Voluntary Wheel Running Has Beneficial Effects in a Rat Model of CKD-Mineral Bone Disorder (CKD-MBD)

Keith G. Avin,1,2,3 Matthew R. Allen,1,3,4 Neal X. Chen,1,3 Shruthi Srinivasan,1,3 Kalisha D. O’Neill,1,3 Ashley D. Troutman,2 Garrison Mast,4 Elizabeth A. Swallow,4 Mary Beth Brown,2 Joseph M. Wallace,1,4 Teresa A. Zimmers,5 Stuart J. Warden,2,4 and Sharon M. Moe1,3,4

1Division of Nephrology and Departments of Medicine, 4Anatomy and Cell Biology, and 5Medicine and General Surgery, Indiana University School of Medicine, Indianapolis, Indiana; 2Department of Physical Therapy, Indiana University School of Health and Human Sciences, Indianapolis, Indiana; and 3Roudebush Veterans Affairs Medical Center, Indianapolis, Indiana

ABSTRACT

Background Reduced bone and muscle health in individuals with CKD contributes to their higher rates of morbidity and mortality.

Methods We tested the hypothesis that voluntary wheel running would improve musculoskeletal health in a CKD rat model. Rats with spontaneous progressive cystic kidney disease (Cy/+ IU) and normal littermates (NL) were given access to a voluntary running wheel or standard cage conditions for 10 weeks starting at 25 weeks of age when the rats with kidney disease had reached stage 2–3 of CKD. We then measured the effects of wheel running on serum biochemistry, tissue weight, voluntary grip strength, maximal aerobic capacity (VO2max), body composition and bone micro-CT and mechanics.

Results Wheel running improved serum biochemistry with decreased creatinine, phosphorous, and parathyroid hormone in the rats with CKD. It improved muscle strength, increased time-to-fatigue (for VO2max), reduced cortical porosity and improved bone microarchitecture. The CKD rats with voluntary wheel access also had reduced kidney cystic weight and reduced left ventricular mass index.

Conclusions Voluntary wheel running resulted in multiple beneficial systemic effects in rats with CKD and improved their physical function. Studies examining exercise interventions in patients with CKD are warranted.

CKD is a worldwide public health epidemic. Modest gains in life expectancy for patients with CKD have shifted attention toward improving the progressive decline in physical function that significantly impairs quality of life in these patients. The impairment in mobility develops concurrently with CKD–Mineral Bone Disorder (CKD-MBD), which is characterized by alterations in serum biochemistries, bone abnormalities, and extraskeletal calcification.1

CKD-MBD is associated with significant morbidity and mortality including fractures and cardiovascular disease.2,3 In addition to bone and muscle, abnormal biochemistries and cardiovascular disease may also contribute to further declines in physical function and mobility. In patients with CKD, every 0.1-m/s decrement in gait speed is associated with a 26% higher risk for death, and gait speed is a stronger predictor of 3-year mortality than kidney function.4

Received April 8, 2019. Accepted June 16, 2019.
Published online ahead of print. Publication date available at www.jasn.org.

Correspondence: Dr. Keith G. Avin, Division of Nephrology, Department of Physical Therapy, School of Health and Human Sciences, IU School of Medicine (Adjunct), 950 W. Walnut Street, R2 202, Indianapolis, IN 46202. Email: keigavin@iu.edu

Copyright © 2019 by the American Society of Nephrology
These dramatic statistics implore the development of techniques to improve musculoskeletal health and physical function in CKD.

Because of well characterized benefits of exercise on multiple organ systems in health and disease, exercise in patients with CKD has been investigated, but results to date are inconclusive. This is likely due to methodologic limitations including small sample size, focusing primarily on patients on dialysis, and varying approaches to exercise prescription. Similarly, the benefits of exercise in animal models of CKD have been inconsistent. In studies using the 5/6th nephrectomy rat, wheel running improved antioxidant and inflammatory signaling in myocardium, hemoglobin concentration, and muscle citrate synthase activity. In contrast, similar rodents found no effect of wheel running on systolic BP or serum creatinine levels. In our slowly progressive model of CKD, the Cy/+ rat, we found detrimental effects with forced exercise in the form of treadmill training, including muscle changes of increased oxidative stress and proinflammatory signaling in skeletal muscle. The inconsistent results of these cumulative data indicate a need for further study, including the study of alternate exercise modalities. The aim of this study was to examine the musculoskeletal and systemic benefits of exercise in the form of voluntary wheel running in a rat model of CKD-MBD.

METHODS

Animals
Cy/+ IU rats are characterized by an autosomal dominant progressive cystic kidney disease that is not orthologous to human autosomal dominant polycystic kidney disease. Cy/+ IU rats (CKD rats) spontaneously develop all of three manifestations of CKD-MBD (i.e., biochemical abnormalities, extraskeletal calcification, and abnormal bone). The Cy/+ IU colony of rats has been bred at Indiana University for >20 years. The animals have a mutation in Ank6, a gene that codes for the protein SamCystin located at the base of cilia. Although specific function is not fully elucidated, Ank6 binds with Ank3 and Bicc1 and thus may alter the nephronophthisis complex. In this rat model, CKD-MBD develops spontaneously, with a much faster progression to ESRD in male animals by 30–40 weeks of age, whereas female rats do not develop azotemia even as old as 21 months, or after oophorectomy (unpublished data). The Cy/+ IU is on a Hans–Sprague Daly background. To remove effects of the background strain, we used normal littermates (NL; Cy/+ +) compared with CKD (Cy+/−); homozygote (Cy−/−) animals die at age 3 weeks.

The rats were bred in-house, and weaned rats were singly housed with free access to tap water and standard chow as previously described. Rats were phenotyped at 10 weeks of age as previously described. At 24 weeks of age, rats were switched to a diet of 18% casein-based protein, 0.7% phosphorous, 0.7% calcium, and 5% fat (Harlan Teklan TD.04539), which leads to more reproducible CKD phenotype. Animals were then randomly divided into four groups (n=12–14/group): (1) NL, (2) NL wheel running, (3) CKD, and (4) CKD wheel running. All procedures were reviewed and approved by the Indiana University School of Medicine Institutional Animal Care and Use Committee, which adheres to the Guide for the Ethical Treatment of Animals to minimize pain and suffering.

Running Wheel
Wheel-running rats were singly housed in cages with a freely accessible, voluntary activity wheel (Model 80850S Scurry Rat Activity Wheel; Lafayette Instruments). Rats were exposed to the wheel without external motivation at 24 weeks of age; collection began at 25 weeks (approximately 50% normal GFR) with free wheel access until study termination at 35 weeks of age (approximately 15% normal GFR). Wheel revolutions were monitored with 86115 Scurry Sensor/Counter, 86130 Interface, and 86165 Scurry Software which was connected to a computer interface for complete data collection (3-second sample rate), analysis, and charting.

Physical Function Measures
Horizontal laser activity was used to determine cage activity. Data were collected for 1-minute time intervals for 24 hours at 25 and 35 weeks of age; data are presented as ambulatory counts via provided software (version 1.4.0; Columbus Instruments). Maximal aerobic exercise capacity (VO2max) was assessed using indirect open-circuit calorimetry on a specially designed rat treadmill and metabolic testing system (Oxymax; Columbus Instruments). Rats were acclimated 1 week before baseline testing, with 5 minutes per day for 4 days. We have determined that this period improves treadmill testing without a training effect. On the day of testing, rats were weighed, then placed on the treadmill for 20 minutes to obtain resting oxygen consumption and O2 saturation values noninvasively. An incremental exercise test immediately followed, with treadmill grade fixed at 25° and speed increased every 3 minutes until exhaustion. Treadmill testing data are presented as time-to-fatigue (minutes) and VO2max (ml/kg per hour). To assess voluntary grip strength, rats were lowered with all four paws on the grid of a testing apparatus (GT3; Harvard Instruments). The tail was gently and steadily pulled horizontally.

Significance Statement
Impaired musculoskeletal health in individuals with CKD reduces their ability to participate in activities and quality of life, and increases the risk of illness, injury, and death. Exercise studies in animals or people with CKD have produced inconsistent results about the potential benefits on the musculoskeletal system and few studies have examined the systemic effects of exercise in CKD. The authors show that 10 weeks of voluntary wheel running in rats with CKD improved multiple systems, including mineral metabolism, left ventricular mass, physical function, and bone health. The results suggest physical activity may help patients with CKD, but further studies are needed to establish the most efficacious modalities and optimal dose.
back until grip was voluntarily released. The maximal force was recorded; each animal performed three reps with up to 5-minute breaks in between each rep. Grip strength data are presented as the maximal force normalized to body weight.

**Body Composition**

Body composition was calculated via quantitative magnetic resonance, with the animals fully alert, yet constrained in a tube placed in the EchoMRI (LLC).

**Tissue Procurement and Blood Biochemistry Testing**

Animals were anesthetized at 35 weeks with isoflurane, and blood was collected via cardiac puncture. Animals were then euthanized by exsanguination. The kidneys, heart, aorta, extensor digitorum longus (EDL), and soleus muscles were removed and weighed. Left ventricular mass index (LVMI) was calculated as the ratio of heart weight to body weight. Blood was collected in lithium heparin–coated blood collection tubes (BD 367884) and centrifuged at 1200 × g for 10 minutes. Plasma was analyzed using colorimetric assays for BUN (DIUR-100), creatinine (BioAssay Systems), calcium, and phosphorous (Pointe Scientific). Intact parathyroid hormone (PTH) and fibroblast growth factor 23 (FGF23) were determined by ELISA (Quidel Corporation). Aortic calcification was assessed by incubating segments of aortic arches in 0.6 N HCl for 48 hours, and the supernatant analyzed for calcium using the o-cresolphthalein complex 1 method (Calcium kit; Pointe Scientific) and normalized by tissue dry weight. The EDL and soleus muscles were collected and stored at −80°C for RNA and protein isolation. The middle third was cut in optimal cutting temperature compound and frozen for 60 seconds in liquid nitrogen–chilled 2-methylbutane. Samples were stored at −80°C until histologic analysis. The heart and kidney were snap-frozen in liquid nitrogen.

**Muscle Cross-Sectional Area**

The EDL and soleus muscles were cryosectioned and muscle fiber cross-sectional area was examined by immunohistochemistry using a goat anti-dystrophin mAb (Developmental Studies Hybridoma Bank at the University of Iowa) to visualize connective tissue. Images at ×20 were taken using a Spot RT Color Camera System mounted on an inverted Nikon Diaphot 200 microscope (Nikon Instruments Inc.). Three images per section, three sections per animal, and six animals per group were analyzed. Thresholding was performed using Metamorph software (Molecular Devices) and average fiber area was analyzed.

**Bone Microarchitecture and Cortical Porosity**

Proximal tibias were scanned using micro–computed tomography (CT; Skyscan 1172) at 12-μm resolution. Trabecular parameters were obtained from a 1-mm region of interest located approximately 0.5 mm below the end of the growth plate. Trabecular bone volume (BV/TV, %), trabecular thickness (Tb.th), trabecular separation (Tb.sp), and trabecular number (Tb.n) were measured following standard recommendations. Of these trabecular properties, BV/TV was considered the primary outcome of interest due to its direct measurement and because it parallels human measures of bone density and bone volume by CT. Cortical porosity was measured in a 1-mm region starting 1 mm below the last trabecular slice.

**Bone Biomechanics**

Bones were thawed, hydrated in saline, and then placed posterior surface down on bottom supports (span = 18 mm). The upper supports (span = 6 mm) were brought down in contact with the specimen’s anterior surface, and then testing was conducted at a displacement rate of 2 mm/min. Femoral midshafts were scanned at 9 μm (Skyscan 1176). Femurs were tested with an 18-mm test span by three-point bending (Test Resources) with the posterior and anterior surfaces in compression and tension, respectively. Once broken, the fracture site location was measured from the distal edge of the third trochanter in order to determine the CT image at the site of fracture location. Geometry values (minimum MOI and AP diameter) were obtained from CtAN (Bruker) and were used to generate mechanical data in a custom MATLAB script. Force versus displacement data were collected at 10 Hz and structural parameters were determined from curves using a customized MATLAB program. Key outcome parameters include ultimate force (highest force obtained during the test), yield force (the force at which permanent damage has been caused), stiffness (the slope of the force/displacement curve in the elastic portion of the test), displacements (the amount of bone displacement divided into three phases: preyield, postyield, and total), and work (the total energy absorbed during the test divided into three phases: preyield, postyield, and total). Material properties were estimated using standard equations that convert force and displacement to stress and strain by accounting for bone geometry. Outcomes for material properties parallel those of the structural properties stress (material estimate of force), strain (material estimate of displacement), modulus (material estimate of stiffness), and resilience/toughness (material estimates of work).

**Statistical Analyses**

GraphPad Prism 8 (GraphPad Inc.) was used to analyze the data. All variables were analyzed using two-way ANOVA, with disease (NL versus CKD) and exercise (cage control versus nonwheel cage control CKD) as the independent variables. Underlying assumptions of normality and variance were assessed via the Brown–Forsythe test; data were log transformed as needed. Post hoc analyses were performed with Fisher’s least significant difference, with the primary a priori questions being the effects of: (1) wheel running in CKD animals (i.e., CKD versus CKD wheel), and (2) CKD (i.e., nonwheel cage control NL versus nonwheel cage control CKD). Data tables provide P values for main effects and interaction (Tables 1–4). Figures present post hoc comparisons for main effect or interaction (Figures 1–4). The main effects post hoc comparisons are presented to
identify whether a particular group is the driving force for an effect. Data are presented as mean±SD.

RESULTS

Running Wheel Distance, but Not Speed, Was Affected by Disease

Two-way ANOVA (disease×time) demonstrated a main effect of time (P<0.05) and disease (P<0.05) for the running wheel average distance per day, when comparing early-stage disease (weeks 25–28 [NL-wheel: 690±203 m; CKD-wheel: 612±234 m]), middle-stage disease (weeks 29–31 [NL-wheel: 694±176 m; CKD-wheel: 560.5±222 m]), and late-stage disease (weeks 32–35 [NL-wheel:555±146 m; CKD-wheel: 425±206 m]). However, average running speed was not different (disease P=0.12; time P=0.25; interaction P=0.57) when comparing early-stage disease (weeks 25–28 [NL-wheel: 17.24±1.2 m/min; CKD-wheel: 17.31±1.4 m/min]), middle-stage disease (weeks 29–31 [NL-wheel: 17.9±1.2 m/min; CKD-wheel: 17.4±1.4 m/min]), and late-stage disease (weeks 32–34 [NL-wheel: 17.4±0.95 m/min; CKD-wheel: 16.7±1.5 m/min]). Thus, animals ran at equivalent speed, although the distance decreased with disease progression and CKD-wheel rats ran less than NL-wheel rats.

Effect of Wheel Running on Serum Biochemistry in CKD Rats

As expected, CKD rats demonstrated common biochemical manifestations of CKD-MBD with significant main effects of disease, with increased PTH (P<0.001), FGF23 (P<0.001), and BUN (P<0.001) (Table 1); calcium was NS (Table 1). Creatinine (P<0.05) and phosphorous (P<0.01) both demonstrated a significant disease×wheel interaction (Table 1). Post hoc comparisons for the wheel effect demonstrated that wheel running reduced PTH (P<0.05) in the CKD group, but not in the NL group. (Figure 1). Post hoc comparisons for phosphorous and creatinine demonstrated that wheel running reduced phosphorous (P<0.05) and creatinine (P<0.01) in the CKD-wheel animals but not the NL-wheel animals (Figure 1). FGF23 was lowered in CKD rats that underwent wheel running, but this difference was not statistically significant (P=0.22, Figure 1). The reduction in creatinine corresponded to a main disease effect of increased cystic kidney weight (P<0.001; Table 1), and wheel effect to reduce cystic kidney weight with wheel running (P<0.01; Table 1). Although the average running distance was less per day in CKD rats, it was sufficient to improve plasma biochemistry by reducing PTH, creatinine, phosphorous, and kidney weight.

Effect of Wheel Running on Physical Function and Strength

Physical performance outcomes of activity (horizontal cage activity), strength (maximum grip strength normalized to body weight), and exercise tolerance (VO_{2max}) were similar across the four groups at 25 weeks of age (Table 2). Wheel running improved a number of physical performance outcomes at 35 weeks of age, including increasing cage activity, grip strength (P<0.001), and time to achieve VO_{2max} (P<0.001) (Table 2). VO_{2max} at 35 weeks of age had a significant interaction (P<0.05; Table 1); post hoc comparisons demonstrated that CKD–nonwheel cage control was lower than NL–nonwheel cage control (P=0.01); no other comparisons were significant.

Effect of Wheel Running on Body Composition

At 35 weeks of age, there was no difference in body weight in the groups (interaction P=0.05, disease P=0.87, exercise P=0.79); given the near significant interaction we explored follow-up comparisons, none of which were significant. Body fat had a significant interaction (P<0.001), with a 36% lower body fat in CKD rats versus NL rats without wheel running (P<0.001), but no difference between the groups that underwent wheel running (P=0.98). Lean tissue had main effects for wheel running (P<0.01) and disease (P<0.001). Wheel running increased skeletal muscle weight for the EDL (P=0.01) and soleus (P<0.01) but there was no disease effect (EDL P=0.09, soleus P=0.38) or interaction (EDL P=0.57, soleus P=0.37). Further, there was no difference between muscle cross-sectional area between NL-control and CKD-control animals in either EDL (P=0.94) or soleus

Table 1. Plasma biochemistry and kidney weight of diseased and wheel-running rats

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normal Control</th>
<th>Wheel</th>
<th>CKD Control</th>
<th>Wheel</th>
<th>Disease</th>
<th>Wheel</th>
<th>D×W</th>
</tr>
</thead>
<tbody>
<tr>
<td>BUN, mg/dl</td>
<td>17.6±1.9</td>
<td>18.5±2.0</td>
<td>42.5±5.0</td>
<td>40.1±5.6</td>
<td>&lt;0.001</td>
<td>0.66</td>
<td>0.11</td>
</tr>
<tr>
<td>Creatinine, mg/dl</td>
<td>0.46±0.06</td>
<td>0.48±0.08</td>
<td>1.26±0.9</td>
<td>0.99±0.58</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Calcium, mg/dl</td>
<td>8.72±1.69</td>
<td>8.34±2.04</td>
<td>9.46±1.49</td>
<td>7.94±1.92</td>
<td>0.98</td>
<td>0.14</td>
<td>0.13</td>
</tr>
<tr>
<td>Phosphorous, mg/dl</td>
<td>4.5±0.77</td>
<td>4.87±0.76</td>
<td>6.14±1.09</td>
<td>5.17±1.31</td>
<td>--</td>
<td>--</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>PTH, pg/ml</td>
<td>119±25</td>
<td>109±29</td>
<td>803±539</td>
<td>521±198</td>
<td>&lt;0.001</td>
<td>&lt;0.05</td>
<td>0.56</td>
</tr>
<tr>
<td>FGF23, pg/ml</td>
<td>391±330</td>
<td>410±445</td>
<td>3644±2757</td>
<td>1913±1338</td>
<td>&lt;0.001</td>
<td>0.22</td>
<td>0.57</td>
</tr>
<tr>
<td>Kidney, g</td>
<td>3.54±0.26</td>
<td>3.32±0.10</td>
<td>7.02±0.80</td>
<td>6.17±1.04</td>
<td>&lt;0.001</td>
<td>&lt;0.01</td>
<td>0.11</td>
</tr>
<tr>
<td>25-wk body weight, g</td>
<td>512±22</td>
<td>468±32</td>
<td>505±25</td>
<td>482±25</td>
<td>0.42</td>
<td>&lt;0.001</td>
<td>0.08</td>
</tr>
<tr>
<td>35-wk body weight, g</td>
<td>546±23</td>
<td>525±45</td>
<td>540±29</td>
<td>527±33</td>
<td>0.87</td>
<td>0.79</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Two-way ANOVA main effects and interaction; post hoc comparisons are in Figure 1. Data presented as mean±SD, n=11–14 per group. Main effects are not presented with significant interaction. D×W, interaction of disease/normal versus wheel/no wheel cage control; --, main effects are not presented with significant interaction.
Effect of Wheel Running on Bone Quality, Porosity, and Mechanics

As we have previously demonstrated, there was a significant disease effect for four of the five bone structure outcomes (Table 3). Wheel running improved bone structure for all five measures: (1) BV/TV (P<0.001), (2) Tb.th (P<0.001), (3) Tb.sp (P<0.001), and (4) Tb.n (P<0.001) (Table 3). The fifth measure of cortical porosity demonstrated a significant interaction (P<0.01; Table 3). Post hoc comparisons for cortical porosity demonstrated greater porosity in CKD–nonwheel cage control compared with NL–nonwheel cage control (P<0.001) that was normalized by wheel running (CKD–nonwheel cage control versus CKD-wheel, P=0.001; Figure 2). Further post hoc comparisons within nonwheel cage control animals found a significant NL–CKD difference for BV/TV, Tb.sp, and Tb.n (i.e., not Tb.th). Wheel running within CKD animals improved BV/TV, Tb.th, Tb.sp, and Tb.n (Figure 2). Bone mechanics demonstrate a significant disease effect for 12 of the 16 parameters (Table 3). Wheel running significantly improved five of the 16 parameters, including total displacement, stiffness, total work, toughness, and postyield work (Figure 3, Table 3). Post hoc comparisons of the main effects demonstrated that within nonwheel cage control animals, there was a significant NL–CKD difference for the outcomes above except total displacement. When comparing within CKD, wheel running only affected one outcome of mechanics (i.e., stiffness). Therefore, the main wheel effect may be driven by the normal wheel animals.

Figure 1. Wheel running significantly improved serum biochemistry and hormones. At 35 weeks of age plasma was assessed for (A) creatinine, (B) phosphorous, (C) PTH, and (D) FGF23. Solid line indicates NL–nonwheel cage control versus CKD–nonwheel cage control; whereas a dotted line indicates CKD–nonwheel cage control versus CKD-wheel. *P<0.05, **P<0.001, ****P<0.001. When comparing within nonwheel cage control animals (solid line), there was a significant NL–CKD difference for all of the outcomes above. When comparing within CKD (dotted line), wheel running improved all of the biochemistries except FGF23. 

(P=0.98); wheel running groups were not analyzed given lack of disease effect.
Table 2. Physical performance outcomes of diseased and wheel-running rats

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normal Control</th>
<th>Normal Wheel</th>
<th>CKD Control</th>
<th>CKD Wheel</th>
<th>Disease Control</th>
<th>Disease Wheel</th>
<th>D×W</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cage activity 25 wk (ambulatory counts)</td>
<td>6902±2165</td>
<td>6876±2217</td>
<td>5931±2158</td>
<td>6991±2024</td>
<td>0.47</td>
<td>0.38</td>
<td>0.37</td>
</tr>
<tr>
<td>Cage activity 35 wk (ambulatory counts)</td>
<td>5553±1863</td>
<td>8011±2400</td>
<td>4643±2218</td>
<td>7729±3696</td>
<td>0.42</td>
<td>&lt;0.001</td>
<td>0.94</td>
</tr>
<tr>
<td>Grip 25 wk (max/body wt)</td>
<td>3.23±0.28</td>
<td>3.30±0.39</td>
<td>3.03±0.56</td>
<td>2.97±0.48</td>
<td>0.10</td>
<td>0.96</td>
<td>0.68</td>
</tr>
<tr>
<td>Grip 35 wk (max/body wt)</td>
<td>2.87±0.35</td>
<td>3.51±0.47</td>
<td>2.82±0.45</td>
<td>3.41±0.71</td>
<td>0.63</td>
<td>&lt;0.001</td>
<td>0.86</td>
</tr>
<tr>
<td>VO₂ 25 wk (ml/kg per h)</td>
<td>3724±316</td>
<td>3489±178</td>
<td>3468±104</td>
<td>3499±253</td>
<td>0.14</td>
<td>0.22</td>
<td>0.11</td>
</tr>
<tr>
<td>VO₂ 35 wk (ml/kg per h)</td>
<td>3632±219</td>
<td>3401±145</td>
<td>3362±300</td>
<td>3437±291</td>
<td>–</td>
<td>–</td>
<td>0.04</td>
</tr>
<tr>
<td>TTM 25 wk (min)</td>
<td>12.1±2.4</td>
<td>14.2±4.0</td>
<td>11.8±2.0</td>
<td>12.6±2.1</td>
<td>0.28</td>
<td>0.11</td>
<td>0.50</td>
</tr>
<tr>
<td>TTM 35 wk (min)</td>
<td>10.4±2.1</td>
<td>12.7±1.9</td>
<td>8.7±1.6</td>
<td>12.6±2.3</td>
<td>0.13</td>
<td>&lt;0.001</td>
<td>0.18</td>
</tr>
</tbody>
</table>

Two-way ANOVA main effects and interaction. Data presented as mean±SD; n=10–12 per group. Main effects are not presented with a significant interaction. D×W, interaction of disease/normal versus wheel/no wheel cage control; –, main effects are not presented with significant interaction; BV/TV, trabecular bone volume; Tb.sp, trabecular separation.

Effect of Wheel Training on the Cardiovascular System

There was a main effect of disease for heart weight, LVMI, and aorta calcification and a main effect of wheel running for heart weight and LVMI (Table 4). Post hoc comparisons demonstrated that CKD–nonwheel cage control rats had increased heart weight (P<0.001), LVMI (P<0.05), and aorta calcification (P<0.05) when compared with NL–nonwheel cage controls. Wheel running reduced heart weight (7%, P<0.01) and LVMI (7%, P<0.01) within CKD comparisons, with a trend toward wheel running attenuating vascular calcification (P=0.07; Figure 4, A–C).

DISCUSSION

Excitingly, this study demonstrated widespread beneficial effects on the phenotype manifestations of CKD-MBD in an animal model by performing 10 weeks of voluntary wheel running. The widespread effects of wheel running include: (1) reduction in circulating PTH and phosphorous and a trend toward reduction in FGF23; (2) improved creatinine and reduced cystic kidney weight; (3) improved cortical porosity, trabecular parameters, and bone mechanical properties; (4) increased skeletal muscle weight, voluntary muscle strength, cage activity, and time-to-fatigue (for VO₂max); and (5) reduced LVMI. The accessibility and pleotropic effects of physical activity upon measures that are analogous to clinically relevant measures in humans make it an attractive and inexpensive intervention. Our results in an animal model of spontaneous CKD-MBD may not be generalizable to humans, or to other rodent models of CKD, and thus further work is needed to validate our results.

Exercise is included under the umbrella of physical activity, and is a planned, structured, and repetitive activity with the goal of improved or maintained physical fitness. Physical activity is an umbrella term for any bodily movement produced by skeletal muscles that requires energy expenditure. The running wheel falls along this spectrum of physical activity and in animals improves muscle insulin sensitivity, skeletal muscle citrate synthase, and visceral fat. Our running wheel daily average (NL-exercise 555 m/d, CKD-exercise 425 m/d; 32–35 weeks of age) was in line with that of previous studies in rats of approximately 300–450 m/d. Although the average daily distance decreased with disease progression, the efficacy appears to be sufficient given the beneficial effect. This is in contrast to our previous study where utilizing forced treadmill in the CKD rat led to negative skeletal muscle effects including oxidative stress and altered muscle cell differentiation. In the treadmill study, the animals performed 10 weeks of treadmill running (at the same age of 25 weeks) beginning at

Table 3. Bone structure and mechanics

<table>
<thead>
<tr>
<th>Outcome Measure</th>
<th>Disease</th>
<th>Wheel</th>
<th>D×W</th>
</tr>
</thead>
<tbody>
<tr>
<td>Structure</td>
<td></td>
<td></td>
<td>0.004</td>
</tr>
<tr>
<td>Cortical porosity</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>BV/TV</td>
<td>0.001</td>
<td>&lt;0.001</td>
<td>0.55</td>
</tr>
<tr>
<td>Tb.th</td>
<td>0.75</td>
<td>0.001</td>
<td>0.15</td>
</tr>
<tr>
<td>Tb.sp</td>
<td>0.04</td>
<td>0.001</td>
<td>0.30</td>
</tr>
<tr>
<td>Tb.n</td>
<td>0.002</td>
<td>&lt;0.001</td>
<td>0.80</td>
</tr>
<tr>
<td>Structural properties</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ultimate force</td>
<td>&lt;0.001</td>
<td>0.40</td>
<td>0.14</td>
</tr>
<tr>
<td>Yield force</td>
<td>&lt;0.001</td>
<td>0.44</td>
<td>0.67</td>
</tr>
<tr>
<td>Stiffness</td>
<td>&lt;0.001</td>
<td>0.04</td>
<td>0.20</td>
</tr>
<tr>
<td>Displacement to yield</td>
<td>0.18</td>
<td>0.46</td>
<td>0.74</td>
</tr>
<tr>
<td>Postyield displacement</td>
<td>0.09</td>
<td>0.09</td>
<td>0.70</td>
</tr>
<tr>
<td>Total displacement</td>
<td>&lt;0.01</td>
<td>0.05</td>
<td>0.44</td>
</tr>
<tr>
<td>Preyield work</td>
<td>&lt;0.001</td>
<td>0.45</td>
<td>0.85</td>
</tr>
<tr>
<td>Postyield work</td>
<td>&lt;0.001</td>
<td>&lt;0.05</td>
<td>0.70</td>
</tr>
<tr>
<td>Total work</td>
<td>&lt;0.001</td>
<td>0.02</td>
<td>0.49</td>
</tr>
<tr>
<td>Material properties</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yield stress</td>
<td>0.18</td>
<td>0.47</td>
<td>0.17</td>
</tr>
<tr>
<td>Modulus</td>
<td>0.97</td>
<td>0.84</td>
<td>0.35</td>
</tr>
<tr>
<td>Preyield strain</td>
<td>&lt;0.01</td>
<td>0.87</td>
<td>0.99</td>
</tr>
<tr>
<td>Total strain</td>
<td>&lt;0.001</td>
<td>0.24</td>
<td>0.20</td>
</tr>
<tr>
<td>Resilience</td>
<td>&lt;0.05</td>
<td>0.96</td>
<td>0.63</td>
</tr>
<tr>
<td>Toughness</td>
<td>&lt;0.001</td>
<td>0.04</td>
<td>0.50</td>
</tr>
<tr>
<td>Resilience</td>
<td>&lt;0.05</td>
<td>0.96</td>
<td>0.63</td>
</tr>
</tbody>
</table>

Two-way ANOVA main effects and interaction; post hoc comparisons are in Figures 2 and 3. n=11–14 per group; main effects are not presented with significant interaction. D×W, interaction of disease/normal versus wheel/no wheel cage control; –, main effects are not presented with significant interaction; BV/TV, trabecular bone volume; Tb.sp, trabecular separation.
Figure 2. Wheel running significantly improved bone quality and porosity. Bone structure: at 35 weeks of age, bone structure was assessed by micro-CT for (A) cortical porosity, (B) proximal BV/TV, (C) Tb.th, (D) Tb.sp, and (E) Tb.n. Only post hoc comparisons are presented in the figure; two-way ANOVA main effects and interaction values are in Table 3. Solid line indicates NL–nonwheel cage control versus CKD–nonwheel cage control; whereas a dotted line indicates CKD–nonwheel cage control versus CKD-wheel. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001. When comparing within nonwheel cage control animals, there was a significant NL-CKD difference for all outcomes except Tb.th. When comparing within CKD animals, wheel running affected all outcomes above.
8 m/min and ending at 18 m/min for 60 minutes, 5 days a week; the daily average distance run was 480 m (8 m/min) and 1080 m (18 m/min). This represents a similar daily average distance between the treadmill and wheel running. However, the discrepant findings may relate to the distinct exercise prescription in these two studies—treadmill running, which is, by nature, “forced” exercise in a rat, compared with voluntary wheel running which reflects interventions aimed at increasing activity time rather than intensity. Further, the treadmill protocol progressively increased speed with disease progression.

**Figure 3.** Wheel running had minimal beneficial effects upon bone mechanics. Bone mechanics: at 35 weeks of age bone mechanics were assessed, including: (A) total displacement, (B) total work, (C) stiffness, and (D) toughness. Only post hoc comparisons are presented in the figure; two-way ANOVA main effects and interaction values are in Table 3. Solid line indicates NL–nonwheel cage control versus CKD–nonwheel cage control; whereas a dotted line indicates CKD–nonwheel cage control versus CKD-wheel. *P<0.05, **P<0.01, ****P<0.001. When comparing within nonwheel cage control animals, there was a significant NL-CKD difference for the outcomes above except total displacement. When comparing within CKD, wheel running only affected one mechanics outcome of stiffness.

**Table 4.** Heart-related outcomes of diseased and wheel-running rats

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normal</th>
<th>Wheel</th>
<th>CKD</th>
<th>Disease</th>
<th>Wheel</th>
<th>D×W</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart weight, g</td>
<td>1.58±0.12</td>
<td>1.48±0.09</td>
<td>1.84±0.08</td>
<td>1.72±0.08</td>
<td>&lt;0.05</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LVMI (heart/body)</td>
<td>2.89±0.21</td>
<td>2.83±0.13</td>
<td>3.42±0.19</td>
<td>3.17±0.26</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Aorta calcification, μmol/mg</td>
<td>4.94±2.37</td>
<td>4.21±0.87</td>
<td>6.91±2.71</td>
<td>5.52±1.38</td>
<td>&lt;0.01</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Two-way ANOVA main effects and interaction. Data presented as mean±SD; n=11–14 per group. D×W, interaction of disease/normal versus wheel/no wheel cage control.
whereas wheel running distance progressively decreased during the course of disease. The forced model may not be the sole reason for the divergent findings, because other studies of forced exercise have found beneficial effects upon skeletal muscle degradation and renal disease in a forced swimming model.\textsuperscript{26,27} However, both of these studies utilized female rats, which confounds the results because females demonstrate less severe disease progression and a more hypertrophic response to exercise.\textsuperscript{28,29} Our findings lend support to the theory that voluntary intermittent exercise has beneficial effects and may be the appropriate prescription for patients with progressive CKD. We do not know whether exercise in CKD animals from this study shares a similar dose-response curve as in pharmacologic interventions. Thus, further studies are needed to optimize frequency, intensity, time, and type.

Bone strength is impaired in patients with CKD and leads to a 2–14-fold increased risk of fracture in patients with CKD grade 2–4.\textsuperscript{3} Bone strength encompasses bone density and bone quality, and the latter includes cortical and trabecular bone architecture, turnover, damage accumulation, and mineralization. In CKD, some or all aspects of bone quality may be impaired (Table 2). The Kidney Disease Improving Global Outcomes guideline recommendations for bone-related treatments of CKD-MBD are centered around normalizing PTH and phosphate; yet, despite intensive PTH-lowering therapies over the past 25 years, there has been no reduction in age-associated hip fracture in patients on dialysis.\textsuperscript{30} Cortical porosity improvements may be due to the lowering of PTH.\textsuperscript{19} However, fragility fractures due to metabolic bone diseases (i.e., osteoporosis) usually occur in trabecular bone.\textsuperscript{31}
Therefore, the discrepancy between PTH therapies and mechanisms of fragility fractures beckons the need for additional targets and interventions.32 Wheel running resulted in marked improvements in cortical porosity, increased trabecular bone volume, and improved trabecular architecture. Importantly, the improvements in bone quality/structure seen with wheel running in CKD positively affected mechanics, including improved total displacement, stiffness, total work, toughness, and postyield work. Thus, voluntary wheel running improved bone quality and could have clinical implications of reducing fragility fracture risk. The clinical implications of this study should be considered in the context of other investigations of exercise-mediated changes in bone health in CKD. Tomayko et al.33 found that 16 weeks of aerobic exercise in a surgically induced mouse model of uremia significantly improved bone volume, Tb.n, trabecular separation, and trabecular connectivity in the proximal femur. Improvements in bone growth and bone mineral levels were also found in related animals models, young/growth-retarded CKD rats and obese Zucker rats, respectively, but not directly applicable to adult CKD.34,35 Interventional studies in humans have found inconsistent results. Gomes et al.36 found that 24 weeks of moderate intensity aerobic exercise did not alter markers of bone metabolism, but there were no direct measures of bone quality or strength and there were not consistent differences between CKD and controls. Therefore, it is not clear the extent to which bone integrity was compromised in this cohort. Morishita et al.37 similarly found a lack of response in bone metabolism markers, but did not perform an interventional study; rather, they assessed 1-week activity levels. Liao et al.38 studied patients on hemodialysis who performed 12 weeks of cycling training, three times per week, for 30 minutes per day. Aerobic exercise prevented the loss of bone density at the femoral neck that was demonstrated in the control group. Although there are a limited number of studies, there is evidence for an exercise-mediated effect upon bone health. Further studies are needed to identify how to optimize exercise for bone health.

The skeletal beneficial effects of wheel running are quite impressive. Using this same rat model of CKD-MBD, we have investigated the efficacy of multiple pharmacologic treatments that target bone. We found that treating with the calcimimetic R-568 alone or with calcium resulted in a desirable change of bone volume, but if calcium was given alone animals further increased extraskeletal calcification.39 Calcitriol failed to improve any skeletal properties.40 Zoledronic acid suppressed the increased trabecular bone remodeling, but only partially improved cortical biomechanical properties.22 Anti-sclerostin antibody had no effect on cortical porosity, but increased trabecular bone volume in animals with low PTH, but not high PTH. Collectively, these results highlight the complexity of bone abnormalities in CKD, and that compared with pharmacologic interventions, voluntary wheel running appears to exert benefits that exceed these pharmacologic agents. The mechanism by which wheel running improves bone is likely multifactorial, including improved metabolism in muscle,9 reduction in serum PTH and/or FGF23, alteration of osteocyte function in bone through mechanotransduction41 or myokines,42,43 altered muscle-bone cross talk,44 and perhaps improved cardiovascular function that could benefit bone blood flow and/or improve overall kidney function.

Interestingly we did not see disease-related changes in muscle weight or cross-sectional area; i.e., there was no presence of sarcopenia in our CKD rats. Our current lack of sarcopenia findings is divergent from our previous study where we found both reduced cross-sectional area and reduced maximal electrically stimulated torque, possibly due to more advanced CKD at the time of testing, and/or different methodology for assessing strength. This variability also reflects data in humans, where sarcopenia is only observed in a minority (4%–29%) of patients and yet is predictive of poor outcomes.45,46 In this study we did not see an improvement in maximal aerobic capacity (i.e., VO2max), suggesting that this measure, which reflects aerobic capacity, may require increased intensity of exercise. However, time to achieve VO2max, which can be considered a measure of running economy or performance, was reduced by 28% in rats with CKD compared with NL animals, with an improvement from wheel running to that of normal animals at baseline (P<0.01). These results are consistent with known effects of low intensity training which improves running economy without a change in VO2max.17

The pleotropic effects of wheel running also had a positive cardiovascular effect, with reduction in LVMI and a trend toward decreased arterial calcification in CKD animals. These changes may have also improved the time to achieve VO2max. A reduction in LVMI may reflect improvement from pathologic to nonpathologic cardiac remodeling in the CKD animals. The positive cardiovascular effect of the running wheel on CKD could also be attributed to reduction of serum levels of FGF23 in CKD animals, because FGF23 can directly induce left ventricle hypertrophy in CKD.47 Overall, the improved cardiovascular health is of interest given the high cardiovascular mortality in CKD.45

In summary, voluntary wheel running resulted in multiple beneficial effects on CKD-MBD and physical function. Wheel running improved kidney and heart weights, voluntary muscle strength, time to max (for VO2max), serum biochemistries, cortical porosity and trabecular microarchitecture, and bone mechanics, and with a trend toward reduced vascular calcification in our rat model of CKD. The findings highlight the potential benefits of physical activity and the need to define a proper dose, especially when contrasted with the apparent adverse effects of forced treadmill training shown in our previous study in this model. These divergent results underscore that more work is needed to identify the proper prescription of physical activity.

ACKNOWLEDGMENTS

The anti-dystrophin mAb developed by Dr. Zimmers was obtained from the Developmental Studies Hybridoma Bank, created by the National Institute of Child Health and Human Development of the National Institute of Neurological Disorders and Stroke through National Institutes of Health grant P4D012022.
National Institutes of Health, and maintained at the Department of Biology, University of Iowa (Iowa City, IA).

Dr. Avin, Dr. Chen, Dr. Allen, Dr. Zimmers, Dr. Warden, and Dr. Moe designed the study. Dr. Avin, Dr. Chen, Mrs. Srinivasan, Mrs. O’Neill, Ms. Troutman, Ms. Swallow, Mr. Mast, and Dr. Brown carried out the experiments. Dr. Avin, Dr. Chen, Dr. Allen, and Dr. Moe analyzed the data. Dr. Avin, Dr. Chen, Dr. Warden, and Dr. Moe drafted and revised the paper. All authors approved the final version of the manuscript.

DISCLOSURES

Dr. Moe reports personal fees from Amgen, grants from Chugai, and grants from Keryx, outside the submitted work.

FUNDING

National Institutes of Health grant K08 DK110429-01 (to Dr. Avin), United States Department of Veterans Affairs Merit award BX003025 (to Dr. Allen), and National Institutes of Health grant RO1 DK110871 (to Dr. Allen).

REFERENCES

43. Greenhill C: Irisin receptor in osteocytes identified. Nat Rev Endocrinol 15: 63, 2019

See related editorial, “From People to Lab Rats to People—Study of Exercise in CKD,” on pages 1777–1778.