Cardiac function is preserved following 4 weeks of voluntary wheel running in a rodent model of chronic kidney disease

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Kuczmarski JM, Martens CR, Kim J, Lennon-Edwards SL, Edwards DG. Cardiac function is preserved following 4 weeks of voluntary wheel running in a rodent model of chronic kidney disease. J Appl Physiol 117: 482–491, 2014. First published July 24, 2014; doi:10.1152/japplphysiol.00344.2014. —The purpose of this investigation was to determine the effect of 4 wk of voluntary wheel running on cardiac performance in the 5/6 ablation-infarction (AI) rat model of chronic kidney disease (CKD). We hypothesized that voluntary wheel running would be effective in preserving cardiac function in AI. Male Sprague-Dawley rats were divided into three study groups: 1) sham, sedentary nondiseased control; 2) AI-SED, sedentary AI; and 3) AI-WR, wheel-running AI. Animals were maintained over a total period of 8 wk following AI and sham surgery. The 8-wk period included 4 wk of disease development followed by a 4-wk voluntary wheel-running intervention/sedentary control period. Cardiac performance was assessed using an isolated working heart preparation. Left ventricular (LV) tissue was used for biochemical tissue analysis. In addition, soleus muscle citrate synthase activity was measured. AI-WR rats performed a low volume of exercise, running an average of 13 ± 2 km, which resulted in citrate synthase activity not different from that in sham animals. Isolated AI-SED hearts demonstrated impaired cardiac performance at baseline and in response to preload/afterload manipulations. Conversely, cardiac function was preserved in AI-WR vs. sham hearts. LV nitrite + nitrate and expression of LV nitric oxide (NO) synthase isoforms 2 and 3 in AI-WR were not different from those of sham rats. In addition, LV H2O2 in AI-WR was similar to that of sham and associated with increased expression of LV superoxide-dismutase-2 and glutathione peroxidase-1/2. The findings of the current study suggest that a low-volume exercise intervention is sufficient to maintain cardiac performance in rats with CKD, potentially through a mechanism related to improved redox homeostasis and increased NO.

Cardiac function; kidney disease; exercise; nitric oxide; and oxidative stress

EXERCISE TRAINING IS AN IMPORTANT adjunct therapy for the treatment and prevention of various cardiovascular diseases (9, 29, 43). Exercise provides sustainable protection against myocardial infarction and hypertension while improving cardiac function, cardiovascular outcomes, and quality of life (9, 29, 43). Yet few clinical and experimental studies have investigated the effect of exercise on cardiovascular function among those suffering with chronic kidney disease (CKD).

Cardiovascular disease (CVD) is the most significant cause of morbidity and mortality in patients with CKD (48a). At the heart, CVD manifests in part as cardiac dysfunction. Left ventricular (LV) systolic dysfunction and diastolic dysfunction are apparent early on in renal disease progression and are important risk factors for the development of heart failure and, ultimately, death (39, 41, 42, 49). Moreover, impaired cardiac function plays an important role in the risk of ischemic cardiac events as well as ensuing poor prognosis in patients with CKD (12, 38, 40). Previous animal studies from our laboratory and others have supported these findings by demonstrating impaired cardiac function in uremic rats that persists following short-duration ischemic insult (6, 27).

The purpose of this investigation was to determine the effect of a short, voluntary wheel-running intervention on cardiac function in the moderate-to-severe, 5/6 ablation-infarction (AI) rat model of CKD. In doing so, kidney disease and associated cardiac dysfunction were allowed to develop and progress over a period of 4 wk. A 4-wk voluntary wheel-running intervention was then initiated to test the hypothesis that wheel running would preserve cardiac function in AI after kidney disease had already been established. The overall cardiovascular benefits of exercise have been attributed to a multifactorial mechanism with effects observed at the heart and peripheral vasculature (9, 18). The underlying molecular mechanisms remain incompletely elucidated, but restoration of redox homeostasis and increased stable nitric oxide (NO) metabolites in the myocardium appear to play important roles (8, 9). Therefore, we also sought to determine whether preserved cardiac function with exercise was associated with alterations in markers of LV oxidative stress and NO.

MATERIALS AND METHODS

Animal care and use. The following experimental protocol was approved by the University of Delaware Animal Care and Use Committee and followed the guidelines established by the National Institutes of Health Office of Laboratory Animal Welfare for the use of animals in research.

Male Sprague-Dawley rats were purchased from Harlan Laboratories (Frederick, MD) and housed in a climate-controlled environment with a 12:12-h light-dark cycle. Food and water were provided ad libitum. Animals were then randomly assigned at 10 wk of age to sham (sedentary nondiseased control) or AI study groups, including one AI subset to remain sedentary (AI-SED) and another to undergo a 4-wk voluntary wheel-running intervention (AI-WR). AI-WR rats were then allowed to acclimate to custom-made running wheels for 1 wk.

At 12 wk of age, sham and AI groups underwent surgery as previously described (27). In short, all animals were anesthetized using isofluorane (1.0–5.0%) and AI animals had 2/3 of the renal artery branches supplying the left kidney ligated followed by removal...
of the right kidney to induce CKD. The kidneys of sham animals were exposed and manipulated with no ligation or ablation being performed. After surgery, all animals were maintained over a total period of 8 wk until being killed at 20 wk of age to allow for moderate-to-severe CKD to develop in AI. The 8-wk period included 4 wk of disease development until AI-WR rats were placed in cages with free access to custom-made running wheels. Citrate synthase activity was measured in soleus muscles isolated from a subset of animals in each group following death using a colorimetric assay (Sigma-Aldrich, St. Louis, MO) (sham n = 5; AI-SED n = 5; AI-WR n = 5; replicates n = 3).

Blood and urine analysis. Urine was collected overnight (16 h) in all groups using metabolic cages 1 wk prior to (baseline) and 4 and 7 wk following surgery. During this time, animals had free access to water but were restricted from food consumption. Urine volume was recorded for calculation of urine flow rate. Blood samples were collected from tail veins (baseline and 4 wk) or the inferior vena cava upon death (8 wk). The tail vein blood draw was carried out while rats were anesthetized using isoflurane (1.0–5.0%) and kept on a heating pad to control body temperature for a period of 10–15 min. Approximately 0.5 ml of whole blood was collected in a 1.5-ml microcentrifuge tube from each animal. The inferior vena cava blood draw was accomplished utilizing a Vacutainer Safety-Lok blood collection set (BD Medical, Franklin Lakes, NJ) to draw approximately 3–4 ml of blood. All blood was centrifuged at 3,000 g for 10 min at 4°C for isolation of serum within an hour of collection. Serum and urine samples were then stored at −80°C until later analysis.

Serum creatinine, blood urea nitrogen (BUN), and protein excretion were used to assess renal function in a subgroup of all animals (sham n = 7; AI-SED n = 7; AI-WR n = 7; replicates n = 3). Serum creatinine (Cayman Chemical, Ann Arbor, MI) and BUN (Bioassay Systems, Hayward, CA) were measured using commercially available colorimetric assays after being filtered with 10 kDa Amicon-Ultra centrifugal filters (Millipore, Billerica, MA). Urine protein concentration was determined using the Bradford method, and excretion was calculated by adjusting for urine flow rate. In addition, urinary excretion of the stable NO metabolites, NO−3 and NO−2 (NO−2 + NO−3: NOx), were measured using the Greiss reaction as an index of systemic NO production (Cayman Chemical). Isolated perfused working heart. Cardiac performance was assessed using an in vitro working heart preparation (Radnoti, Monrovia, CA) at baseline and in response to altering preload and afterload to construct LV function curves, as previously described (27). Animals (sham n = 10; AI-SED n = 11; AI-WR n = 10) were anesthetized using an ip injection of ketamine-xylazine (100 mg/kg) to construct LV function curves, as previously described (27). Ani-
in pressure level. If a significant interaction existed, two separate one-way ANOVAs were conducted. All statistical tests were performed using SPSS statistical software 22 (IBM) and GraphPad Prism (La Jolla, CA) with two-tailed probability values reported. Alpha was set at 0.05, and data are presented as means ± SE. An a priori power analysis was performed to determine the number of animals needed (n = 10 per group, 80% power) to examine differences in cardiac function in AI-SED and AI-WR groups compared with the sham group.

RESULTS

Animal characteristics. Animal characteristics are presented in Table 1. As shown, both groups of AI animals had decreased body mass and increased heart mass relative to sham animals at 8 wk postsurgery (P < 0.05). In contrast, no significant differences were observed in lung mass. By design, renal function (Table 2) was not different at baseline but became significantly impaired in AI-SED and AI-WR animals at 4 and 8 wk following surgery as indicated by elevated protein excretion and increased BUN. Serum creatinine was also elevated at 8 wk in AI animals, but was not different in AI-SED or AI-WR compared with sham animals at 4 wk (P > 0.05). In conjunction with impaired renal function, urinary NOx excretion was reduced at 4 and 8 wk following surgery in both AI groups, whereas all animals demonstrated significant reductions in NOx over the course of the study from baseline to 4 and 8 wk postsurgery (Table 2; all P < 0.05). Urine flow rate was not different between AI groups and sham animals at baseline (sham 4.9 ± 0.4; AI-SED 4.0 ± 0.5; AI-WR 5.5 ± 1.0 ml·24 h⁻¹·100 g of body mass⁻¹) but became significantly elevated in AI animals relative to sham at 4 wk (sham 4.1 ± 0.8; AI-SED 8.8 ± 0.5; AI-WR 7.2 ± 0.7 ml·24 h⁻¹·100 g of body mass⁻¹) and 8 wk (sham 2.9 ± 0.6; AI-SED 10.0 ± 1.2; AI-WR 8.5 ± 1.3 ml·24 h⁻¹·100 g of body mass⁻¹). In addition, urine flow rate was increased relative to baseline at 4 wk in AI-SED animals and both AI groups at 8 wk (all P < 0.05).

Wheel running and baseline cardiac function. AI-WR animals ran an average of 13 ± 2 km total (range 4–22 km) over the 4-wk intervention period, indicating that the volume of activity performed was low. Running behavior was consistent

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### Table 1. Animal characteristics and baseline cardiac function

<table>
<thead>
<tr>
<th>Animal characteristics*</th>
<th>Sham</th>
<th>AI-SED</th>
<th>AI-WR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass, g</td>
<td>441 ± 6</td>
<td>347 ± 19‡</td>
<td>385 ± 9‡</td>
</tr>
<tr>
<td>Heart mass/tibia length, mg/mm</td>
<td>39 ± 1</td>
<td>45 ± 2‡</td>
<td>45 ± 2‡</td>
</tr>
<tr>
<td>Lung mass/tibia length, mg/mm</td>
<td>46 ± 1</td>
<td>49 ± 2‡</td>
<td>48 ± 2</td>
</tr>
<tr>
<td>Baseline cardiac function†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>295 ± 13</td>
<td>271 ± 21</td>
<td>272 ± 14</td>
</tr>
<tr>
<td>Coronary flow, ml/min</td>
<td>17 ± 1</td>
<td>12 ± 1‡</td>
<td>13 ± 1‡</td>
</tr>
<tr>
<td>Cardiac output, ml/min</td>
<td>43 ± 2</td>
<td>28 ± 3‡</td>
<td>35 ± 3‡</td>
</tr>
<tr>
<td>Stroke volume, μl/beat</td>
<td>147 ± 7</td>
<td>101 ± 10†</td>
<td>129 ± 9†</td>
</tr>
<tr>
<td>Systolic pressure, mmHg</td>
<td>84 ± 2</td>
<td>77 ± 2‡</td>
<td>80 ± 2‡</td>
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<tr>
<td>Diastolic pressure, mmHg</td>
<td>39 ± 1</td>
<td>44 ± 1‡</td>
<td>42 ± 1‡</td>
</tr>
<tr>
<td>Cardiac work, SP/CO</td>
<td>3,629 ± 237</td>
<td>2,125 ± 278‡</td>
<td>2,787 ± 239</td>
</tr>
<tr>
<td>Stroke work, SP/CO</td>
<td>12,411 ± 790</td>
<td>7,827 ± 918‡</td>
<td>10,356 ± 920</td>
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<tr>
<td>RPP, HR/SP</td>
<td>24,622 ± 117</td>
<td>20,476 ± 1,357‡</td>
<td>21,514 ± 1,099</td>
</tr>
<tr>
<td>Maximum rate of ΔP (+dP/dt), mmHg/s</td>
<td>1,230 ± 50</td>
<td>902 ± 53‡</td>
<td>1,057 ± 55</td>
</tr>
<tr>
<td>Minimum rate of ΔP (−dP/dt), mmHg/s</td>
<td>795 ± 55</td>
<td>−746 ± 46‡</td>
<td>−810 ± 46‡</td>
</tr>
</tbody>
</table>

AI-SED, 5/6 ablation-infarction sedentary rats; AI-WR, 5/6 ablation-infarction wheel-running rats; CO, cardiac output; ΔP, change in pressure; dP/dt, change in pressure over time; HR, heart rate; RPP, rate pressure product; SP, systolic pressure; SV, stroke volume. Values are means ± SE. *Sham n = 20, AI-SED n = 17, AI-WR n = 17. †Sham n = 10, AI-SED n = 11, AI-WR n = 10. ‡P < 0.05 vs. sham; §P = 0.05 for ANOVA.

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### Table 2. Renal function and urinary NOx excretion

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>4 Weeks</th>
<th>8 Weeks</th>
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</thead>
<tbody>
<tr>
<td>Serum creatinine, mg/dl</td>
<td></td>
<td></td>
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<tr>
<td>Sham</td>
<td>1.01 ± 0.11</td>
<td>1.23 ± 0.13</td>
<td>0.72 ± 0.05</td>
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<tr>
<td>AI-SED</td>
<td>1.01 ± 0.07</td>
<td>1.80 ± 0.28</td>
<td>2.39 ± 0.36†</td>
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<tr>
<td>AI-WR</td>
<td>0.95 ± 0.07</td>
<td>1.76 ± 0.18*</td>
<td>2.09 ± 0.21†</td>
</tr>
<tr>
<td>Blood urea nitrogen (mg/dl)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>24 ± 1</td>
<td>29 ± 3</td>
<td>22 ± 2</td>
</tr>
<tr>
<td>AI-SED</td>
<td>26 ± 5</td>
<td>50 ± 5*†</td>
<td>56 ± 6*†</td>
</tr>
<tr>
<td>AI-WR</td>
<td>25 ± 1</td>
<td>57 ± 5*†</td>
<td>62 ± 5*†</td>
</tr>
<tr>
<td>Protein excretion, mg·24 h⁻¹·100 g of body mass⁻¹</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>5.5 ± 0.8</td>
<td>4.9 ± 0.9</td>
<td>5.9 ± 1.0</td>
</tr>
<tr>
<td>AI-SED</td>
<td>5.9 ± 0.6</td>
<td>41.3 ± 4.9†</td>
<td>54.7 ± 6.6†</td>
</tr>
<tr>
<td>AI-WR</td>
<td>6.0 ± 0.8</td>
<td>35.9 ± 5.2†</td>
<td>54.9 ± 7.6†</td>
</tr>
<tr>
<td>Urinary NOx excretion, nmol·24 h⁻¹·100 g of body mass⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>1,595 ± 260</td>
<td>1,112 ± 222†</td>
<td>1,002 ± 154†</td>
</tr>
<tr>
<td>AI-SED</td>
<td>1,496 ± 242</td>
<td>303 ± 62†</td>
<td>400 ± 74†</td>
</tr>
<tr>
<td>AI-WR</td>
<td>1,588 ± 298</td>
<td>368 ± 87†</td>
<td>239 ± 43†</td>
</tr>
</tbody>
</table>

Different measures of renal function and urinary NOx excretion in sham (n = 7) and AI groups (AI-SED: n = 7, AI-WR: n = 7) at baseline and 4 and 8 wk following surgery. NOx, nitrite + nitrate. Values are means ± SE. *P < 0.05 vs. sham; †P < 0.05 vs. baseline.
across the duration of the intervention as indicated by weekly running totals recorded in a subset of animals (n = 6; week 1, 2.7 ± 0.7 km; week 2, 2.7 ± 0.6 km; week 3, 2.1 ± 0.5 km; week 4, 1.8 ± 0.7 km; all P > 0.05 vs. week 1). Soleus muscle citrate synthase activity was significantly attenuated in AI-SED animals (sham 580 ± 46 vs. AI-SED 455 ± 9 mmol·ml⁻¹·min⁻¹; P < 0.05 vs. sham) and was not different from sham animals with voluntary wheel running (AI-WR 530 ± 29 mmol·ml⁻¹·min⁻¹; P > 0.05 vs. sham). Wheel running coincided with preserved baseline CO as well as other measures of in vitro cardiac function (Table 1). Stroke volume in isolated perfused hearts from AI-WR and sham animals were not different, suggesting inotropic-mediated effects of wheeling running. In addition, systolic dysfunction and diastolic dysfunction were not evident in isolated AI-WR hearts as shown by preserved systolic and diastolic pressure and rates of pressure development. In contrast, AI-SED animals demonstrated impaired in vitro CO and systolic and diastolic function. Coronary flow was also significantly decreased in AI-SED but not AI-WR hearts, indicating preserved myocardial perfusion in these animals.

CO was impaired in AI-SED hearts in response to both alterations in preload (Fig. 1, A and C) and afterload (Fig. 1, B and D), an effect not observed in AI-WR hearts with the exception of one preload level. Preloads of 9.5, 13.5, and 21.5 cmH₂O elicited impaired CO in AI-SED (P < 0.05) but not AI-WR (P > 0.05) hearts while an afterload was maintained at 80 cmH₂O, whereas at a preload of 17.5 cmH₂O, CO was impaired in both groups (P < 0.05). Afterloads of 60, 70, and 80 cmH₂O (all P < 0.05 AI-SED vs. sham), 90 cmH₂O (P = 0.05 AI-SED vs. sham), and 100 cmH₂O (P = 0.12 AI-SED vs. sham) also elicited impaired CO in AI-SED but not AI-WR hearts with preload set at 13.5 cmH₂O (all P > 0.05 for AI-WR vs. sham). Furthermore, isolated hearts from two AI-SED animals were unable to achieve aortic overflow during the 100 cmH₂O pressure level. Area under the curve, as an index of the overall response of CO to afterload (Fig. 1D) and preload (Fig. 1C) manipulation, also indicated significantly impaired functional responses in AI-SED but preserved function in AI-WR.

Differences in CO, or lack thereof, in AI groups during afterload and preload manipulation may be predominately attributed to changes in stroke volume (Fig. 2, A and C); however, heart rate (Fig. 2D) did increase in both AI groups with increasing afterload, suggesting some chronotropic-induced increases in CO during the afterload manipulation.

Biochemical markers of oxidative stress. Differential LV NO production and nitric oxide synthase expression were observed in AI-SED and AI-WR animals. LV NOx (P = 0.05; Fig. 3D) as well as NOS-3 (P < 0.05; Fig. 3, A and B) was reduced in AI-SED animals, whereas NOS-2 was increased

Fig. 1. Left ventricular (LV) function curves and mean area under the curve (AUC) data depicting the response of cardiac output (CO) to preload and afterload manipulations. As shown, in vitro CO was significantly impaired in 5/6 ablation-infarction sedentary (AI-SED) animals but not in ablation-infarction wheel-running (AI-WR) animals vs. sham animals during preload (A and C) and afterload manipulations (B and D). Values are means ± SE. *P < 0.05 vs. sham; +P = 0.05 vs. sham; αP = 0.12 vs. sham; #P < 0.05 vs. 9.5 cmH₂O value.

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Conversely, LV NOx, NOS-3, and NOS-2 (Fig. 3, A–D) in AI-WR animals were not different from those of sham animals ($P > 0.05$). LV nitrotyrosine was not significantly different between AI groups and sham animals (Fig. 3E). Similar decreases in LV SOD-1 ($P < 0.001$; Fig. 4A) and increases in LV GPx-1/2 ($P < 0.05$; Fig. 4C) were observed in AI-SED and AI-WR animals. In contrast, LV catalase expression was similar between sham and AI-WR animals (Fig. 4D), and greater differences were observed in LV SOD-2 in AI-WR relative to sham animals ($P < 0.01$), although SOD-2 was also elevated in the LV of AI-SED animals ($P < 0.05$; Fig. 4B). LV NOX-2 was not significantly different between AI groups and sham animals (Fig. 5B), whereas LV Nox-4 was elevated in AI-WR ($P < 0.05$) and AI-SED (Fig. 5C) groups, approaching statistical significance ($P = 0.12$). LV H$_2$O$_2$ was increased in AI-SED but not AI-WR animals, suggesting decreased production in the myocardium with wheel running (Fig. 5D).

**DISCUSSION**

The present investigation is the first to demonstrate preserved cardiac function following a low-volume, 4-wk, voluntary wheel-running intervention in the AI model of moderate-to-severe CKD. Isolated perfused hearts from sedentary AI animals demonstrated significantly impaired car-
Cardiac performance at baseline and in response to alterations in preload and afterload. Conversely, measures of cardiac performance were not different from those of sham animals in AI-WR rats that ran an average of only 13 ± 2 km total (4.8 km/day) over the 4-wk intervention period. Taken together, a short-term, low-volume exercise intervention is sufficient to maintain cardiac function in kidney-diseased rats.

Previous studies investigating cardiac dysfunction in experimental CKD have yielded mixed results, potentially related to the severity of the model used (27, 44). Nevertheless, systolic dysfunction and diastolic dysfunction are apparent in uremic cardiomyocytes, and isolated perfused hearts from CKD rats demonstrate depressed CO at baseline and with manipulation of preload and afterload (25, 27, 33). Other studies have demonstrated in vitro and in vivo functional impairments in animals with CKD that persist following ischemia-reperfusion injury (6, 27). Here, we support previous findings and demonstrate significantly impaired cardiac function in AI-SED animals. The AI model of CKD is an accelerated moderate-to-severe uremic model characterized by decreased systemic NO production and endothelial dysfunction (2, 31, 45). AI rats in the current study had elevated levels of BUN, significant cardiac hypertrophy, and decreased urinary NOx excretion in the absence of changes in lung mass. Therefore, uremia and concomitant hemodynamic effects may be contributing to the cardiac dysfunction observed in the absence of significant fluid accumulation in the lungs.

Experimental evidence is limited regarding the effect of increased physical activity or exercise training on cardiac function in CKD. Exercise studies conducted in CKD have predominately focused on its ability to limit kidney disease progression. Kidney function is not altered or improved with aerobic exercise in humans and animal models (3, 13, 22, 24, 26, 35, 36), an effect that may be dependent upon the modality of exercise utilized (30, 36). However, diastolic function has been shown to improve following 12 mo of a lifestyle and exercise intervention in patients with CKD (23). Furthermore, in a study conducted by da Silva Luiz et al., a high-intensity aerobic swimming intervention maintained myocardial function in nephrectomized rats while improving papillary muscle contractility (13). In the current study, several measures of cardiac function were preserved in AI animals that underwent a short, 4-wk period of low-volume voluntary wheel running. AI-WR animals only ran an average of 13 ± 2 km total but had preserved soleus muscle citrate synthase activity levels in
response to this level of stimulus. The average running distance of AI-WR animals is comparable to that observed in rodent models of aging, but less than other models of CKD, and despite this, cardiac performance was preserved (1, 16). Furthermore, unpublished data from our laboratory suggests that cardiac function is impaired at 4 wk in AI rats (AI-SED CO at 4-wk 31 ± 5 ml/min, n = 4; P < 0.05 vs. sham at 8 wk 43 ± 2 ml/min, n = 10). Therefore, cardiac function may be im-

Fig. 4. Representative blots and quantification of LV antioxidant enzymes. Western blot analysis of LV superoxide dismutase-1 (SOD-1) (A), SOD-2 (B), glutathione peroxidase-1/2 (GPx1/2) (C), and catalase (D) is shown. Antioxidant enzyme expression was similar in the LV of AI-SED and AI-WR animals. However, LV SOD-2 was elevated in AI-WR more so than AI-SED relative to shams, and LV catalase was not significantly different from that of sham animals in AI-WR animals. Values are means ± SE. *P < 0.05 vs. sham, **P < 0.01 vs. sham, ***P < 0.001 vs. sham.

**Fig. 5. LV NADPH-oxidase expression and hydrogen peroxide (H₂O₂) production.** Quantification and representative blots of LV NADPH-oxidase-2 (Nox-2) (A), Nox-4 (B), and H₂O₂ production (D). LV H₂O₂ was not different from that of sham animals with wheel running; however, LV NADPH-oxidase-4 expression was elevated in AI-WR animals and no difference was observed in LV NOX-2 expression between AI groups and sham. Values are means ± SE. *P < 0.05 vs. sham.
proved with the 4-wk voluntary wheel-running intervention despite further progression of kidney disease.

CO was preserved in AI-WR animals and persisted during preload and afterload manipulations, with all isolated hearts from AI-WR animals able to withstand these perturbations. In contrast, 2 out of 11 isolated AI-SED hearts were unable to achieve aortic overflow at the highest level of afterload. Increased CO in AI-WR animals is potentially the result of inotropic effects because stroke volume was preserved in the absence of changes in heart mass. In addition, systolic dysfunction and diastolic dysfunction were not apparent in the AI-WR group, and wheel running did not alter renal function in these animals.

Enhanced redox homeostasis and increased NO production, NO bioavailability, or both may be partly responsible for the preserved cardiac function observed with wheel running in AI. In the absence of adequate sequestration by antioxidant enzymes, excessive reactive oxygen species (ROS) production has various harmful effects on the heart. ROS can disrupt the cardiac Ca$^{2+}$ transport system, elicit mitochondrial injury, and cause cell death that are implicated in cardiac dysfunction (20). In addition, ROS diminish the bioavailability of NO, which is essential for normal cardiac function in humans and animal models (4, 48).

ROS are elevated systemically and in the myocardium in experimental kidney failure, and elicit cardiac pathogenesis likely via NO-dependent and -independent mechanisms (11, 27, 34, 45). Nonphagocytic NADPH-oxidases (Noxs) that are responsible for enzymatic production of superoxide (O$_2^-$) and hydrogen peroxide (H$_2$O$_2$) are increased in the uremic heart and contribute to LV hypertrophy and cardiac dysfunction (27, 34). ROS have also been linked to cardiac fibrosis in uremia and impaired endothelial function in the peripheral vasculature (14, 21). Furthermore, a deficiency in NO is apparent in CKD and related to hypertension and systolic dysfunction (2, 4). ROS are believed to contribute to decreased NO in kidney disease by oxidizing critical cofactors essential for its synthesis, increasing endogenous nitric oxide synthase (NOS) inhibitors, and reacting with NO to form the damaging radical, peroxynitrite (ONOO$^-$) (2).

AI-SED animals in the current study presented with elevated levels of LV H$_2$O$_2$ and nonsignificant increases in Nox-4, whereas no difference was observed in expression of Nox-2. This supports previous findings and potentially reflects the source of ROS production and related AI cardiac disease (27). Nox-2 represents a membrane-associated multisubunit protein complex that is analogous to phagocytic Nox and produces O$_2^-$, whereas Nox-4 may be predominately localized in the mitochondria in cardiomyocytes and is a major source of O$_2^-$, H$_2$O$_2$, or both, in response to pressure overload-induced cardiomyopathy (28). Consistent with this finding, the superoxide dismutase-2 (SOD-2; MnSOD) isoform, which is responsible for sequestering O$_2^-$ to produce H$_2$O$_2$ in the mitochondria, was increased in the LV of AI-SED animals; conversely, cytosolic SOD-1 (CuZn SOD) was reduced, suggesting a mitochondrial source of ROS production (15). Other antioxidant enzymes including LV glutathione peroxidase-1/2 (GPx) and catalase were also increased in the AI-SED group but were apparently unable to adequately decrease overall myocardial H$_2$O$_2$ levels.

Contrary to AI-SED rats, AI-WR animals had LV H$_2$O$_2$ levels not different from those of sham animals, despite reduced SOD-1 and significantly elevated Nox-4. The unchanged levels of LV catalase, sustained increase in GPx, and increased SOD-2 with wheel running may explain this finding. Also, Nox-4 is considered a unique Nox isoform that has been shown to be vascular protective by inducing angiogenesis and preventing apoptosis (46). In this regard, Nox-4 may also represent a novel mediator of the low-volume exercise-induced cardioprotective effects observed. Overall, ROS production was decreased in the heart with wheel running as associated with elevated antioxidant defense demonstrating an attenuation of the prooxidant environment in CKD.

Consistent with enhanced redox homeostasis; LV NO production was increased in the AI-WR group with differential expression of NOS isoforms. NO signaling in the myocardium is complex, with exogenous and endogenous sources of NO (32). However, in general, NO is produced enzymatically in the myocardium from three NOS isoforms (NOS-1 to NOS-3) and nonenzymatically from the stable NO metabolites, NO$_2^-$ and NO$_3^-$ (NOx) (8, 32). AI-SED animals demonstrated similar NOS expression profiles to that of heart failure; overall LV NOx levels were reduced with decreased constitutive NOS-3 and increased inducible NOS-2 (7, 32). NOS-2 has been associated with impaired function and mortality in the failing myocardium and may be an important component of the cardiac dysfunction observed in AI-SED animals (7). Decreases in LV NOx observed in AI-SED animals may be due to increased ROS production through inhibition of NOS-3 as well as increased ONOO$^-$. Specifically, LV nitrotyrosine, as a marker of ONOO$^-$, was not different between AI-SED and sham animals. However, decreased LV NOx in the AI-SED group suggests that O$_2^-$ is likely the dominant substrate for ONOO$^-$ in these animals and NO bioavailability is decreased. NOS-2 may be the primary isoform responsible for this observation, because its overexpression has been shown to induce ONOO$^-$, cardiomyopathy, and sudden cardiac death (37).

AI-WR rats had LV NOx, NOS-3, and NOS-2 levels maintained at the same level as sham animals. The slightly improved cardiac NO production observed could be related to the improved redox homeostasis in this group. Increased NO in the heart was not accompanied by increased systemic NO production as indicated by urinary NOx excretion. The validity of urinary NOx as a marker of systemic NO production as opposed to primarily kidney NO production may explain this discrepancy. Nevertheless, increased LV NO observed in AI-WR could elicit a number of different effects related to improved cardiac function. Specifically, coronary flow rate was preserved with wheel running, suggesting improved myocardial perfusion. In addition, cardiac hypertrophy was not further augmented in the AI-WR group, a finding that contrasts previous studies conducted in other models of CVD (29). LV NO may have conferred these effects because it is suggested to prevent cardiac hypertrophy and mediate coronary vasodilation via guanosine 3',5'-cyclic monophosphate (cGMP)-dependent mechanisms (17). Finally, NO is involved in regulation of various Ca$^{2+}$ handling proteins through S-nitrosylation and cGMP, and could contribute to increased stroke volume (contractility) in AI-WR animals (17, 19). Interestingly, in the absence of sympathetic-induced β-adrenergic stimulation, endogenous inhibition of NO prevents the positive inotropic effects of increased preload in isolated perfused hearts (47).
Clinical perspectives. CKD affects ~11% of the United States adult population (~26 million Americans), and thus represents a major public health concern (10). Patients with CKD are at increased risk of developing CVD and dying from it, and are more likely to die of CVD than progress to end-stage renal disease (48a). CVD manifests at the heart as cardiac dysfunction related to congestive heart failure, ischemic heart disease, and death (12, 38–42, 49). In this study, we demonstrated preserved cardiac function in a rat model of CKD following a 4-wk period of low-volume exercise. Therefore, habitual physical activity, and chronic exercise, are likely ideal adjunct treatment strategies for cardiac dysfunction and associated mortality in renal disease. Indeed, exercise has already been shown to improve quality of life and reduce hospitalization in a number of other cardiovascular diseases (9, 18, 29, 43). Consequently, further clinical study is needed to confirm our findings and demonstrate the beneficial effects of exercise in patients with CKD.

Conclusion. In conclusion, cardiac function is preserved in AI following a low-volume exercise intervention. Preserved cardiac performance following 4 wk of voluntary wheel running was associated with increased expression of LV NOx and the constitutive NOS-3 isoform as well as reduced inducible NOS-2. In addition, LV H2O2 was lower in AI-WR animals and was associated with increased expression of SOD-2 and GPx1/2. Therefore, preserved cardiac function in AI with wheel running may be mediated in part by improved redox homeostasis and increased NO. Future investigation is needed to determine the effectiveness of pharmaceuticals or nutraceuticals that elicit similar effects as exercise in treating impaired cardiac function in CKD. Recently, a paper published by Correa et al. demonstrated that curcumin, a natural pigment with antioxidant capacity, maintained both cardiac and mitochondrial function in AI animals (11). Additional study could focus on the efficacy of similar nutraceuticals and more novel, targeted antioxidants to treat and prevent oxidative-related cardiac dysfunction in CKD.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


