25 mg testosterone) implanted subcutaneously. Pellets were purchased from Innovative Research of America (Sarasota, FL) and previously shown to produce physiological levels of estrogen or testosterone.

Statistics: Repeated measures ANOVA using Graphpad Prism 9.0 was used to determine differences in PE. Student t-test or one-way ANOVA was also used in some comparisons to determine differences between groups.

Figure #1 Metabolic Cages

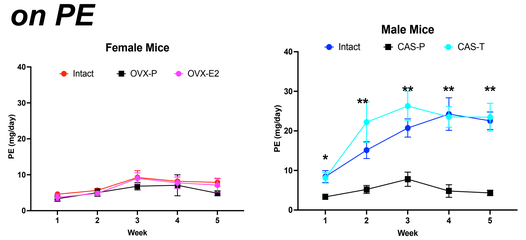
Results

Figure #2. PE in Intact and Gonadectomized Mice



In females, only one period showed a significant difference between intact and OVX groups. In males, significant differences between intact and CAS mice occurred in all weeks besides week 1. * p<0.01, ** p<0.001, *** p<0.0001.

Figure #3. Effect of Sex Steroids on PE



In females, no differences between groups occurred through the protocol. In males, significant differences were measured in each week between the CAS-P group and the other two groups. No differences were measured between the intact and CAS-T groups.
* p<0.01, ** p<0.001, *** p<0.0001
OVX-P & OVX-E2: ovariectomized mice with placebo pellet and ovariectomized mice with E2 pellet, respectively.
CAS-P & CAS-T: castrated mice with placebo pellet and castrated mice with testosterone pellet, respectively.

Figure #4. Kidney-to-Body Weight

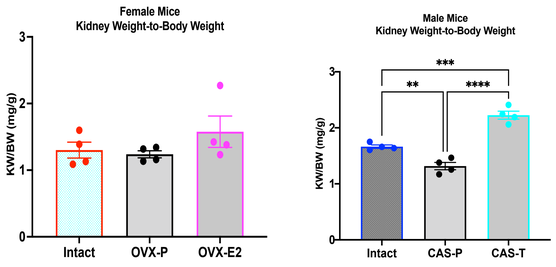


Figure shows that in females, the KW/BW was not different between groups. In males, differences occurred between groups. CAS-T > Intact > CAS-P.

Conclusions

This study was conducted to determine if sex differences exist regarding urinary protein excretion in mice consuming a high protein diet and to determine if the sex steroids play a role in these differences. Our results clearly show that in healthy mice, males excrete more protein than females. Food intake data, not shown in the results, was not significantly different between males and females or among the intact and gonadectomized mice. Thus, the PE data in this study appeared to be produced by the specific actions at the kidney.

Figure 2 clearly shows that intact males excrete higher PE compared to gonadectomized males which had similar PE values as the females. Figure 3 clearly shows that administration of exogenous testosterone to gonadectomized male mice increased PE to the level of intact mice. Administration of exogenous E2 in gonadectomized female mice did not increase PE.

It is known that males have larger kidneys than females. Figure 4 shows that testosterone increases the size of the kidney. This testosterone-induced kidney hypertrophy may have significant consequences on the pathophysiology of the renal system.

We conclude that sex differences exist in urinary protein excretion in mice consuming high protein diet. Estrogen has little if any effect on PE whereas testosterone significantly increases PE.

Testosterone also causes kidney hypertrophy which might be directly or indirectly responsible for the higher PE.

Studies are underway to determine the specific nature of the protein excreted in the urine from this high protein consumption. We suspect that at least some of this protein is albumin, which under normal conditions should not appear in the urine. Future studies will also focus on the potential effect of testosterone to induce albuminuria – a key indicator of kidney disease.

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