Gender Differences of Renal CYP-Derived Eicosanoid Synthesis in Rats Fed a High-Fat Diet

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Background: Renal cytochrome P450 (CYP)-derived eicosanoids, hydroxyeicosatetraenoic acids (HETEs), epoxygenes (EETs), and dihydroxyeicosatrienoic acids (DHETs), have been shown to affect renal function and blood pressure (BP). We recently reported that high fat (HF) diet treatment in male rats increases BP and decreases production of these eicosanoids in the kidneys. However, at what level the downregulation of renal eicosanoid synthesis occurs and whether the HF diet has any effects on the regulation of renal eicosanoid synthesis in female rats are not known. The purpose of this study was to determine renal CYP-derived eicosanoid synthesis and its association with BP regulation in HF male and female rats.

Methods: In the first set of experiments, male and female rats were fed the HF or control diet for 10 weeks. In the second set of experiments, male and female rats were fed the HF diet for 10 days. In the third set of experiments, HF-fed and control female rats were treated with 5α-dihydrotestosterone for 4 weeks. After treatment, BP, urinary sodium, sodium balance, eicosanoid production, and CYP enzyme expression were determined.

Results: An elevation of BP and a decrease of renal cortical eicosanoid production were found in HF male rats, but no BP and eicosanoid production changes were observed in HF female rats. The HF treatment also caused a significant decrease of eicosanoid production and a decrease of CYP4A and 2C23 expression in the proximal tubules of HF male rats. Moreover, the HF diet treatment in male rats caused an increase in cumulative sodium balance and an elevation of BP, whereas no change in cumulative sodium balance and BP was observed in female rats. The treatment of 5α-dihydrotestosterone increased BP and 20-HETE production in the renal microvessels, but no effect on urinary sodium excretion and renal microvessel EET production in both control and HF-fed female rats.

Conclusions: These results demonstrate that there are gender-specific differences in regulation of renal eicosanoid synthesis, sodium balance, and BP caused by HF treatment, and it appears that androgens play some role in upregulation of renal eicosanoid synthesis in both HF and control female rats. Am J Hypertens 2005; 18:530–537 © 2005 American Journal of Hypertension, Ltd.

Key Words: Gender, cytochrome P450, arachidonic acid, eicosanoid, 20-hydroxyeicosatetraenoic acid, epoxyeicosatrienoic acid, kidney, fat, obesity.

In the rat kidney, arachidonic acid is metabolized by cytochrome P450 (CYP) enzymes into different eicosanoids. These eicosanoids include hydroxyeicosatetraenoic acids (19-HETE and 20-HETE [the major metabolite]) and epoxygenes (5,6-, 8,9-, 11,12-, and 14,15 EET). These EET molecules can be further hydrolyzed by epoxide hydrolase to the corresponding dihydroxyeicosatrienoic acid (DHET). The 20-HETE synthesis is catalyzed by isoforms of the CYP4A gene family (eg, CYP4A1, CYP4A2, CYP4A3, and CYP4A8).


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its potential impact on essential hypertension; however, the exact mechanisms whereby obesity could cause essential hypertension are not fully understood. To better understand these mechanisms, several animal models, including genetic models such as the Zucker rat and the non-genetic high fat (HF) diet-induced obese rat, have been established. Advantages for using the Zucker rat include that they are commercially available and that some pathophysiological changes of kidneys, such as glomerulosclerosis and necrosis, can be observed in young rats. However, this animal model has different neurohumoral changes compared to the obese human. For example, the plasma renin activity is decreased in obese Zucker rats, whereas obese humans have increased plasma renin activity. In contrast to this genetic model, the HF diet rat model has characteristics similar to human obesity-induced hypertension, such as increased plasma renin activity, increased plasma norepinephrine response to intravenous glucose, increased plasma leptin concentration, and decreased growth hormone secretion and synthesis. On the basis of these results, we have chosen to use HF rats as our experimental model.

Recent studies in HF animal models and obese humans have shown that obesity is associated with increases in renal blood flow, arterial blood pressure (BP), renal tubular reabsorption, and glomerular filtration rate. Because of the important role of the kidneys in the regulation of long-term BP, and because renal CYP-derived eicosanoids have been shown to possess biological effects on renal function, we hypothesized that these metabolites can potentially contribute to these physiologic changes during obesity. These biological effects include inhibition of ion transport in the nephron and vasoconstriction or vasodilation of renal arterioles. In the proximal tubules, 20-HETE inhibits Na⁺-K⁺-ATPase activity by inhibiting the phosphorylation of the α-subunit of Na⁺-K⁺-ATPase by protein kinase C. Similarly, EETs have been reported to inhibit Na⁺-K⁺-ATPase activity and sodium transport in the proximal tubules. In the renal vasculature, 20-HETE causes vasoconstriction, whereas EETs cause vasodilation of renal arterioles. In addition, 20-HETE has been shown to inhibit the opening of the calcium-activated potassium channels to depolarize smooth muscle cells, increase intracellular calcium, and constrict vascular smooth muscle. The role of 20-HETE and EETs in the regulation of BP has been further implied by studies showing that inhibition of 20-HETE synthesis and stimulation of EETs formation affect arterial pressure. These studies demonstrate, therefore, that these eicosanoids possess biological effects that may greatly contribute to the regulation of renal function and BP during obesity.

A sex-dependent mechanism in the regulation of BP has been observed in human and animal models. Recent studies have shown that BP is greater in men than in women at the same age. This is one reason why men are at greater risk for cardiovascular and renal disease than age-matched women. Similar observations have been made in animal models. For example, male spontaneously hypertensive rats have higher BP than age- and strain-matched female rats. Although the mechanisms responsible for the gender-specific differences in BP are not clear, several investigators have shown that the alterations of renal α2-adrenoceptor and leptin receptors may be involved in the sexual dimorphism of HF-induced hypertension.28,29 However, whether CYP-derived eicosanoids are involved in the sexual differences of BP regulation in HF rats is still not clear.

The present study was designed to measure BP and renal eicosanoid production in HF and control male and female rats to investigate whether the HF diet causes sodium retention in male and female rats, and to investigate the role of androgens in the regulation of renal eicosanoid synthesis and BP in HF-fed and control female rats. This study provides valuable information to evaluate the role of gender and CYP-derived metabolites in the regulation of renal function and BP during HF diet treatment.

**Methods**

**Materials**

The [1-¹⁴C]arachidonic acid (56 mCi/mmol) was obtained from DuPont-New England Nuclear (Boston, MA). All reagents for Western blot analysis were purchased from Amersham Bioscience (Piscataway, NJ). All solvents were HPLC grade. All EETs and 20-HETE standards were purchased from Cayman Chemicals (Ann Arbor, MI).

**Animals**

Male and female Sprague-Dawley rats were purchased from Harlan (Indianapolis, IN). Rats were split into two groups; high fat (HF) rats were fed a modified chow containing 36% fat (15.2% saturated and 20.8% unsaturated), 35% carbohydrate, and 0.4% salt (Bio-Serv, Frenchtown, NJ), and control rats were fed normal rat chow containing 10% fat (10% saturated and 90% unsaturated), 25% carbohydrate, and 0.3% salt. In some experiments, 9-week-old control or HF female rats, which were fed the HF diet for 6 weeks, were treated with 5α-dihydrotestosterone (40 mg/kg/d, intraperitoneally) for 4 weeks. This dosage is based on a previous study by Nakagawa et al. All animals were maintained on a 12-h light–dark cycle and were housed two to a cage. All animal protocols were approved by the Institutional Animal Care and Use Committee and were in accordance with the protocols for animal use outlined in the Guides for the Care and Use of Laboratory Animals.

**Isolation of Renal Microsomal Fractions, Proximal Tubules, and Microvessels**

Treated and control rats were anesthetized with sodium pentobarbital (50 mg/kg, intraperitoneally). The kidneys were removed and renal cortex was homogenized in buffer containing 100 mmol/L Tris-HCl and 1.15% KCl at pH 7.4.
7.4. Homogenates were centrifuged at 10,000 g for 30 min. Microsomes were obtained by centrifugation of the supernatant at 100,000 g for 90 min and resuspended in 0.25 mol/L sucrose buffer and stored at –80°C. The proximal tubules were isolated according to the methods of Hatzinger and Stevens31 and Chaudhari and Kirschbaum.32 This procedure uses proteolytic digestion and Percoll gradient centrifugation. For the isolation of renal microvessels, kidneys were excised, placed in ice-cold Tyrode buffer, and coronally sectioned. The renal papilla was removed to expose the microvessels, and segments of the interlobar artery were microdissected and freed from cortical and connective tissue. The purity of the microdissected microvessel preparation was determined as described previously.33

Activities of Arachidonic Acid Metabolism in Renal Microsomes, Proximal Tubules, and Renal Microvessels

Renal cortical and proximal tubular microsomes (150 μg) isolated from treated and control rats were incubated with [1-14C]arachidonic acid (0.4 μCi, 7 nmol) and NADPH (1 mmol/L) in 0.3 mL of potassium phosphate buffer (100 mmol/L, pH 7.4) containing 10 mmol/L MgCl2 for 30 min at 37°C. The reaction was terminated by acidification to pH 3.5–4.0 with 2 mol/L formic acid, and arachidonic acid metabolites were extracted with ethyl acetate. The ethyl acetate was evaporated under nitrogen, and the metabolites were resuspended in 50 μL of methanol and injected onto the HPLC column. Reverse-phase HPLC was performed on a 5 μm ODS-Hypersil column, 4.6 × 200 mm (Hewlett Packard, Palo Alto, CA) using a linear gradient of acetonitrile:water:acetic acid ranging from 50:50:0.1 to 100:0:0.1 at a flow rate of 1 mL/min for 30 min. The elution profile of the radioactive products was monitored by a flow detector (In/us System Inc., Tampa, FL). The identities of arachidonic acid metabolites (20-HETE, DHETs, and EETs) were confirmed with authentic standards. The activity of the formation of these metabolites was estimated based on the specific activity of the standards. The activity of the formation of these metabolites was estimated based on the specific activity of the standards. The activity of the formation of these metabolites was estimated based on the specific activity of the standards. The activity of the formation of these metabolites was estimated based on the specific activity of the standards.
anti-chicken β-actin antibody (Sigma, St. Louis, MO) for 10 h. The secondary antibody was horseradish peroxidase-coupled, rabbit anti-mouse antibody (1:5000). Immunoreactive β-actin was detected as described. The ECL films of Western blot analysis were scanned, and densitometry analysis was performed with Scion Image software using gray color scale as a standard.

Statistical Analysis
Data are expressed as mean ± SE. All data were analyzed by a one-way analysis of variance or the Student t test for unpaired samples. Statistical significance was set at P < .05.

Results
Effect of HF Diet on BP and Arachidonic Acid Metabolism in Male and Female Rats
To study the effect of the HF diet on gender-specific BP regulation in rats, we measured mean arterial pressure (MAP) after HF diet treatment in male and female rats. Three-week-old male and female rats were fed the control or HF diet. Fig. 1A shows that the HF diet significantly increased MAP in male rats after 6 weeks of treatment, and that this elevation in BP was maintained for 5 weeks. In contrast, HF diet treatment showed no significant impact on MAP in female rats. Moreover, there was a 46% decrease in renal cortical ω-hydroxylase and epoxygenase activities in HF male rats compared with control rats, whereas no significant changes in renal cortical ω-hydroxylase and epoxygenase activities were observed in female rats (Fig. 1B). These results demonstrate that there are gender-specific differences in BP regulation and renal eicosanoid synthesis between HF male and female rats.

Arachidonic Acid Metabolism and CYP4A and 2C23 Expression in the Proximal Tubules of HF Male Rats
Because there is significant downregulation of renal eicosanoid synthesis in cortical microsomes and no significant change in the renal microvessels, we examined arachidonic acid metabolism in the proximal tubules of male rats after being fed the HF diet for 10 weeks. As shown in Fig. 2A, HF diet treatment caused a 30% decrease in ω-hydroxylase activity and a 38% decrease in epoxygenase activity in the proximal tubules of HF male rats. To examine whether CYP4A and 2C23 were responsible for the reduction of synthesis of these eicosanoids as previously described, we conducted Western blot analysis for CYP4A, 2C23, and CYP2J isoforms in the proximal tubules (Fig. 2B). The expression of CYP4A and 2C23 in the proximal tubules of HF male rats was lower than in control male rats, whereas no significant difference was seen for CYP2J proteins (Fig. 2B). Densitometric analysis normalized to β-actin revealed a significant decrease of 48% for CYP4A (1.72 ± 0.13 v 0.9 ± 0.06 arbitrary units, n = 3, P < .05) and 40% for CYP2C23 (1.79 ± 0.15 v 1.07 ± 0.15 arbitrary units, n = 3, P < .05) in the proximal tubules. These results suggest that downregulation of the expression of the CYP4A and 2C23 isoforms are responsible for the decrease of eicosanoid synthesis in the proximal tubules of HF male rats.

Effect of the HF Diet on Urinary Sodium Excretion, Urine Volume, Cumulative Sodium Balance, and BP in Male and Female Rats
To examine whether the HF diet has an effect on sodium retention in male and female rats, 8-week-old female and male rats were fed the control or HF diet. The MAP was measured after 6 to 10 weeks of treatment as described in the Methods section. After 10 weeks of treatment, renal cortical microsomes were prepared for analysis of arachidonic acid metabolism. Epoxygenase activity was determined from the sum of DHETs and EETs formation, and ω-hydroxylase activity was determined from 20-HETE formation. Results are mean ± SE (n = 5, *P < .05 from control, #P < .05 untreated male from female rats). See text for abbreviations.
mimic the results in Fig. 1A, which showed a significant elevation of BP in male rats after 6 weeks of treatment with the HF diet. These results were similar to those observed in HF dogs,\textsuperscript{36} and provide evidence that the HF diet is an important factor in causing sodium retention and elevation of BP in male rats.

**Effect of 5α-dihydrotestosterone on Arachidonic Acid Metabolism, BP, CYP Expression, and Urinary Sodium Excretion in HF-fed and Control Female Rats**

To investigate the role of androgens in the regulation of BP in HF and control female rats, 9-week-old control and HF-fed female rats, fed the control or HF diet for 6 weeks, were treated with 5α-dihydrotestosterone (40 mg/kg/d, intraperitoneally) for 4 weeks. At this dosage, Nakagawa et al.\textsuperscript{30} have shown that the treatment of 5α-dihydrotestosterone can cause the elevation of testosterone in the plasma of female rats. As shown in Table 1, 5α-dihydrotestosterone treatment increased BP and renal microvessel α-hydroxylase activity by 76% and 65% in HF and control female rats, respectively, but showed no significant effect on epoxygenase activity and on urinary sodium excretion. To further study the effect of 5α-dihydrotestosterone on renal CYP-derived eicosanoid produc-

FIG. 2  Effect of HF diet on arachidonic acid metabolism and the expression of CYP2C23, CYP4A, and CYP2J in the proximal tubules of male rats. (A) Epoxygenase and α-hydroxylase activity in the proximal tubular microsomes isolated from male rats fed the HF diet for 10 weeks. Results are mean ± SE (n = 4, *P < .05 from control). (B) Representative immunoblots of CYP4A, CYP2C23, CYP2J, and β-actin expression in the proximal tubules isolated from control (Con) and rats fed the high fat diet (HF). Renal microsomes (10 μg) from cortical microsomes were separated on an 8% SDS-polyacrylamide gel, transferred to a nitrocellulose membrane, and blotted with goat anti-rat CYP4A1 (1:5000), rabbit anti-CYP2C23 (1:5000), rabbit anti-human CYP2J2 (1:2000), or mouse anti-chicken β-actin antibody (1:5000). Immunoreactive proteins were detected using ECL Plus detection system. See text for abbreviations.

FIG. 3  Effect of HF diet treatment on cumulative sodium balance in male (A) and female (B) rats for 10 days. Cumulative sodium balance was determined as described in the Methods section. Results are mean ± SE (n = 6, *P < .05 from control).
tion and the expression of CYP \( \omega \)-hydroxylase and epoxygenase, we measured arachidonic acid metabolism and performed Western blot analysis on the renal cortex of HF-fed female rats treated with 5\( \alpha \)-dihydrotestosterone. As shown in Fig. 4A, arachidonic acid \( \omega \)-hydroxylase activity in renal cortical microsomes was increased by 73%, but no effect on epoxygenase activity was seen. In addition, the expression of cortical CYP4A enzymes in the 5\( \alpha \)-dihydrotestosterone treatment group was higher than that of HF female rats (Fig. 4B). Densitometric analysis revealed a marked 2.8-fold increase in CYP4A protein levels in the 5\( \alpha \)-dihydrotestosterone treatment group (0.75 \pm 0.08 in treated group \( v 0.26 \pm 0.03 \) arbitrary unit in control group, \( n = 3, P < 0.05 \)), but no significant effect on the expression of CYP2J (0.84 \pm 0.08 in treated group \( v 0.95 \pm 0.07 \) arbitrary unit in control group, \( n = 3 \)) and CYP2C23 (1.51 \pm 0.03 in treated group \( v 1.62 \pm 0.2 \) arbitrary unit in control group, \( n = 3 \)).

**Table 1.** Effect of 5\( \alpha \)-dihydrotestosterone on mean arterial pressure, urinary sodium excretion, and renal microvessel CYP-derived eicosanoid production in HF-fed and control female rats

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>5( \alpha )-T-treated</th>
<th>Female Rats</th>
<th>Control</th>
<th>5( \alpha )-T-treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mm Hg)</td>
<td>114 ± 5</td>
<td>128 ± 3*</td>
<td>118 ± 3</td>
<td>131 ± 2*</td>
<td></td>
</tr>
<tr>
<td>Urinary Na (mEq/24 h)</td>
<td>1.1 ± 0.08</td>
<td>1.0 ± 0.02</td>
<td>1.1 ± 0.04</td>
<td>1.0 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>MV ( \omega )-hydroxylase (pmol/mg/min)</td>
<td>25 ± 6</td>
<td>44 ± 8*</td>
<td>23 ± 4</td>
<td>38 ± 7*</td>
<td></td>
</tr>
<tr>
<td>MV epoxygenase (pmol/mg/min)</td>
<td>32 ± 9</td>
<td>30 ± 8</td>
<td>30 ± 6</td>
<td>27 ± 5</td>
<td></td>
</tr>
</tbody>
</table>

MAP = mean arterial pressure; 5\( \alpha \)-T = 5\( \alpha \)-dihydrotestosterone; MV = microvessel.

\( P < 0.05 \).

**Discussion**

In a previous study, we demonstrated significant changes in renal microsomal CYP4A and CYP2C23 expression, CYP-derived eicosanoid synthesis, and BP after 10 weeks of HF diet treatment in 3-week-old male rats.\(^{35}\) The HF diet treatment increased BP and reduced the expression of CYP4A and CYP2C23. The decreased expression of CYP4A and CYP2C23 corresponded to decreased synthesis of 20-HETE, EETs, and DHETs. However, the exact site of the downregulation of eicosanoid synthesis is unknown. In the present study, we demonstrated that the downregulation site of the arachidonic acid metabolic pathway that yields 20-HETE, EETs, and DHETs occurs in the proximal tubules, the major site for eicosanoid synthesis in the renal cortex. This conclusion is based on the observation that microsomal \( \omega \)-hydroxylase and epoxygenase activities in the proximal tubules are significantly decreased in male rats fed the HF diet. The expression levels of CYP4A and CYP2C23 are also significantly decreased in proximal tubular microsomes.

This finding may have very important functional implications for these eicosanoids in the regulation of renal function and BP in obese male rats. For example, research results by Hall and colleagues\(^{36}\) have demonstrated that an increase in cumulative sodium balance is associated with the elevation of MAP in an obese dog model, and that sodium retention is due to an augmentation of tubular reabsorption. The 20-HETE is one of the major non-cyclooxygenase metabolites of arachidonic acid in the rat kidney. The main site of its synthesis along the nephron is the proximal tubules, where it is estimated that 85% of total 20-HETE formation occurs.\(^ {37}\) Similarly, the proximal tubules are also a rich source of EET synthesis, providing more than 50% of the EET produced by...
the nephron in rats.\textsuperscript{37} Interestingly, both 20-HETE and EETs can inhibit Na\textsuperscript{+}-K\textsuperscript{+}-ATPase activity in the proximal tubules.\textsuperscript{3} Because 20-HETE and EETs have been shown to play an important role in the inhibition of sodium transport in the proximal tubules,\textsuperscript{1,2} the downregulation of the synthesis of these metabolites in the proximal tubules may cause the augmentation of sodium reabsorption in the kidneys, and result in the elevation of BP in HF rats. We observed significant sodium retention (Fig. 3A) and elevation of BP after 10 days of HF diet treatment in male rats, whereas no sodium retention was found in female rats (Fig. 3B). These results provide additional evidence that the HF diet causes sodium retention, which may underlie the elevation of BP in HF male rats.

Many results have shown that the CYP4A enzymes are considered to be the major renal arachidonic acid ω-hydroxylases in the rat and thereby the primary contributors of 20-HETE synthesis. For example, several reports have demonstrated that the CYP4A isoforms, in their recombinant or purified forms, can catalyze the ω-hydroxylation of arachidonic acid into 20-HETE,\textsuperscript{4,6} and CYP4A1 is the low K\textsubscript{m} form for ω-hydroxylase.\textsuperscript{4} Moreover, studies using CYP4A isoforms, a possibility of the involvement of other CYP4 proteins such as the CYP4F isoforms, for renal 20-HETE synthesis has been suggested.\textsuperscript{39} The CYP4F isoforms share about 40\% amino acid sequence similarity to CYP4A isoforms, and CYP4F1, CYP4F4, and CYP4F5 are expressed in renal tissues.\textsuperscript{40,41} Recently, Xu et al\textsuperscript{39} have demonstrated that recombinant CYP4F1 and CYP4F4 have very high catalytic activity for 20-HETE synthesis (K\textsubscript{m} about 9 and 11 min\textsuperscript{−1}). However, the involvement of these two isoforms for renal 20-HETE synthesis is still questionable because some of renal ω-hydroxylase inhibitors have very high median inhibitory concentration (IC\textsubscript{50}) for CYP4F-catalyzed 20-HETE formation. For example, DDMS (N-methylsulfonyl-12,12-dibromododec-11-enamide) is a very specific inhibitor for renal microsomal 20-HETE formation with an IC\textsubscript{50} about 2 μmol/L.\textsuperscript{42} In our previous publication, we have shown the IC\textsubscript{50} of DDMS for CYP4A-catalyzed 20-HETE formation to be about 1 μmol/L.\textsuperscript{4} However, the IC\textsubscript{50} of DDMS for CYP4F4-catalyzed 20-HETE formation is about 145 μmol/L, and no inhibition was found for CYP4F1. Nevertheless, we cannot rule out the possibility of the involvement of these CYP4F isoforms for 20-HETE synthesis under some specific circumstances. The development of specific antibodies and inhibitors for the CYP4F isoforms will be very important to clear up these issues. Although many CYP enzymes can carry out the epoxidation of arachidonic acid,\textsuperscript{2} epoxidation of arachidonic acid has been attributed to members of the CYP2C and CYP2J families.\textsuperscript{7,29} For example, Holla et al\textsuperscript{40} have demonstrated that CYP2C11 has the highest, CYP2C23 is in the middle, and CYP2C24 has the lowest epoxide activity. Based on kidney epoxygenase profiles and antibody inhibition studies it has been established that CYP2C23 is the predominant epoxide enzyme in the rat kidney. Our present results indicate that downregulation of CYP4A and CYP2C23 (Fig. 2B) corresponds to the decreased activities of ω-hydroxylation and epoxidation of arachidonic acid in the proximal tubules (Fig. 2A). These results suggest that the CYP 4A and 2C23 isoforms contribute significantly in the synthesis of these metabolites in the proximal tubules, and that these isoforms may play an important role in the regulation of renal function and BP in HF-fed male rats.

In the present study, we have shown that treatment of HF-fed and control female rats with 5α-dihydrotestosterone causes hypertension and induces renal microvessel 20-HETE production in both groups of rats (Table 1), which are quite different from that observed in HF-fed male rats.\textsuperscript{35} These results are similar to the observation by Nakagawa et al\textsuperscript{30} who showed the same alterations with hypertension in male and female rats after 5α-dihydrotestosterone treatment. It appears thus first that androgens seem not play a major role in the renal alterations with hypertension induced by the HF diet in male rats. Because 5α-dihydrotestosterone has similar effects on BP, urinary sodium excretion, and renal microvessel CYP-derived eicosanoid production in both HF-fed and control female rats (Table 1), androgens may not be important factors in the sexual dimorphism of HF-induced hypertension. Because it has been shown that androgens can regulate CYP4A expression,\textsuperscript{30,43} the present study is primarily focused on androgens rather than estrogens. However, we cannot rule out that estrogens may play an important role in protecting females from developing hypertension. Additional experiments involving estrogen replacement in ovariectomized female rats will be needed to further study the sexual dimorphism in HF-induced hypertension.

In summary, this study demonstrates gender-specific regulation of renal eicosanoid synthesis and BP caused by feeding rats the HF diet. These data also demonstrate that the HF diet can cause downregulation of renal eicosanoid synthesis and the expression of CYP4A and CYP2C23 in the proximal tubules of male rats, and cause sodium retention and increase BP as well. In addition, androgens may not be the major contributors to the sexual dimorphism of HF-induced hypertension.

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