

Chronic Exercise Protects against the Progression of Renal Cyst Growth and Dysfunction in Rats with Polycystic Kidney Disease

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ABSTRACT

QIU, J., Y. SATO, L. XU, T. MIURA, M. KOHZUKI, and O. ITO. Chronic Exercise Protects against the Progression of Renal Cyst Growth and Dysfunction in Rats with Polycystic Kidney Disease. *Med. Sci. Sports Exerc.*, Vol. 53, No. 12, pp. 2485–2494, 2021. **Introduction:** Polycystic kidney disease (PKD) is a genetic disorder characterized by the progressive enlargement of renal epithelial cysts and renal dysfunction. Previous studies have reported the beneficial effects of chronic exercise on chronic kidney disease. However, the effects of chronic exercise have not been fully examined in PKD patients or models. The effects of chronic exercise on the progression of PKD were investigated in a polycystic kidney (PCK) rat model. **Methods:** Six-week-old male PCK rats were divided into a sedentary group and an exercise group. The exercise group underwent forced treadmill exercise for 12 wk (28 m·min⁻¹, 60 min·d⁻¹, 5 d·wk⁻¹). After 12 wk, renal function and histology were examined, and signaling cascades of PKD progression, including arginine vasopressin (AVP), were investigated. **Results:** Chronic exercise reduced the excretion of urinary protein, liver-type fatty acid-binding protein, plasma creatinine, urea nitrogen, and increased plasma irislin and urinary AVP excretion. Chronic exercise also slowed renal cyst growth, glomerular damage, and interstitial fibrosis and led to reduced Ki-67 expression. Chronic exercise had no effect on cAMP content but decreased the renal expression of B-Raf and reduced the phosphorylation of extracellular signal-regulated kinase (ERK), mammalian target of rapamycin (mTOR), and S6. **Conclusion:** Chronic exercise slows renal cyst growth and damage in PCK rats, despite increasing AVP, with the downregulation of the cAMP/B-Raf/ERK and mTOR/S6 pathways in the kidney of PCK rats. **Key Words:** POLYCYSTIC KIDNEY DISEASE, PCK RATS, CHRONIC EXERCISE, CYST GROWTH, RENAL PROTECTION

Polycystic kidney disease (PKD) is a genetic disorder characterized by the progressive enlargement of renal epithelial cysts. Autosomal dominant PKD (ADPKD) is the most common hereditary cystic kidney disease with a prevalence estimated to be 1:400 and 1:1000 (1). Renal cysts have already formed from the embryonic period, but most

patients are asymptomatic until the 30s and 40s. Because of the compensation of nephrons, the glomerular filtration rate remains usual until the renal cyst enlarges to substantial pathological changes. Total kidney volume is a major factor predicting disease progression in ADPKD, and it is also used as a standard biomarker to indicate disease progression (1). After renal cyst enlargement, glomerular sclerosis and renal function deteriorate progressively, and finally, the end-stage renal disease requiring renal replacement therapy occurs in approximately 50% of patients by the age of 60 yr (1,2). ADPKD is the fourth leading cause of end-stage kidney disease among chronic kidney disease (CKD) in adults (2). However, adequate and satisfactory therapies for ADPKD have not been clarified.

ADPKD is caused by mutations in *PKD1* (encoding polycystin 1) or *PKD2* (encoding polycystin 2), whereas autosomal recessive PKD is caused by mutations in *PKHD1* (encoding fibrocystin) (2). Polycystin 1, polycystin 2, and fibrocystin are all localized in the primary cilia and are required for the regulation of Ca²⁺ influx in response to ciliary bending. Primary cilia abnormalities are associated with lowered intracellular Ca²⁺ (2). Low intracellular Ca²⁺-related

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abnormal signaling leads to the induction of cyst epithelial cell proliferation, which is a key feature of cyst growth (3).

Low intracellular Ca^{2+} activates adenylyl cyclase and increases intracellular cAMP levels. Next, cAMP and protein kinase A signaling upregulates the B-Raf and extracellular signal-regulated kinase (ERK) pathway in renal cyst epithelial cells (4). The finding that increased cAMP signaling is a crucial driver of cyst growth has led to the development of a therapy based on arginine vasopressin (AVP) type 2 receptor (V2R). AVP is a pivotal hormone for maintaining body fluid homeostasis and secreted by osmotic, hemodynamic, or stress stimuli. AVP has antidiuretic effects in the collecting ducts via V2R and increases vascular tone via AVP type 1 receptor. V2R is coupled with the stimulatory G protein (Gs), and V2R antagonists, including tolvaptan, reduce renal cAMP content and slow cyst growth and renal function decline in ADPKD patients and rodent PKD models (5). However, tolvaptan cannot slow liver cyst growth in ADPKD patients and polycystic kidney (PCK) rats (2,5) because AVP stimulates cAMP production in the kidney but not in the liver. In addition to the cAMP/B-Raf/ERK pathway, it has been reported that the mammalian target of rapamycin (mTOR)/S6 pathway promotes cyst growth by enhancing the proliferation, size, and metabolism of renal tubular cells (6).

Lifestyle modifications that slow the progression of CKD have long been a topic of research interest. Clinical studies have reported the renal protective effects of exercise therapy in CKD patients (7–10). Resistance training increases the glomerular filtration rate (7,8). Combination with aerobic and resistance training slows the decline in glomerular filtration rate (7,8) and ameliorates albuminuria (9). Walking lowers overall mortality and the risk of renal replacement therapy (10). We have also reported that chronic exercise at moderate intensity has renal protective effects in CKD model rats with 5/6 nephrectomy, diabetic nephropathy, and salt-sensitive hypertension (11–15). With respect to the mechanisms of the beneficial effects of chronic exercise, a newly discovered exercise-induced myokine, irisin, has been reported to have renal protective effects (16). Both endurance and resistance exercises increase irisin in skeletal muscles and plasma (17). Moreover, recombinant irisin administration prevents renal damage and fibrosis in mice with folic acid nephropathy, unilateral ureteral obstruction, and 5/6 nephrectomy (16).

ADPKD patients with a glomerular filtration rate ≥ 60 ($\text{mL}\cdot\text{min}^{-1}$ per 1.73 m^{-2}) have a low exercise capacity (18). Similarly, we recently reported a low exercise capacity in PCK rats (19), which have polycystic hepatorenal diseases and resemble human ADPKD (20). Chronic exercise at a moderate intensity for 12 wk improved the low exercise capacity and, unexpectedly, slowed liver cyst growth and fibrosis in PCK rats (19). However, it is controversial whether chronic exercise has renal protective effects in PKD patients and/or models because acute or chronic exercise stimulates the posterior pituitary gland to secrete AVP, thus

increasing AVP levels (21). Therefore, we examined whether chronic exercise could slow renal cyst growth and progression of glomerular sclerosis and renal dysfunction, affecting AVP secretion and the signaling cascades responsible for cellular proliferation in PCK rats.

METHODS

Experimental animals. Five-week-old male PCK and Sprague–Dawley (SD) rats were obtained from Charles River Laboratories Japan (Yokohama, Japan). PCK rat was developed spontaneously in the SD rat strain and identified as an orthologous rodent model of human PKD (20). PKD and liver disease result from a frameshift mutation (exon 36 is deleted by a change of A to T in intron 35) in the *Pkhd1* gene, located on rat chromosome 9. The renal cysts develop from collecting ducts, thick ascending loops of Henle, and distal tubules, and renal function such as serum creatinine and urea nitrogen worsen beyond 18 wk of age (22).

All rats had free access to tap water and were fed a standard rat diet (Labo MR Stock; Nossan Kogyo Co., Yokohama, Japan). All animal experiments were approved by the Tohoku University Committee for Animal Experiments and were performed in accordance with the Guidelines for Animal Experiments and Related Activities of Tohoku University (permit no. 2018-084).

Exercise protocol. After 1 wk of acclimatization, PCK rats were divided into the sedentary group (Sed-PCK, $n = 10$) or the exercise group (Ex-PCK, $n = 10$). The Sprague–Dawley (SD) rats were set as a control group (Con-SD, $n = 10$). The Ex-PCK group underwent forced treadmill exercise with moderate intensity, using treadmills (KN-73; Natsume Industries, Tokyo, Japan) for 12 wk ($28\text{ m}\cdot\text{min}^{-1}$, $60\text{ min}\cdot\text{d}^{-1}$, $5\text{ d}\cdot\text{wk}^{-1}$) (12). Six-week-old PCK rats were adapted to $28\text{ m}\cdot\text{min}^{-1}$ training speed, described in our previous study. Rats were exercised for $10\text{ min}\cdot\text{d}^{-1}$ at an initial treadmill speed of $20\text{ m}\cdot\text{min}^{-1}$ at 0% grade. The treadmill speed was increased gradually to $28\text{ m}\cdot\text{min}^{-1}$, and the duration of exercise was increased to $60\text{ min}\cdot\text{d}^{-1}$ for 1 wk. After acclimation to the treadmill, rats could run voluntarily with gait speeds. All rats completed every single training session for 60 min in the 12-wk training protocol.

Plasma and urinary parameters. The rats were housed individually in metabolic cages (Model ST; Sugiyama-General, Tokyo, Japan) for 3 d to acclimatize to the conditions. Food and water intake were measured, and urine was collected on ice for 24 h. Systolic blood pressure was measured using the tail-cuff method (MK-2000A; Muromachi Kikai, Tokyo, Japan). The rats were euthanized with sodium pentobarbitone ($100\text{ mg}\cdot\text{kg}^{-1}$ i.p.), and blood samples were collected from the ventral aorta. Urine and blood samples were centrifuged for 10 min at 2000g, and the supernatant was collected. Plasma and urine aliquots were rapidly frozen and stored at -80°C until analysis.

Urinary protein and plasma glucose, total cholesterol, triglyceride, urea nitrogen, and creatinine were measured using standard autoanalysis techniques (SRL Inc., Tokyo, Japan).

The urinary level of liver-type fatty acid-binding protein (L-FABP), which is a biomarker of proximal tubular stress and a valuable marker for tubulointerstitial damage in PCK rats, was measured using a highly sensitive enzyme-linked immunosorbent assay (CMIC, Tokyo, Japan) (23). Plasma AVP levels are fluctuated by anesthetics or stress (24), and the indwelling catheter into the femoral artery may affect treadmill running. Therefore, we measured AVP concentration in the 24-h urine by radioimmunoassay (SRL, Tokyo, Japan) and calculated urinary AVP excretion for 24 h as described previously (25). Plasma irisin was measured using an enzyme immunoassay kit (Phoenix Pharmaceuticals Inc., Burlingame, CA) described previously (26).

Histological analysis. After the rats were killed, kidneys were excised and decapsulated. The left kidney was immediately frozen in liquid nitrogen, and the right kidney was sliced perpendicularly to the sagittal axis at approximately 5-mm intervals. Slices from the midportion of the kidneys were fixed in 10% buffered formalin overnight, and the tissue was then embedded in paraffin. Sections (3 μm thick) were stained with hematoxylin and eosin (HE), periodic acid-Schiff (PAS), and Masson's trichrome (MT) following standard protocols. The whole kidney area and the cyst area in the HE-stained sections were determined using ImageJ analysis software (National Institutes of Health, Bethesda, MD) (27). Glomerular injury was evaluated in PAS-stained glomeruli using the index of

glomerular sclerosis (14). The percentage of interstitial fibrosis area was estimated in MT-stained tissue, except for the cyst areas, glomeruli, and blood vessels, as described previously (12,15).

Immunohistochemical analysis. Deparaffinized kidney sections (5 μm thick) were immunostained with antibodies against desmin (ab8470; Abcam, Cambridge, UK), Ki-67 (no. 418071; Nichirei Biosciences, Tokyo, Japan), p-mTOR (no. 293133; Santa Cruz Biotechnology, Santa Cruz, CA), and p-ERK (no. 4376; Cell Signaling Technology, Danvers, MA) according to the instructions for analyzing under a light microscope (Eclipse 80i microscope; Nikon, Tokyo, Japan). For each section, 30 randomly chosen fields were photographed using a digital color camera (DS-Fi2-U3 color camera, Nikon). Using ImageJ, the stained percentage of the target area was then estimated after selecting a glomerular area with desmin staining (14). The percentage of cells positive for Ki-67 was calculated from the total number of cells containing epithelial cysts and noncystic tubules from each kidney section using ImageJ, as described previously (28).

Western blot analysis. The frozen kidney of each rat was thawed, dissected into the cortex and medulla, and then homogenized in 100 $\text{mmol}\cdot\text{L}^{-1}$ potassium buffer (pH 7.25) containing 30% glycerol, 1 $\text{mmol}\cdot\text{L}^{-1}$ dithiothreitol, and 0.1 $\text{mmol}\cdot\text{L}^{-1}$ phenylmethylsulfonyl fluoride (15). Protein expression and phosphorylation were examined using Western

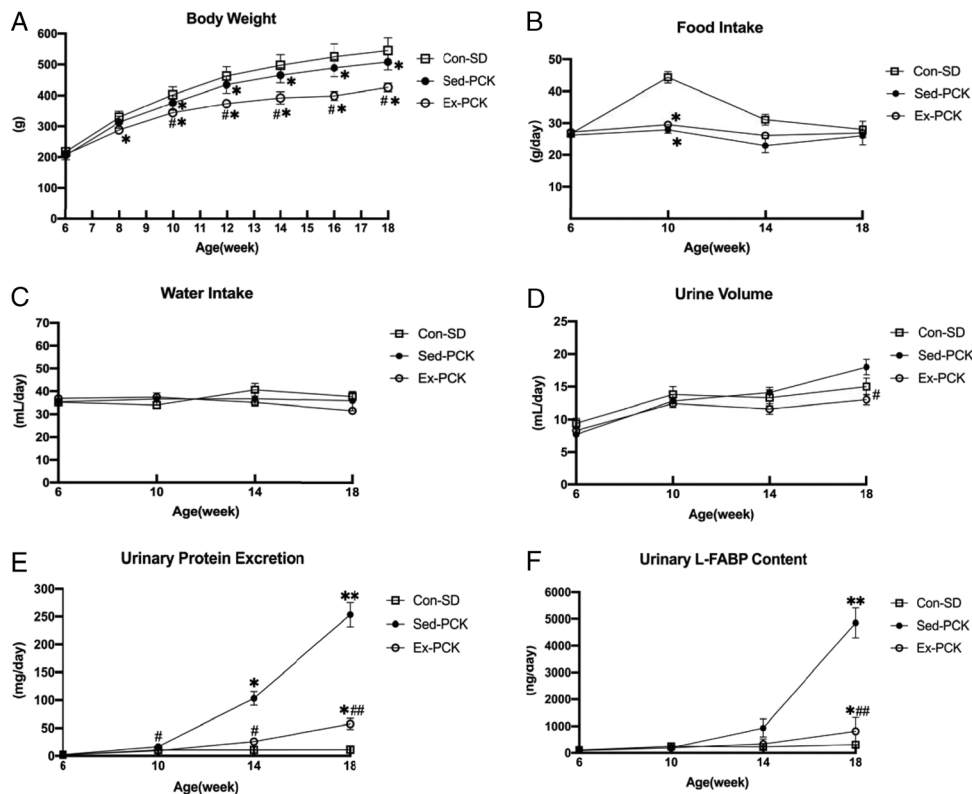


FIGURE 1—Effects of chronic exercise on general parameters and urinary parameters in PCK rats. Time courses of body weight (A), food intake (B), water intake (C), urine volume (D), urinary protein excretion (E), and urinary L-FABP excretion (F) were compared among the Con-SD (rectangle dots), Sed-PCK (closed dots), and Ex-PCK (round dots) groups ($n = 10$ in each group). Data are presented as mean \pm SEM. * $P < 0.05$, ** $P < 0.01$ compared with the Con-SD group; # $P < 0.05$, ## $P < 0.01$ compared with the Sed-PCK group.

TABLE 1. Blood pressure and plasma parameters.

	Con-SD	Sed-PCK	Ex-PCK
Systolic blood pressure (mm Hg)	106 ± 7	104 ± 3	96 ± 4
Glucose (mg·dL ⁻¹)	169.1 ± 6.8	139.7 ± 7.8*	125.5 ± 20.1**
Total cholesterol (mg·dL ⁻¹)	67.9 ± 5.1	148.2 ± 8.6**	114.1 ± 7.6**,#
Triglyceride (mg·dL ⁻¹)	59.7 ± 6.0	69.3 ± 5.3	49.8 ± 3.7##
Urea nitrogen (mg·dL ⁻¹)	16.8 ± 0.5	18.2 ± 0.9	15.9 ± 0.3#
Creatinine (mg·dL ⁻¹)	0.28 ± 0.01	0.35 ± 0.02**	0.30 ± 0.01#
Irisin (ng·dL ⁻¹)	1134.8 ± 29.7	1070.0 ± 41.0	1578.1 ± 106.0*##

Data are presented as mean ± SEM. *n* = 10 in each group.

P* < 0.05, *P* < 0.01 compared with the Con-SD group.

#*P* < 0.05, ##*P* < 0.01 compared with the Sed-PCK group.

Con-SD, control Sprague-Dawley rats; Sed-PCK, sedentary polycystic kidney rats; Ex-PCK, exercise polycystic kidney rats.

blot analysis, as described previously (19). Antibodies against Raf-B (no. 5284, Santa Cruz), ERK (no. 4695, Cell Signaling Technology), p-ERK (no. 4376, Cell Signaling Technology), mTOR (no. 2983, Cell Signaling Technology), p-mTOR (no. 2971, Cell Signaling Technology), S6 (no. 2217, Cell Signaling Technology), and p-S6 (no. 2211, Cell Signaling Technology) were used. Secondary HRP-conjugated mouse antirabbit (no. 2357, Santa Cruz) and rabbit antimouse (no. 516102, Santa Cruz) antibodies were then used. Relative band intensities were quantified using ImageJ and normalized using β-actin (A2228; Sigma-Aldrich, St. Louis, MO) as an internal standard.

cAMP assay. The frozen kidneys were ground to a fine powder with liquid nitrogen in a stainless-steel mortar. After the liquid nitrogen had evaporated, the tissues were assayed for cAMP using an enzyme-linked immunosorbent assay kit (Enzo Life Sciences Inc., Farmingdale, NY) (29). Results are expressed in picomole per milligram of tissue protein.

Statistical analysis. Data are expressed as the mean ± SEM. Statistical comparisons between the groups were performed using

the two-tailed unpaired *t*-test or one-way ANOVA. All analyses were carried out using GraphPad Prism software (version 8.4; GraphPad Inc., La Jolla, CA). *P* values < 0.05 were considered statistically significant.

RESULTS

General parameters and urinary parameters. PCK rats as a slow progression model of PKD and Sprague-Dawley (SD) rats as a control model were used to assess general parameters and urinary parameters in the kidney. Bodyweight was similar between the control SD (Con-SD) and the sedentary PCK (Sed-PCK) groups but was significantly lower in the exercise PCK (Ex-PCK) group than in the Sed-PCK group after 10 wk of age (*P* < 0.05) (Fig. 1A). There were no differences in food or water intake among the three groups (Figs. 1B and 1C). Urine output was similar between the Con-SD and the Sed-PCK groups but was significantly lower in the Ex-PCK group than in the Sed-PCK group at the end of the experiment (*P* = 0.023) (Fig. 1D). Urinary protein and L-FABP excretions were significantly increased in the Sed-PCK group after 14 wk of age compared with the beginning of the experiment and were significantly higher in the Sed-PCK group than in the Ex-PCK group by the end of the experiment (*P* < 0.0001 and *P* = 0.002, respectively) (Figs. 1E and 1F).

Plasma parameters. Table 1 shows the plasma parameters of the groups. Total cholesterol and creatinine were significantly higher in the Sed-PCK group than in the Con-SD group (*P* < 0.0001 and *P* = 0.004, respectively), and plasma glucose was significantly lower in the Sed-PCK group than in the Con-SD group (*P* = 0.011). Glucose, total cholesterol, triglyceride, urea nitrogen, and creatinine were significantly lower in

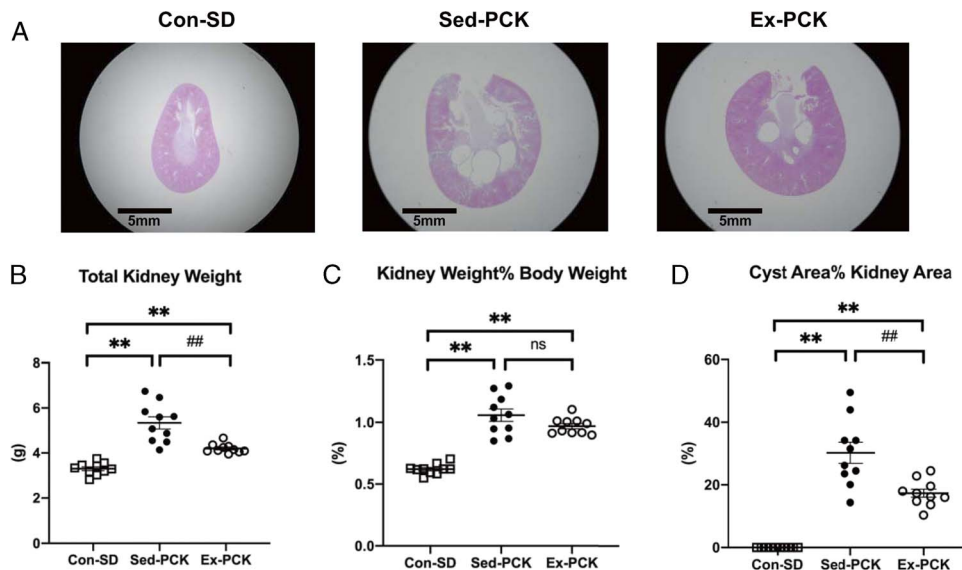


FIGURE 2—Effects of chronic exercise on kidney cysts in PCK rats. A, Representative images of kidney specimens stained with HE in the Con-SD, Sed-PCK, and Ex-PCK groups. Total kidney weight (B), kidney-to-body weight ratio (C), and cystic index (D) were compared among the Con-SD (rectangle dots), Sed-PCK (closed dots), and Ex-PCK (round dots) groups (*n* = 10 in each group). Data are presented as mean ± SEM. ***P* < 0.01 compared with the Con-SD group; ##*P* < 0.01 compared with the Sed-PCK group; ns: no significant difference.

the Ex-PCK group than in the Sed-PCK group ($P = 0.0002$, $P = 0.0084$, $P = 0.0085$, $P = 0.0375$, and $P = 0.0326$, respectively). Plasma irisin was similar between the Con-SD and the Sed-PCK groups but was significantly higher in the Ex-PCK group than in the Sed-PCK or Con-SD group ($P = 0.0008$ and $P = 0.0022$, respectively).

Kidney weight and morphology. Figure 2A shows representative images of the HE-stained kidney from the three groups. Renal cysts were observed in the outer medulla of both the Sed-PCK and the Ex-PCK groups, and cyst sizes were smaller in the Ex-PCK group than in the Sed-PCK group. Total kidney weight was significantly lower in the Ex-PCK group than in the Sed-PCK group ($P = 0.0023$) (Fig. 2B), but the kidney-to-body weight ratio was not significantly different between the two PCK groups ($P = 0.13$) (Fig. 2C). The cystic index was significantly higher in the Sed-PCK group than in the Con-SD group ($P < 0.0001$) and significantly lower in the Ex-PCK group than in the Sed-PCK group ($P = 0.0044$) (Fig. 2D).

Glomerular damage and renal interstitial fibrosis.

Figure 3A shows representative images of PAS-stained and desmin-immunostained glomeruli and MT-stained kidneys in each group. Glomerular sclerosis, podocyte injury, and renal interstitial fibrosis were observed in the Sed-PCK group. The index of glomerular sclerosis was significantly higher in the Sed-PCK group than in the Con-SD group ($P < 0.0001$) and significantly lower in the Ex-PCK group than in the Sed-PCK group ($P = 0.048$) (Fig. 3B). The desmin-positive staining area in the glomeruli was significantly larger in the Sed-PCK group than in the Con-SD group ($P < 0.0001$) and significantly smaller in the Ex-PCK group than in the Sed-PCK group ($P < 0.0001$) (Fig. 3C). The renal interstitial fibrosis area was significantly higher in the Sed-PCK group than in the Con-SD group ($P < 0.0001$) and smaller in the Ex-PCK group than in the Sed-PCK group ($P = 0.0007$) (Fig. 3D).

Cell proliferation and signaling cascades. Figure 4A shows representative images of the kidney immunostained for Ki-67 from the Sed-PCK and Ex-PCK groups. Ki-67-positive

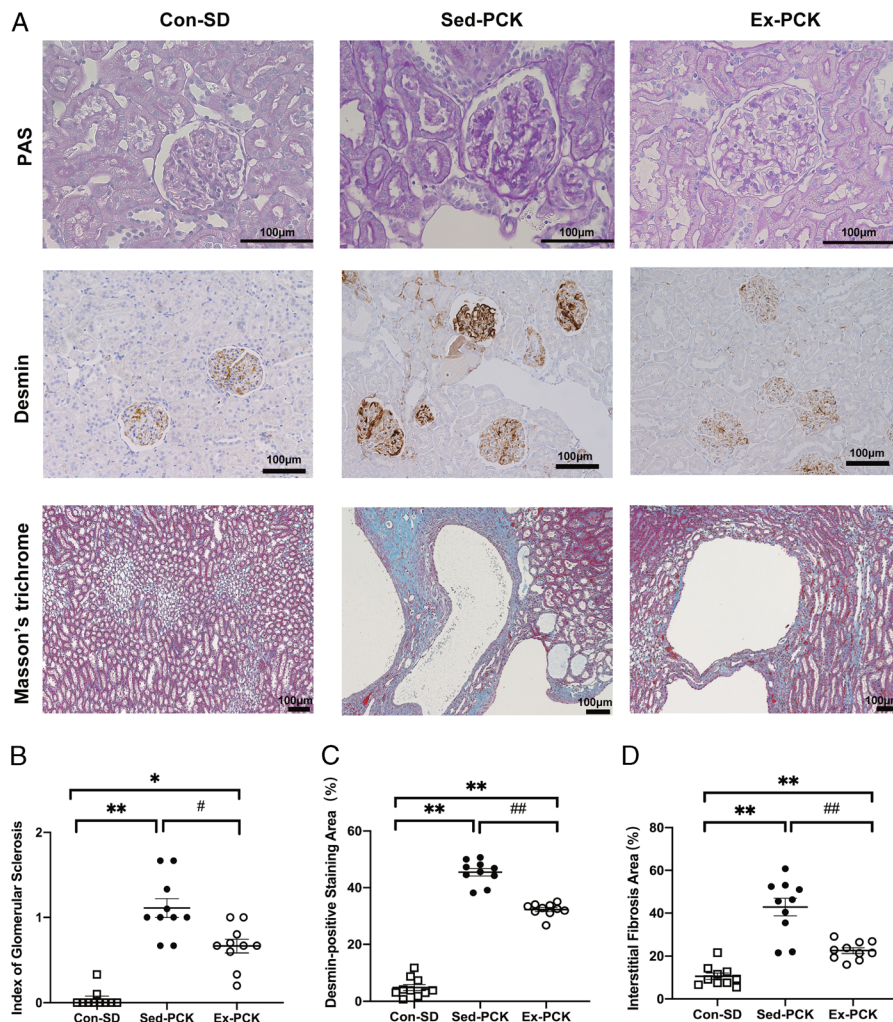


FIGURE 3—Effects of chronic exercise on glomerular sclerosis, podocyte injury, and renal interstitial fibrosis in PCK rats. A, Representative images of PAS-stained, desmin-immunostained glomeruli, and MT-stained kidneys in the Con-SD, Sed-PCK, and Ex-PCK groups. Index of glomerular sclerosis (B), desmin-positive staining area (%) (C), and interstitial fibrosis area (%) (D) in the Con-SD (rectangle dots), Sed-PCK (closed dots), and Ex-PCK (round dots) groups ($n = 10$ in each group). Data are presented as mean \pm SEM. * $P < 0.05$, ** $P < 0.01$ compared with the Con-SD group; # $P < 0.05$, ## $P < 0.01$ compared with the Sed-PCK group.

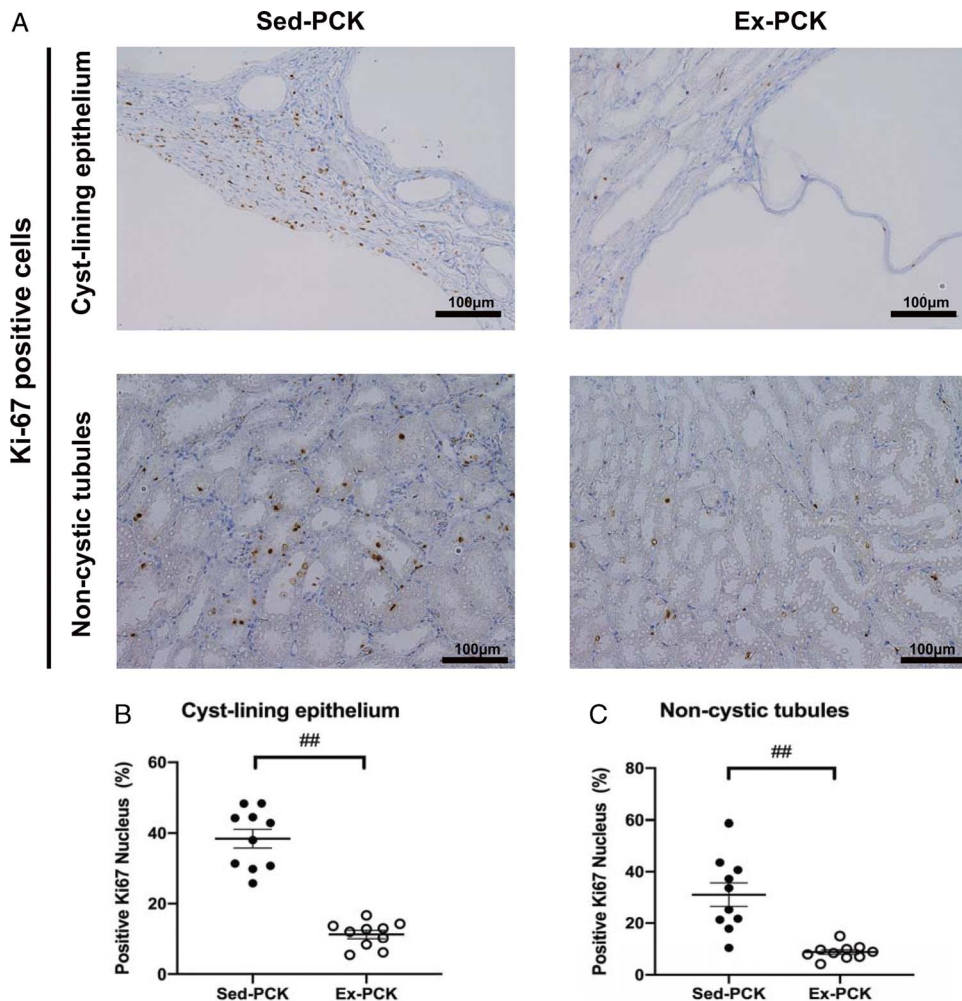


FIGURE 4—Effects of chronic exercise on cell proliferation in the kidneys of PCK rats. **A**, Representative images of kidney specimens immunostained for Ki-67 in the Sed-PCK and Ex-PCK groups. **B**, Ki-67 labeling index in the cyst-lining epithelium of the Sed-PCK (closed dots) and Ex-PCK (round dots) groups ($n = 10$ in each group). **C**, Ki-67 labeling index in the noncystic tubules of the Sed-PCK (closed dots) and Ex-PCK (round dots) groups ($n = 10$ in each group). Data are presented as mean \pm SEM. $##P < 0.01$ compared with the Sed-PCK group.

cells were highly expressed in the cyst-lining epithelium, interstitium, and noncystic tubules of the Sed-PCK group. Chronic exercise led to fewer Ki-67-positive cells. The Ki-67 labeling index was significantly lower in the cyst-lining epithelium and noncystic tubules in the Ex-PCK group compared with the Sed-PCK group ($P < 0.0001$ and $P = 0.0008$, respectively) (Figs. 4B and 4C).

Urinary AVP excretion was significantly higher in the Sed-PCK group than in the Con-SD group ($P = 0.0343$) and was considerably higher in the Ex-PCK group than in the Sed-PCK group ($P = 0.0174$) (Fig. 5A). Renal cAMP content was significantly higher in the Sed-PCK group than in the Con-SD group ($P = 0.0147$), but it was not significantly different between the Sed-PCK and the Ex-PCK groups ($P = 0.924$) (Fig. 5B). Renal B-Raf expression was significantly higher in the Sed-PCK group than in the Con-SD ($P = 0.0022$) and significantly lower in the Ex-PCK group than in the Sed-PCK group ($P = 0.0002$) (Fig. 5C).

Figures 6A and 6B show representative images of kidneys immunostained for phosphorylated (p-) ERK and p-mTOR, respectively, from each group. The p-ERK and the p-mTOR

proteins were highly expressed in the cyst-lining epithelium and noncystic tubules in the Sed-PCK group, and chronic exercise decreased their expressions (Figs. 6A and 6B). Renal ERK and mTOR phosphorylation were significantly higher in the Sed-PCK group than in the Con-SD group ($P < 0.0001$ and $P = 0.0046$, respectively), and S6 phosphorylation also tended to be higher in the Sed-PCK group compared with the Con-SD group (Figs. 6C, 6D, and 6E). Renal ERK, mTOR, and S6 phosphorylation were significantly lower in the Ex-PCK group than in the Sed-PCK group ($P = 0.0005$, $P = 0.0071$, and $P = 0.0033$, respectively).

DISCUSSION

Chronic exercise has renal protective effects in CKD patients and models (11–15); however, the renal protective effects of chronic exercise have not yet been reported in PKD patients or models. The present study revealed that chronic exercise at a moderate intensity slowed the progression of renal cyst growth, glomerular damage, interstitial fibrosis, and renal

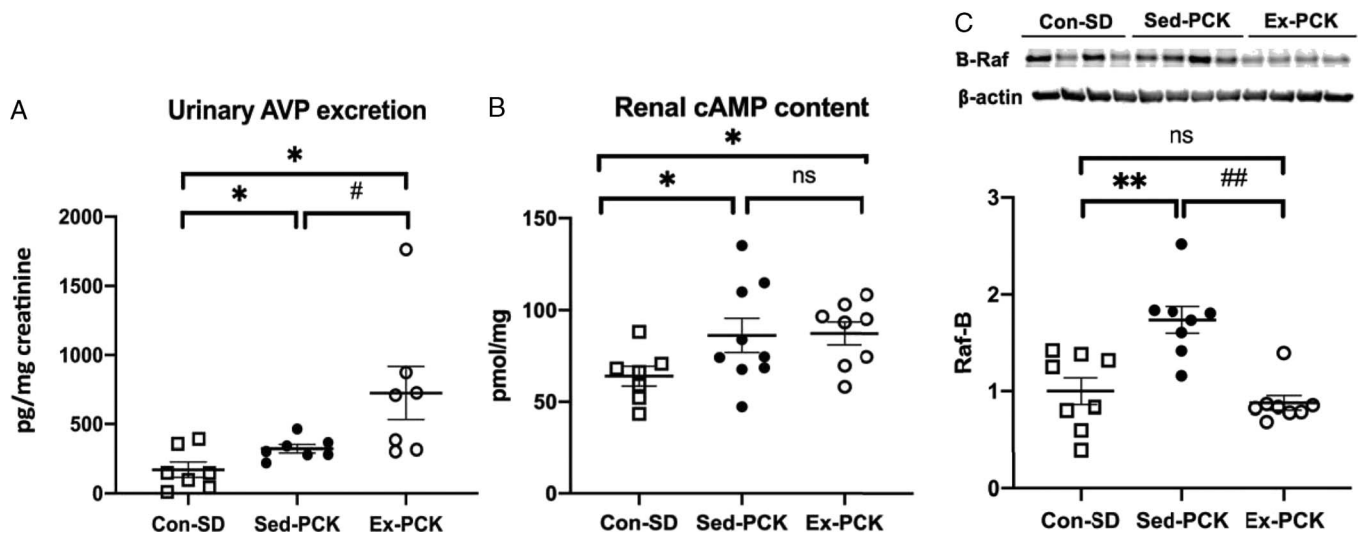


FIGURE 5—Effects of chronic exercise on urinary AVP excretion, renal cAMP content, and renal B-Raf expression in PCK rats. **A**, Urinary AVP excretion in the Con-SD (rectangle dots), Sed-PCK (closed dots), and Ex-PCK (round dots) groups ($n = 10$ in each group). **B**, Renal cAMP content in the Con-SD (rectangle dots), Sed-PCK (closed dots), and Ex-PCK (round dots) groups ($n = 8$ in each group). **C**, Western blotting analysis of B-Raf expression in the Con-SD (rectangle dots), Sed-PCK (closed dots), and Ex-PCK (round dots) groups ($n = 8$ in each group). Top panels show representative immunoblotting. Each lane was loaded with a protein sample prepared from four different rats per group. The ratio in the Con-SD group was assigned a value of 1. Data are presented as mean \pm SEM. * $P < 0.05$, ** $P < 0.01$ compared with the Con-SD group; # $P < 0.05$, ## $P < 0.01$ compared with the Sed-PCK group; ns, no significant difference.

dysfunction in PCK rats, despite increasing AVP. Chronic exercise also inhibited excessive cell proliferation, with the downregulation of the cAMP/B-Raf/ERK and mTOR/S6 pathways in renal epithelial cells. To the best of our knowledge, the present study is the first to report that chronic exercise has therapeutic potential against renal cyst growth. In addition, this study will provide a new understanding of how the early start of chronic exercise could effectively prevent PKD progression.

We chose the exercise protocol in the present study based on our previous study of CKD model rats with 5/6 nephrectomy (12), in which proteinuria and glomerular sclerosis were significantly attenuated after 12 wk of chronic exercise. We confirmed that when PCK rats run at a speed of $28 \text{ m}\cdot\text{min}^{-1}$ on the treadmill, oxygen consumption ($\dot{V}O_2$) corresponds to approximately 65% of the maximal $\dot{V}O_2$, which is assumed to be aerobic exercise at a moderate intensity (19). In contrast to the present results, Darnley et al. (30) reported that treadmill exercise ($14 \text{ m}\cdot\text{min}^{-1}$, $30 \text{ min}\cdot\text{d}^{-1}$, $3 \text{ d}\cdot\text{wk}^{-1}$) for 6 wk did not lead to any changes in serum urea nitrogen or creatinine in Han:SPRD-*cy* rats. Similarly, in our pilot studies, chronic exercise for 8 wk did not significantly affect renal cyst growth in PCK rats (data not shown). Thus, the intensity, time, frequency, and duration of the exercise protocol may be important to obtain benefits in PKD models.

In agreement with our previous studies (11–14), chronic exercise lowered proteinuria and plasma creatinine and attenuated glomerular sclerosis and podocyte injury in PCK rats. Chronic exercise for 8 wk significantly decreased urinary protein excretion (Fig. 1E) without significant effects on renal cyst growth in PCK rats (data not shown). Therefore, glomerular protection may be a primary effect of chronic exercise rather than secondary to slowing renal cyst growth. Chronic exercise also inhibited the increase in urinary L-FABP

excretion, suggesting that chronic exercise might strongly attenuate proximal tubular stress and the progression of the tubulointerstitial disorder in PCK rats (23).

As an indicator of cell proliferation, chronic exercise decreased the number of Ki-67-positive cells in the kidneys of PCK rats, indicating the inhibition of excessive cell proliferation. As well as in the kidney, chronic exercise has also been recently reported to slow the progression of cyst growth and fibrosis in the liver of PCK rats (19). However, chronic exercise inhibits liver cyst growth with an activating AMP-activated protein kinase (AMPK) (19); an AMPK activator, metformin, inhibits cystic growth in the liver but not in the kidney of PCK rats (31). On the other hand, tolvaptan can slow cystic growth in the kidney but not in the liver of PCK rats (2,5). These results suggested that the inhibitory effects of chronic exercise on cystic growth may be mediated by different mechanisms in the kidney and liver of PCK rats.

The present study indicates that chronic exercise increases AVP in PCK rats. In agreement with these results, AVP synthesis and secretion have been previously reported to increase during exercise (32). Sustained moderate exercise (at an intensity threshold of 40%–65% of $\dot{V}O_{2\text{max}}$) increased plasma AVP (33). Furthermore, chronic exercise with a treadmill for 5 wk increased plasma AVP in Wistar rats (34). The present study also indicates that chronic exercise did not change renal cAMP content and did decrease the cAMP-inducible B-Raf expression in PCK rats, despite increasing AVP, suggesting that chronic exercise might inactivate adenylate cyclase via the inhibitory G protein (Gi). Previous studies indicate that norepinephrine and α_2 -adrenergic receptor (α_2 -AR) agonists inhibit the AVP-activated adenylate cyclase, cAMP content, and water transport via the Gi in the rat collecting ducts (35,36). Therefore, it is possible that chronic exercise might

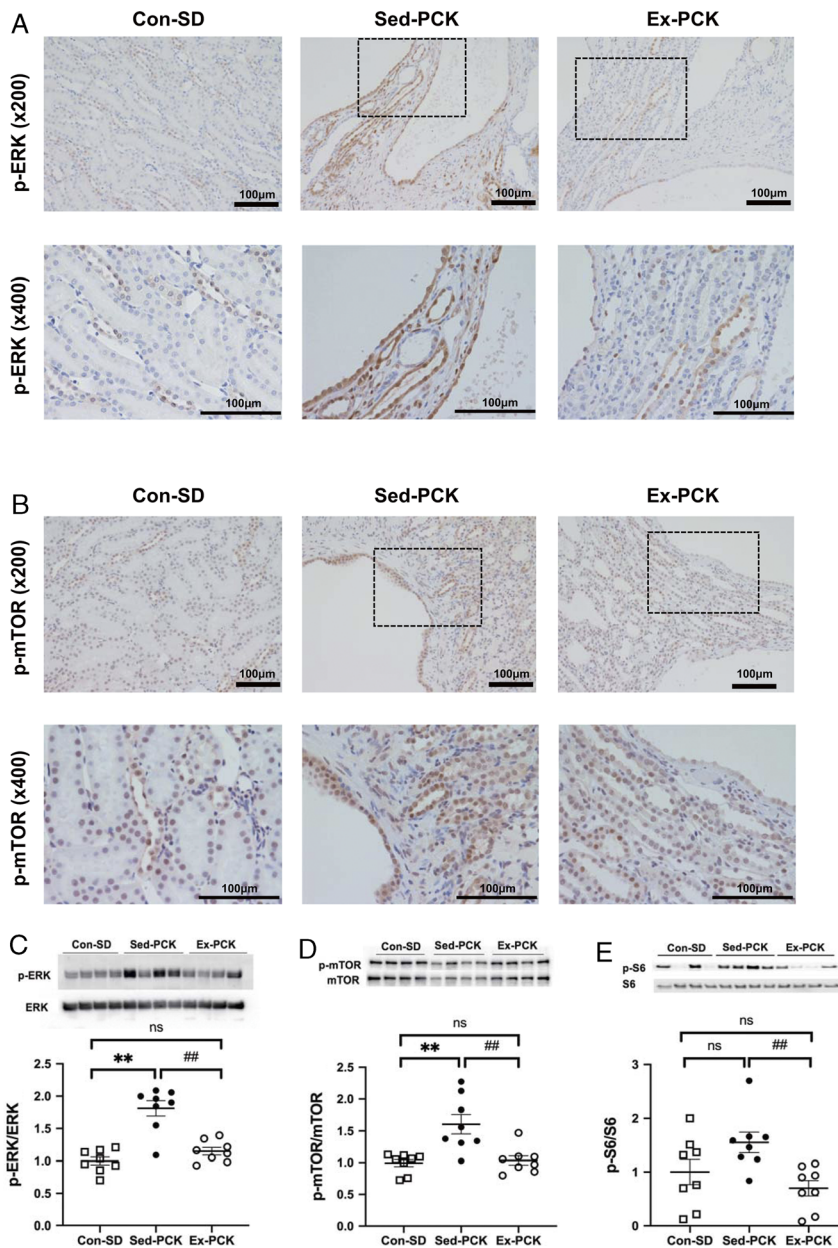


FIGURE 6—Effects of chronic exercise on the phosphorylation of ERK, mTOR, and S6 in PCK rats. Representative images of kidney specimens immunostained for p-ERK (A) and p-mTOR (B) in the Con-SD, Sed-PCK, and Ex-PCK groups. Western blotting analysis of p-ERK (C), p-mTOR (D), and p-S6 expression (E) in the Con-SD (rectangle dots), Sed-PCK (closed dots), and Ex-PCK (round dots) groups ($n = 8$ in each group). Top panels show representative immunoblotting. Each lane was loaded with a protein sample prepared from four different rats per group. Ratios of the relative band intensity of the phosphorylated protein to that of the total protein were calculated. The ratio in the Con-SD group was assigned a value of 1. Data are presented as mean \pm SEM. ****** $P < 0.01$ compared with the Con-SD group; **##** $P < 0.01$ compared with the Sed-PCK group; ns, no significant difference.

stimulate renal sympathetic activity and activate α_2 -AR in the collecting ducts to slow the progression of renal cyst growth with reducing the renal cAMP content in PCK rats. In this regard, our preliminary study indicates that chronic treatment of the α_2 -AR agonist clonidine slows the progression of renal cyst growth in PCK rats (data not shown).

Previous studies indicate that even normal plasma AVP levels increase B-Raf expression and ERK phosphorylation in the kidneys of PCK rats and that inhibition of AVP by V2R antagonists and hydration can downregulate the B-Raf/ERK pathway (25,37). The present study indicates that chronic

exercise downregulates not only the B-Raf/ERK pathway but also the mTOR/S6 pathway in the kidneys of PCK rats. Both mTOR and ERK are involved in excessive cell proliferation and cyst growth in the renal tubules and cholangiocytes of PCK rats (38). However, neither tolvaptan nor AEZ-131, an ERK inhibitor, affected S6 phosphorylation in the kidney of PCK rats, and the suppressive effects of tolvaptan and an mTOR inhibitor, rapamycin, on renal cyst growth were additive (39). The suppressive effects of chronic exercise on excessive cell proliferation and renal cyst growth in the present study might therefore be mediated by the downregulation of both the B-Raf/

ERK and the mTOR/S6 pathways in the kidneys of PCK rats. Several types of exercise affect mTOR and ERK in the skeletal muscle, adipose tissue, liver, and vasculature (40–42). However, the effects of exercise on mTOR or ERK have not previously been reported in the kidney, especially in the renal tubules. We recently reported that chronic exercise downregulates mTOR and ERK phosphorylation in the liver and cholangiocytes in PCK rats (19). In agreement with the results from PCK rats, chronic exercise with a treadmill inactivated mTOR and suppressed excessive cell proliferation in hepatocellular carcinoma in PTEN-deficient mice (43) and carcinoma-implanted rats (44).

The present study also demonstrates that chronic exercise increases plasma irisin in PCK rats. Irisin mediates the beneficial effects of exercise, such as by promoting the brown adipose formation and by improving the metabolism, and also has a beneficial role in kidney and heart diseases (16,45,46). In contrast to the present study, the effects of chronic exercise on plasma irisin are controversial in humans. Plasma irisin is increased by chronic exercise in obese people, the elderly, and patients with metabolic dysfunction, but not in healthy subjects (47). Plasma irisin levels were significantly decreased in CKD patients and were inversely correlated with blood urea nitrogen and creatinine levels (48). Skeletal muscle-specific PGC-1 α overexpression increased irisin production and plasma irisin levels and attenuated renal damage in mice with folic acid nephropathy, unilateral ureteral obstruction, and 5/6 nephrectomy (16). Moreover, recombinant irisin administration attenuated renal damage in the mouse kidney disease models (16). Future study is necessary to examine whether irisin slows renal cyst growth and renal damage in PCK rats.

There are several limitations in the present study. First, although chronic exercise for 12 wk has renal protective effects, it does not determine the current exercise protocol suggests superlative exercise intensity and frequency for renal cyst growth in PCK rats. In addition, the present results may not be directly applicable to ADPKD patients. Second, the present

study did not examine the effects of chronic exercise in SD rats. Our previous study reported that chronic exercise for 12 wk did not affect renal function or urine albumin excretion in SD rats (49). Third, young rats were only used in this study. An early start of exercise therapy can be expected to suppress the progression of cyst growth and renal dysfunction in ADPKD, which can be early diagnosed from family history. However, because most ADPKD patients with renal dysfunction are adults, further studies are required to examine the renal effects of chronic exercise in age PCK rats. Fourth, several studies call into question the use of commercial irisin ELISA kits (50), which have prominent cross reactivity with nonspecific proteins in human and animal sera. This study relied on a commercial irisin ELISA kit, which may result in contradictory data concerning plasma irisin.

In conclusion, chronic exercise slows the progression of PKD pathologies, such as renal dysfunction, renal cyst growth, glomerular damage, and renal interstitial fibrosis in PCK rats. Despite increasing AVP, chronic exercise also inhibits excessive cell proliferation, with the downregulation of the cAMP/B-Raf/ERK and mTOR/S6 pathways in the kidney of PCK rats. Chronic exercise may be a novel therapeutic approach against cyst growth and renal dysfunction in patients with ADPKD, for which effective and satisfactory therapy has not been clarified.

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The authors declare no conflicts of interest associated with this manuscript.

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