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Gender differences in adenine-induced chronic kidney disease and cardiovascular complications in rats

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1School of Biomedical Science, The University of Queensland, Brisbane, Australia; 2Centre for Kidney Disease Research, School of Medicine, Translational Research Institute, The University of Queensland, Brisbane, Australia; and 3School of Health, Nursing and Midwifery, The University of Southern Queensland, Toowoomba, Australia

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Diwan V, Small D, Kauter K, Gobe GC, Brown L. Gender differences in adenine-induced chronic kidney disease and cardiovascular complications in rats. Am J Physiol Renal Physiol 307: F1169–F1178, 2014. First published September 10, 2014; doi:10.1152/ajprenal.00676.2013.—Gender contributes to differences in incidence and progression of chronic kidney disease (CKD) and associated cardiovascular disease. To induce kidney damage in male and female Wistar rats (n = 12/group), a 0.25% adenine diet for 16 wk was used. Kidney function (blood urea nitrogen, plasma creatinine, proteinuria) and structure (glomerular damage, tubulointerstitial atrophy, fibrosis, inflammation); cardiovascular function (blood pressure, ventricular stiffness, vascular responses, echocardiography) and structure (cardiac fibrosis); plasma testosterone and estrogen concentrations; and protein expression for oxidative stress [heme oxygenase-1, inflammation (TNF-α), fibrosis (transforming growth factor-β), ERK1/2, and estrogen receptor-α (ER-α)] were compared in males and females. Adenine-fed females had less decline in kidney function than adenine-fed males, although kidney atrophy, inflammation, and fibrosis were similar. Plasma estrogen concentrations increased and plasma testosterone concentrations decreased in adenine-fed males, with smaller changes in females. CKD-associated molecular changes in kidneys were more pronounced in males than females except for expression of ER-α in the kidney, which was completely suppressed in adenine-fed males but unchanged in adenine-fed females. Both genders increased blood pressure, ventricular stiffness, and cardiac fibrosis with the adenine diet. Cardiovascular changes with adenine were similar in males and females, except males developed concentric, and females eccentric cardiac hypertrophy. In hearts from adenine-fed male and female rats, expression of ER-α and activation of the ERK1/2 pathway were increased, in part explaining changes in cardiac hypertrophy. In summary, adenine-induced kidney damage may be increased in males due to the suppression of ER-α.

chronic kidney disease; cardiovascular disease; inflammation; estrogen receptor-α; ERK1/2

ESTROGEN IS PRESENT AT HIGHER concentrations in premenopausal women than in postmenopausal women and men (20). Estrogen has antioxidant, anti-inflammatory, cardioprotective (20), and renoprotective (18) properties. Estrogen-regulated genes are involved in extracellular matrix metabolism and upregulated the expression of angiotensinogen and angiotensin type 2 receptors (40). Physiological concentrations of estradiol induced release of nitric oxide (NO) (42), which can affect the glomerular filtration rate (GFR) and induce apoptosis and hence may influence renal disease progression (10, 33). In kidney diseases, men progressed to end-stage kidney disease at a faster rate than women before menopause (47). In a Chinese population divided into three groups as healthy, at-risk, and chronic kidney disease (CKD) groupings within men or women, men showed elevated blood pressure, total cholesterol, triglycerides, and LDL and decreased GFR compared with women (46). This suggests that increased estrogen concentrations may protect premenopausal women against the development of CKD and cardiovascular disease (46, 47). Animal studies suggest that females are protected against kidney disease compared with age-matched males (23). Female rats showed less renal injury from ischemia-reperfusion than males (3). In the cisplatin-induced rat model of nephrotoxicity, male rats showed greater increases in blood urea nitrogen (BUN) and creatinine than female rats (14). In ½ nephrectomized rats, males and ovariectomized females showed greater increases in BUN and creatinine concentrations than intact females; these changes were reversed by administration of estradiol (18). Estrogen therapy reduced malondialdehyde, glutathione, and myeloperoxidase concentrations in hearts from these rats, suggesting antioxidant and anti-inflammatory actions in CKD-associated cardiovascular disease (18). These studies suggest that CKD in females progresses more slowly than in males because of higher estrogen concentrations.

In the heart, estrogen regulated the level and activity of ion channels and modulated cardiac repolarization, cardiac contractility, and contractile reserve (28). Gender differences have been recorded in cardiovascular disease, a serious complication associated with CKD (13, 24). Female rats showed greater left ventricular remodeling than males in chronic aortic regurgitation (12), but males showed greater left ventricular remodeling than females in valvular disease (36). Females showed estrogen-mediated protection from cardiovascular disease, supported by the increased rates of cardiovascular disease in postmenopausal women (37).

Estrogen activates two different estrogen receptors, referred to as estrogen receptor α and β (ER-α and ER-β), but the classic estrogen effects are mediated by ER-α (27). Human myocardial ER-α expression and mRNA are upregulated in human aortic stenosis and heart failure, in both male and female patients (26), suggesting a possible role in cardiovascular diseases. However, in kidney disease, ER-α may provide protection against apoptosis of podocytes and glomerulosclerosis (11, 21). These studies suggest that higher estrogen and...
ER-α concentrations may underlie the slower progression of CKD in female than in male subjects. Therefore, this study was designed to elucidate the gender differences in CKD and related cardiovascular disease and to evaluate the expression and effect of ER-α pathway. We have established a model of CKD with associated cardiovascular damage using 0.25% adenine in the diet of male rats for 16 wk (10). In this model, marked kidney and heart damage were found after 16 wk of adenine supplementation. In the present study, we compared the development of kidney disease and associated cardiovascular damage with 0.25% adenine in age-matched young adult male and female rats.

MATERIALS AND METHODS

Animal Studies

All experimental protocols were approved by the Animal Ethics Committee of the University of Southern Queensland, under the guidelines of the National Health and Medical Research Council of Australia. Twenty-four male and 24 female Wistar rats, aged 9–10 wk, were divided into groups of 12 rats treated for 16 wk either as controls with powdered rat chow or with 0.25% adenine (Carbosynth, Compton, Berkshire, UK) in powdered rat chow (10).

Systolic Blood Pressure Measurement

Systolic blood pressure was measured in rats under light intraperitoneal (ip) sedation with an injection of zolotel (15 mg/kg tiletamine, 15 mg/kg zolazepam im) every 4 wk. Measurements were taken using an MLT1010 Piezo-Electric Pulse Transducer (ADInstruments, Sydney, Australia) and an inflatable tail cuff connected to a MLT844 Physiological Pressure Transducer (ADInstruments) and Power Lab data acquisition unit (ADInstruments) (32). Systolic blood pressure was averaged from at least four measurements in each rat at each time point.

Echocardiography

Echocardiography was performed at the end of 16 wk by trained cardiac sonographers at the Small Animal Theater of The Prince Charles Hospital (Brisbane, Australia). Rats were anesthetized via injection with zolotel (15 mg/kg tiletamine, 15 mg/kg zolazepam im) mixed with ilum xylazil (10 mg/kg xylazine). Echocardiographic images of rats were obtained using a Hewlett Packard Sonos 5500 (12-MHz-frequency fetal transducer) at an image depth of 3 cm using two focal zones. Measurements of left ventricular posterior wall thickness and internal diameter were made using two-dimensional M-mode taken at the midpapillary level (4).

Collection of Urine and Plasma

Rats were kept in metabolic cages after 16 wk for 24 h to collect urine. The urine was used for the estimation of protein by Bradford assay (43). Before the termination of experiments, rats were anesthetized and blood was collected for plasma, which was stored at −20°C until further analysis (32). BUN and plasma creatinine (Pcr), uric acid, sodium, potassium, calcium, and lactate dehydrogenase (LDH) were determined by The University of Queensland Veterinary Pathology Services, using an Olympus AU400 autoanalyzer (32). Clearances of creatinine and BUN were calculated (34).

Isolated Heart Preparation

The ventricular diastolic stiffness of the rats in all treatment groups was assessed. Terminal anesthesia was induced via an ip injection of pentobarbitone sodium (Lethabar, 100 mg/kg). Blood (5 ml) was immediately drawn from the abdominal aorta after administration of heparin (1,000 IU, Sigma-Aldrich, Sydney, Australia) through the right femoral vein. Retrograde perfusion was initiated at constant pressure (100 cm water) with modified Krebs-Henseleit buffer containing (in mM) 119.1 NaCl, 4.75 KCl, 1.19 MgSO4, 1.19 KH2PO4, 25.0 NaHCO3, 11.10 glucose, and 2.16 CaCl2 maintained at 37°C and bubbled with 95% O2-5% CO2. A latex balloon catheter was inserted into the left ventricle for measurement of isovolumic left ventricular function via connection to a Capto SP844 physiological pressure transducer (MLT844/D) linked to a PowerLab recording system. Hearts were paced at 250 beats/min by attaching two electrodes to the surface of the right atria. End-diastolic pressure was initially set to 10 mmHg by balloon inflation, and all hearts received an equilibration period of ~5 min. End-diastolic pressure was measured for 3 min at 5-mmHg increments beginning at 0 mmHg up to a maximum of 30 mmHg. Measurements of diastolic pressure and systolic pressure were made after 2 min of each 3-min recording for further calculation of diastolic stiffness and left ventricular developed pressure. Myocardial diastolic stiffness was defined by the stiffness constant (k, dimensionless), which is the slope of the linear relationship between the tangent elastic modulus (E, dynes/cm2) and stress (s, dynes/cm2) (1).

Organ Bath Studies for Vascular Response

Thoracic aortic rings (~4 mm in length) were suspended in an organ bath chamber with a resting tension of 10 mN and bathed in a modified Tyrode solution containing (in mM) 136.9 NaCl, 5.4 KCl, 1.05 MgCl2, 1.8 CaCl2, 22.6 NaHCO3, 0.42 NaH2PO4, 5.5 glucose, 0.28 ascorbic acid, and 1.0 EDTA. The Tyrode solution was bubbled with carbogen (95% O2-5% CO2), and the temperature was maintained at 37 ± 0.5°C. Force of contraction was measured isometrically with Grass FT03C force transducers connected via amplifiers to a Macintosh computer via the MacLab system. Cumulative concentration-response (contraction) curves were measured for noradrenaline (Sigma-Aldrich), and concentration-response (relaxation) curves were measured for acetylcholine (Sigma-Aldrich) and sodium nitroprusside (Sigma-Aldrich) in the presence of a submaximal (70%) contraction to noradrenaline (6). For the stock solutions of the drugs, 0.1 M solutions in water of noradrenaline, acetylcholine, and sodium nitroprusside were prepared for serial dilutions to 1 μM. Each of the concentrations was used to measure the cumulative response for each drug. Data obtained were analyzed using one-way ANOVA followed by Tukey’s test as post hoc analysis. P < 0.05 was considered significant.

Estrogen and Testosterone Measurements

Estrogen concentrations were measured with a 17β-estradiol high-sensitivity ELISA kit from Enzo Life Sciences (catalog no. ADI-900-174, Sapphire Bioscience) with 0.13 pg/ml sensitivity. Testosterone concentrations were measured with a testosterone ELISA kit from Enzo Life Sciences (catalog no. ADI-900-065, Sapphire Bioscience) with 6.47 pg/ml sensitivity.

Histology

Immediately after removal, slices of kidney and heart (left ventricle) were fixed in 10% buffered formalin for 3 days, with a change of formalin solution every day. The samples were then dehydrated and embedded in paraffin wax. Thin sections (4 μm) of the kidney and left ventricle were cut and stained with hematoxylin and eosin (H&E) or Masson’s trichrome stain (kidney) and picrosirius red (heart) for collagen deposition (32). An Aperio Scanscope XT histology slide scanner (Vista, Aperio Technologies) for imaging was used for H&E and Masson’s trichrome, and image morphometry was analyzed using ImagePro Plus image-analysis software. Cardiac fibrosis using picrosirius red was evaluated by National Institutes of Health ImageJ software. Percent tubulointerstitial atrophy and percent tubules with casts/cell debris were assessed morphometrically in four fields/kidney at ×20 digitally scanned magnification. Apoptosis was counted using...
Table 1. Kidney functional parameters and plasma biochemistry in 0.25% adenine-fed male and female rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>0.25% Adenine</th>
<th>Females</th>
<th>0.25% Adenine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water intake, ml/day</td>
<td>51 ± 6</td>
<td>91 ± 4*</td>
<td>31 ± 1</td>
<td>41 ± 1*†</td>
</tr>
<tr>
<td>Urine output, ml/24 h</td>
<td>23 ± 2</td>
<td>79 ± 2*</td>
<td>17 ± 1</td>
<td>23 ± 1*†</td>
</tr>
<tr>
<td>BUN, mmol/l</td>
<td>6 ± 0.6</td>
<td>57 ± 5*</td>
<td>6 ± 0.4</td>
<td>8 ± 0.3*†</td>
</tr>
<tr>
<td>Creatinine, μmol/l</td>
<td>42 ± 3</td>
<td>268 ± 23*</td>
<td>46 ± 1</td>
<td>61 ± 1*†</td>
</tr>
<tr>
<td>Potassium, mmol/l</td>
<td>3.8 ± 0.2</td>
<td>5.0 ± 0.3*</td>
<td>4.0 ± 0.1</td>
<td>4.5 ± 0.3*</td>
</tr>
<tr>
<td>LDH, U/l</td>
<td>307 ± 31</td>
<td>487 ± 47*</td>
<td>328 ± 56</td>
<td>391 ± 59*</td>
</tr>
<tr>
<td>Uric acid, μmol/l</td>
<td>38 ± 1</td>
<td>64 ± 1*</td>
<td>47 ± 6</td>
<td>70 ± 8*</td>
</tr>
<tr>
<td>Testosterone, ng/ml</td>
<td>16.0 ± 0.1</td>
<td>13.3 ± 0.2*</td>
<td>6.7 ± 0.3†</td>
<td>6.5 ± 0.3†</td>
</tr>
<tr>
<td>Estrogen, pg/ml</td>
<td>141 ± 12</td>
<td>346 ± 8*</td>
<td>157 ± 4</td>
<td>189 ± 8*†</td>
</tr>
<tr>
<td>Protein in urine, mg/l</td>
<td>150 ± 7</td>
<td>303 ± 19*</td>
<td>134 ± 2</td>
<td>238 ± 3*†</td>
</tr>
<tr>
<td>Interstitial fibrosis, % in kidneys</td>
<td>5 ± 0.5</td>
<td>36 ± 3*</td>
<td>4.5 ± 0.4</td>
<td>32 ± 3.5*</td>
</tr>
</tbody>
</table>

Values are means ± SE. BUN, blood urea nitrogen; LDH, lactate dehydrogenase. *P < 0.05 vs. control. †P < 0.05 vs. adenine-fed male rats.

Table 2. Histopathological assessment of kidneys from males and females on 0.25% adenine diet

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Males (0.25% Adenine)</th>
<th>Females (0.25% Adenine)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Atrophy</td>
<td>91.0 ± 1.4</td>
<td>44.3 ± 5.3*</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Apoptotic cells/bodies in tubular epithelium</td>
<td>5.5 ± 0.9</td>
<td>7.1 ± 1.8</td>
<td>0.1605</td>
</tr>
<tr>
<td>% Area with casts in tubules</td>
<td>68.3 ± 4.1</td>
<td>31.4 ± 4.7*</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Glomeruli with thickened Bowman’s capsule</td>
<td>2.4 ± 0.4</td>
<td>1.6 ± 0.3</td>
<td>0.1524</td>
</tr>
<tr>
<td>Inflammatory infiltrate</td>
<td>3.3 ± 0.3</td>
<td>1.5 ± 0.2*</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05 vs. male adenine-fed rats.
kidneys from male and female adenine-fed male rats showed tubular atrophy with flattening of the epithelium in the proximal tubules and vacuolation of tubular cells, increased infiltration of inflammatory cells, increased cell debris or proteinaceous cast material in the tubular lumen (Fig. 1, Table 2), and increased collagen deposition (details in Table 1 compared with control rat kidneys). There was no mesangial thickening/sclerosis in glomeruli of males and females fed 0.25% adenine, but there was sometimes thickening of Bowman's capsule (scored as numbers of glomeruli with thickened Bowman’s capsules/field in 4 fields/kidney) (Table 2).

**Cardiovascular Function and Structure**

Systolic blood pressure increased in both male and female adenine-fed rats compared with their respective control groups, but the increase in adenine-fed female rats was less than in adenine-fed male rats (Fig. 2). Male adenine-fed rats had decreased left ventricular internal diameter, both in systole and diastole, with decreased end-systolic, end-diastolic volumes, and stroke volume, suggesting concentric cardiac hypertrophy (5). Male adenine-fed rats had increased relative wall thickness. Female adenine-fed rats showed eccentric cardiac hypertrophy, demonstrated by increased left ventricular diameter, both in systole and diastole, with increased end-systolic, end-diastolic, and stroke volumes. Female adenine-fed rats, unlike male rats, had decreased relative wall thickness, suggesting ballooning of the left ventricle, with a decrease in ejection fraction (Table 3).

In isolated thoracic aortic rings, male adenine-fed rats had impaired noradrenaline-mediated contraction and sodium nitroprusside- and acetylcholine-mediated relaxation (Fig. 3) compared with control rats. Female adenine-fed rats only showed impaired acetylcholine- and sodium nitroprusside-mediated relaxation. There was no change in noradrenaline-mediated contraction in adenine-fed female rats compared with control female rats. The acetylcholine-mediated relaxation in adenine-fed female rats was less impaired compared with adenine-fed male rats (Fig. 3). Both adenine-fed male and
female rats had increased left ventricular stiffness constant (Table 3) compared with their respective controls. There was increased infiltration of inflammatory cells and cardiac fibrosis in hearts from both male and female adenine-fed rats (Fig. 4).

**Plasma Estrogen and Testosterone Concentrations**

Plasma estrogen concentrations increased in adenine-fed rats, with a greater increase in males than in females (Table 1). Plasma concentrations of testosterone decreased in adenine-fed rats compared with control males, but were unchanged in female rats.

**Protein Expression in the Kidney and Heart**

Both male and female rat kidneys showed increased expression of HO-1, TNF-α, and TGF-β (Fig. 5). The percentage increases in expression of HO-1, TNF-α, and TGF-β were less in kidneys from female adenine-fed rats than in male adenine-fed rats (Fig. 5). Kidney ER-α expression in male adenine-fed rats was completely suppressed compared with no change in kidney ER-α expression in female adenine-fed rats (Fig. 6). In contrast, hearts from both male and female adenine-fed rats had increased expression of ER-α. However, the percent increase in females was smaller than in males (Fig. 6). The hearts from both male and female adenine-fed rats had decreased expression of ERK 1/2 compared with control male rats. However, female kidneys did not show any change in ERK 1/2 expression compared with control female rats (Fig. 8).

**DISCUSSION**

The cellular and molecular characteristics of gender differences in CKD and the associated cardiovascular damage are not well defined. Preclinical and clinical evidence suggests progression of kidney disease is slower in females compared with males (15, 23, 47). The links between the pathogenesis of CKD and cardiovascular disease are known, but the molecular mechanisms linking these diseases are not clear (25). In the present study, we have used an adenine diet model of CKD with associated cardiovascular changes (10) to demonstrate cellular and molecular contributions to gender differences. We suggest that gender-specific differences in kidney ER-α expression possibly related to changes in plasma estrogen and testosterone concentrations may correlate with differences in kidney function in adenine-fed males and females.

Female adenine-fed rats showed minimal decline in kidney function, demonstrating only increased PCR with normal BUN. However, these females had increased proteinuria and evidence of early structural characteristics of CKD such as infiltration of inflammatory cells, fibrosis, tubular atrophy, and tubules with cell debris compared with control female rats. Similar changes were shown previously in 5/6 nephrectomized rats, where males and females developed glomerular injury after 90 days (23). Adenine-fed female rats also showed changes in protein expression compared with control female rats, with increased expression of HO-1 (oxidative stress), TNF-α (inflammation), TGF-β (fibrosis), and ERK 1/2 (cell growth) as important mechanisms in the progression of CKD (10). Male adenine-fed rats showed increased kidney injury defined by decreased function, structural damage, and molecular changes compared with female adenine-fed rats. The differences in renal function due to ER-α may be secondarily mediated by any or all of these factors, or the observed differences in renal function may be independent of ER-α. Further work is necessary to define these possibilities.

ER-α knockout mice showed more kidney damage than wild-type mice (11, 21) with more collagen deposition and increased expression of TGF-β in kidneys (21), suggesting that increased ER-α expression is protective in kidney diseases.

Table 3. Functional and structural changes in male and female rat hearts at 16 wk on 0.25% adenine diet

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>0.25% Adenine</td>
</tr>
<tr>
<td>Left ventricular diameter (systole, mm)</td>
<td>3.6 ± 0.2</td>
<td>2.4 ± 0.2*</td>
</tr>
<tr>
<td>Left ventricular diameter (diastole, mm)</td>
<td>8.1 ± 0.3</td>
<td>7.5 ± 0.2*</td>
</tr>
<tr>
<td>Relative wall thickness</td>
<td>0.45 ± 0.02</td>
<td>0.62 ± 0.04*</td>
</tr>
<tr>
<td>End systolic volume, μl</td>
<td>49 ± 8</td>
<td>18 ± 4*</td>
</tr>
<tr>
<td>End diastolic volume, μl</td>
<td>570 ± 60</td>
<td>437 ± 34*</td>
</tr>
<tr>
<td>Stroke volume, μl</td>
<td>527 ± 60</td>
<td>422 ± 34*</td>
</tr>
<tr>
<td>Ejection fraction, %</td>
<td>93.2 ± 1.3</td>
<td>96.4 ± 1.1</td>
</tr>
<tr>
<td>Fractional shortening, %</td>
<td>59.2 ± 1.7</td>
<td>61.9 ± 2.3</td>
</tr>
<tr>
<td>Ventricular stiffness, κ</td>
<td>23.5 ± 0.53</td>
<td>32.9 ± 1.4*</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05 vs. control.
the present study, kidneys of adenine-fed males had negligible expression of ER-α compared with kidneys from control males in contrast to the maintained ER-α expression in kidneys from adenine-fed females. This markedly different response in females compared with males may help explain the decreased structural and functional changes in kidneys from adenine-fed females. ER-α in kidneys are expressed in tubules, brush borders, and glomeruli (17). In adenine-fed male rats, there was more damage to tubules, brush borders, and glomeruli (10), suggesting that this damage led to decreased expression of ER-α in male rat kidneys. Testosterone concentrations decreased in CKD males (2), as shown in adenine-fed male rats in the present study. Clearance of some hormones may decrease because of the decline in kidney function in CKD patients, which may lead to increased plasma concentrations (2). The reduced clearance might have resulted in increased concentration of estrogen in adenine-fed male and female rats. In N-nitro-L-arginine methyl ester/ANG II-induced proteinuria and kidney injury, ovariectomy reduced the injury in female rats. Estrogen replacement in these rats together with sham-operated rats increased proteinuria and structural injury (31). These reports support the results in our study where adenine-fed male rats showed marked kidney damage and proteinuria with higher plasma estrogen concentrations compared with female adenine-fed rats. In cultured ovine endothelial cells, 6-h exposure to estrogen increased ER-α expression (16) and long-term treatment with estrogen increased ER-α expression in vasculature (16). This supports increased expression of ER-α in hearts of adenine-fed rats with increased plasma estrogen concentrations.

In CKD patients, cardiovascular events are prevalent and increase mortality (9, 38). However, evidence for gender differences in cardiovascular disease is contradictory. Cardiovascular disease may be independent of the progression of kidney disease (8), and gender may not affect progression (36), even when obesity is a factor in development of the disease (35). Estrogens in females have been reported as protective against cardiovascular disease (37), but moderate dosing of estrogens worsened cardiac function and remodeling in ovariectomized female rats with left anterior artery ligation-induced myocardial infarction (48). In our previous study, male adenine-fed rats developed high blood pressure in addition to kidney damage, and both could contribute to the cardiac hypertrophy (10). In the present study, both males and females showed cardiac hypertrophy, with males showing concentric rather than eccentric hypertrophy as in the females. Concentric hypertrophy is an outcome of pure pressure overload while eccentric hypertrophy can be either volume overload or a
mixture of volume overload and pressure overload (5). In the present study, male rats showed a larger increase in blood pressure, explaining pressure overload (5). The increase in water intake in males was accompanied by increased urine output, which suggests that volume overload was minimal, with pressure overload as the possible reason for concentric hypertrophy. Female rats showed an increase in water intake that was not compensated for by increased urine output, suggesting that both volume overload (7) and pressure overload caused the eccentric hypertrophy (5). Similar changes in left
ventricular geometry have been demonstrated in obese male and female patients (35).

Clinical reports demonstrate increases in expression of ER-α in cardiac hypertrophy, aortic stenosis, failing hearts, and pressure overload in both genders (26, 30). Activation of ER-α induced phosphorylation of ERK 1/2 (44). In our study where cardiac hypertrophy was noted, we found close links between ER-α and the ERK 1/2 pathway. Similar results have been found by others: the ERK 1/2 pathway was activated in hearts in diabetic cardiomyopathy (41), and in recovery from myocardial infarction induced by left anterior descending artery ligation in ovariectomized females (48); in the myometrium, activation of ER-α increased the phosphorylation of ERK 1/2 which was decreased by ER-α inhibition (44); membrane ER-α activated the ERK 1/2 pathway in breast cancer cells, which further activated nuclear ER-α transcriptional activity, thus promoting ER-dependent transcription (39); ER-dependent transcription may be involved in hypertrophy (41); and higher levels of ER-α resulted in increased ERK 1/2 activation in breast cancer cells (49). Activation of the ERK 1/2 pathway has been found in both concentric and eccentric hypertrophy (19). In the present study, both genders showed increased expression of ER-α with increased phosphorylation of ERK 1/2, which could be the molecular pathway for cardiac hypertrophy associated with CKD in both genders.

**Conclusions**

Male rats developed more severe kidney damage with adenine treatment than female rats. The decreased kidney expression of ER-α and changes in plasma estrogen and testosterone concentrations in males may be associated with this increased damage, as ER-α was maintained and plasma hormone concentrations less affected in female adenine-fed rats. In both males and females, adenine induced structural changes in the kidneys associated with molecular changes, demonstrated by
increased oxidative stress, inflammation, and fibrosis. Both genders demonstrated increased blood pressure and left ventricular hypertrophy, but with different geometry. Cardiac hypertrophy may be mediated by ER-α and activation of the ERK 1/2 pathway.

GRANTS
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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS
Author contributions: V.D., K.K., G.C.G., and L.B. provided conception and design of research; V.D. and D.M.S. performed experiments; V.D. analyzed data; V.D., D.M.S., K.K., G.C.G., and L.B. interpreted results of experiments; V.D. prepared figures; V.D. and L.B. drafted manuscript; V.D., K.K., G.C.G., and L.B. edited and revised manuscript; V.D., K.K., G.C.G., and L.B. approved final version of manuscript.

REFERENCES


