SEX DIFFERENCES IN PROXIMAL TUBULE-DERIVED ANGIOTENSINOGEN AND RENIN AND PRORENNIN RECEPTORS IN THE COLLECTING DUCTS DURING HIGH DIETARY SALT

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Sex disparities in cardiovascular and renal diseases (CVRD) have been suggested because:

- The severity of hypertension and progression to end organ damage occurs more rapidly in men than in women.
- The responses to renin-angiotensin system (RAS) blockade differ between men and women.
- The RAS plays an important role in the control of blood pressure (BP); whether it plays a role in the sexual dimorphism of BP and related renal injury is not yet completely understood.
- When Ang II levels are not suppressed, salt intake exacerbates hypertension and renal injury.
We have previously demonstrated that rats with Ang II-dependent hypertension exhibit:

- Upregulation of renin produced by the principal cells of the collecting duct (CD)
- Augmented renin activity in their renal medullary tissues

The demonstration that the synthesis, secretion and urinary excretion of proximal tubule (PT)-derived angiotensinogen (AGT) is augmented in several forms of hypertension, has prompted us to propose that **PT-derived AGT and CD renin may contribute to the development and progression of hypertension.**

However, little is known about these RAS components in the kidney and even less in regard their role on the sex disparities observed in CVRD.
Study Objectives

- To determine whether intrarenal RAS components, particularly AGT derived from PT cells and renin and prorenin receptors expressed in distal nephron segments, exhibit sex differences during baseline conditions.

- To examine if the effects high salt diet on these RAS components differ between male and female rats, thus contributing to sex disparities in hypertension.
Material and Methods

- Male and female age-matched Sprague-Dawley rats, BW: 175-200g
  - Male Normal Salt Diet (NS; n=5)
  - Male High Salt Diet, 8% NaCl (HS; n=5)
  - Female Normal Diet Normal (NS; n=5)
  - Female High Salt Diet, 8% NaCl (HS; n=5)

- Systolic blood pressure (tail cuff/method)

- BW, food and water intake, urine output.

- Plasma Renin Activity (PRA), 17-β estradiol levels (Assay Designs, EIA)

- Proteinuria, AGT (ELISA), and RENIN content measured in 24-h collected urine samples

- After 2 weeks of study, kidneys were harvested and microdissected into cortexes and medullas for:
  - AGT, Renin, Prorenin Receptor for mRNA and protein levels
Changes in Systolic Blood Pressure and Proteinuria in Male and Female Rats with Dietary Salt

Systolic Blood Pressure
(mmHg)

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<tr>
<th></th>
<th>NS-Male</th>
<th>HS-Male</th>
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<tr>
<td>-1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
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<tr>
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*p<0.001 vs. male normal salt
#p<0.05 vs. female normal salt
¥p<0.001 vs. male high salt
AGT Expression is decreased in Female Rats

Changes with Dietary Salt

AGT mRNA levels

**p<0.01 vs. male normal salt

* p<0.01 vs. male high salt
**PRA and Urine Renin Content in Male and Female Rats with Changes in Dietary Salt**

Plasma Renin Activity

- **Normal Salt**
  - Male
  - Female

- **High Salt**
  - Male
  - Female

<table>
<thead>
<tr>
<th></th>
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<td><strong>7</strong></td>
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<tr>
<td>High Salt</td>
<td>4</td>
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**p<0.05 vs. male normal salt**

**p<0.05 vs. male high salt**

Urinary Renin Excretion

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<thead>
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<tr>
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<td><strong>3</strong></td>
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<tr>
<td>High Salt</td>
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</tr>
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</table>

**p<0.001 vs. male normal salt**

**p<0.05 vs. female normal salt**
Collecting Duct Renin Gene Expression with Changes in Dietary Salt in Male and Female Rats

Renin mRNA

NS= normal salt diet
HS= high salt diet
*p<0.001 vs. male NS
*¥p<0.001 vs. female NS
(P)RR gene expression in renal medullary tissues from rats under NORMAL and HIGH salt diet

(P)RR mRNA Levels in Renal Medulla

* p< 0.001 vs. male normal salt
¥ p< 0.01 vs. female normal salt
Summary

- Baseline SBP was not different between male and female Sprague-Dawley rats.

- HS diet alone did not increase BP in either male or female rats; however it was able to induce proteinuria in both genders and to a greater extent in male than in female rats.

- Renin mRNA levels were significantly higher in male than in female rats during normal salt diet; however when salt consumption was increased, RENIN transcript, as well as prorenin protein levels increased significantly in female rats but not in male rats.

- AGT mRNA levels, as well as its urinary excretion were significantly higher in male than female rats regardless of the content of salt in the diet.

- Although during baseline conditions (NS diet), the (P)RR mRNA levels were two times higher in female rats than in the males; a diet with high content of salt decreased (P)RR mRNA levels in female but not in male rats. This effect may suggest a protective mechanism exhibited by female but not male rats.
Conclusions

- These data reveal that there are sex differences in the endogenous intrarenal RAS, particularly for proximal tubule-derived AGT, as well as for renin and (P)RR in distal nephron segments.

- Importantly, although chronic consumption of a high salt diet does not increase BP it induces proteinuria in both genders but with a greater impact in males.

- Augmentation of AGT and RENIN content in the urine of male rats exposed to HS indicates a greater predisposition to develop kidney injury than female rats; suggesting that in men renal tissue may be more susceptible to be damaged by salt than in women.
Further studies in this direction will not only contribute to our understanding of why females have a lower incidence of CVRD compared with males but may also lead to the development of gender specific therapeutics that may offer protection against diseases that show clear sex differences in their etiology and progression.
Acknowledgment

- Hiroyuki Kobori, MD., PhD
  - AGT measurements in urine (ELISA)
- Dale M. Seth, MS
  - Renin measurements in urine and renal tissue

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Muchas Gracias
Plasma Ang II Levels

Plasma Ang II (fmol/mL)

M ns  F ns  M HS  F HS
Prorenin Receptor (P)RR

- Cloned by G. Nguyen et al. in 2002
- Expressed in mesangial cells, podocytes, and distal tubules.
- Binds renin and prorenin
- Exerts a non-proteolytic activation of prorenin
- Elicits intracellular signaling activation via MAPKs

Nguyen G. et al., Curr Opin Nephrol Hypertens, Volume 16(2). March 2007. 129–133
Working hypothesis
Ang II Urinary Excretion Rates (fmol/day) in chronic Ang II-infused Rats

Male

Female

- M NS
- M HS
- M Ang II
- M Ang II + HS
- M Ang II + HS + Cand

- F NS
- F HS
- F Ang II
- F Ang II + HS
- F Ang II + HS + Cand