

Chronic exercise protects against the progression of renal cyst growth and dysfunction in rats with polycystic kidney disease

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Short Title: Exercise is renoprotective in PCK rats

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Abstract

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 weeks (28 m/min, 60 min/day, 5 days/week). After 12 weeks, kidney function and histology were examined, protein expressions were analyzed, and signaling cascades of PKD were examined.

Results: Chronic exercise reduced the excretion of urinary protein, liver-type fatty acid-binding protein, plasma creatinine, urea nitrogen, and increased plasma irisin and urinary arginine vasopressin (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 down-regulation of the cAMP/B-Raf/ERK and mTOR/S6 pathways in the kidney of PCK rats.

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Keywords

Polycystic kidney disease; PCK rats; chronic exercise; cyst growth; renal protection

1 **Introduction**

2 Polycystic kidney disease (PKD) is the most prevalent of all genetic disorders, which is
3 characterized by progressive enlargement of epithelial cysts in the kidney. Autosomal dominant
4 PKD (ADPKD) is caused by mutations in *PKD1* (encoding polycystin-1) or *PKD2* (encoding
5 polycystin-2), whereas autosomal recessive PKD (ARPKD) is caused by mutations in *PKHD1*
6 (encoding fibrocystin). Polycystin-1, polycystin-2, and fibrocystin are all localized in the primary
7 cilia and are required for the regulation of Ca^{2+} influx in response to ciliary bending. Primary cilia
8 abnormalities are associated with lowered intracellular Ca^{2+} (1). Low intracellular Ca^{2+} -related
9 abnormal signaling leads to the induction of cyst epithelial cell proliferation, which is a key feature
10 of cyst growth (2).

11 Low intracellular Ca^{2+} activates adenylyl cyclase and increases intracellular cAMP levels. Next,
12 cAMP and protein kinase A signaling upregulates the B-Raf and extracellular signaling-regulated
13 kinase (ERK) pathway in renal cyst epithelial cells (3). The finding that increased cAMP signaling
14 is a crucial driver of cyst growth has led to the development of arginine vasopressin (AVP) type 2
15 receptor (V2R)-based therapy. Antagonists of V2R, including tolvaptan, reduce renal cAMP content
16 by inhibiting V2 receptors which coupled with the stimulatory G protein (Gs) and slow cyst growth

17 and the decline of renal function in ADPKD patients and rodent PKD models (4). In addition, it has
18 been reported that the mammalian target of rapamycin (mTOR) and S6 pathway promotes cyst
19 growth by enhancing the proliferation, size, and metabolism of renal tubular cells (5).

20 Lifestyle modifications that slow the progression of chronic kidney disease (CKD) have long
21 been a topic of research interest. Clinical studies have reported that chronic exercise slows the
22 decline in glomerular filtration rate (6, 7), decreases albuminuria (8), delays the initiation of dialysis,
23 and diminishes overall mortality in CKD patients (9). We have also reported that chronic exercise
24 at moderate intensity has renal protective effects in CKD model rats with 5/6 nephrectomy, diabetic
25 nephropathy, and salt-sensitive hypertension (10-14). With respect to the mechanisms of the
26 beneficial effects of chronic exercise, a newly discovered exercise-induced myokine, irisin, has been
27 reported to have renal protective effects (15). Both endurance and resistance exercises increase irisin
28 in skeletal muscles and plasma (16). Moreover, recombinant irisin administration prevents renal
29 damage and fibrosis in mice with folic acid nephropathy, unilateral ureteral obstruction, and 5/6
30 nephrectomy (15).

31 ADPKD patients with a glomerular filtration rate ≥ 60 (mL/min/1.73 m²) have a low exercise
32 capacity (17). Similarly, we recently reported a low exercise capacity in polycystic kidney (PCK)

33 rats (18), which have polycystic kidney and liver diseases and resemble human ADPKD (19, 20).
34 Chronic exercise at a moderate intensity for 12 weeks improved the low exercise capacity and,
35 unexpectedly, slowed liver cyst growth and fibrosis in PCK rats (18). However, it is controversial
36 whether chronic exercise has renal protective effects in PKD patients and/or models, because acute
37 or chronic exercise stimulates the posterior pituitary gland to secrete AVP, thus increasing AVP
38 levels (21). Therefore, we examined the effects of chronic exercise on the progression of PKD, as
39 well as on the signaling cascades responsible for cellular proliferation in PCK rats.

40

41 **Methods**

42 **Experimental animals**

43 Five-week-old male PCK and Sprague-Dawley rats were obtained from Charles River
44 Laboratories Japan (Yokohama, Japan). All rats had free access to tap water and were fed a
45 standard rat diet (Labo MR Stock, Nosan Kogyo Co., Yokohama, Japan). All animal experiments
46 were approved by the Tohoku University Committee for Animal Experiments and were performed
47 in accordance with the Guidelines for Animal Experiments and Related Activities of Tohoku
48 University (permit no. 2018-084).

49 **Exercise protocol**

50 After 1 week of acclimatization, PCK rats were divided into the sedentary group (Sed-PCK,
51 $n=10$) or the exercise group (Ex-PCK, $n=10$). The Sprague-Dawley (SD) rats were set as a control
52 group (Con-SD, $n=10$). The Ex-PCK group underwent forced treadmill exercise with moderate
53 intensity, using treadmills (KN-73; Natsume Industries, Tokyo, Japan) for 12 weeks with the
54 following protocol: 28 m/min, 60 min/day, and 5 days/week (11).

55 **Plasma and urinary parameters**

56 The rats were housed individually in metabolic cages (Model ST; Sugiyama-General, Tokyo,
57 Japan) for 3 days to acclimatize to the conditions. Food and water intake were measured, and urine
58 was collected on ice for 24h. Systolic blood pressure was measured using the tail-cuff method (MK-
59 2000A; Muromachi Kikai, Tokyo, Japan). The rats were euthanized with sodium pentobarbitone
60 (100 mg/kg, i.p.) and blood samples were collected from the ventral aorta. Urine and blood samples
61 were centrifuged for 10 min at $2\ 000 \times g$, and the supernatant was collected. Plasma and urine
62 aliquots were rapidly frozen and stored at -80°C until analysis.

63 Urinary protein and plasma glucose, total cholesterol, triglyceride, urea nitrogen, and creatinine
64 were measured using standard auto-analysis techniques (SRL Inc., Tokyo, Japan). L-FABP was

65 measured using a highly sensitive enzyme-linked immunosorbent assay (CMIC, Tokyo, Japan) (22).
66 Plasma AVP levels are fluctuated by anesthetics or stress (23), and the indwelling catheter into the
67 femoral artery may affect treadmill running. Therefore, we measured AVP concentration in the 24h
68 urine by radioimmunoassay (SRL, Tokyo, Japan) and calculated urinary AVP excretion for 24h
69 described previously (24, 25). Plasma irisin was measured using an enzyme immunoassay kit
70 (Phoenix Pharmaceuticals Inc, Burlingame, CA, USA).

71 **Histological analysis**

72 After the rats were sacrificed, kidneys were excised and decapsulated. The left kidney was
73 immediately frozen in liquid nitrogen and the right kidney was sliced perpendicularly to the sagittal
74 axis at approximately 5 mm intervals. Slices from the midportion of the kidneys were fixed in 10%
75 buffered formalin overnight, and the tissue was then embedded in paraffin. Sections (3 μ m thick)
76 were stained with hematoxylin and eosin (HE), periodic acid–Schiff (PAS), and Masson’s trichrome
77 (MT) following standard protocols. The whole kidney area and the cyst area in the HE-stained
78 sections were determined using ImageJ analysis software (National Institutes of Health, Bethesda,
79 MD) (26). Glomerular injury was evaluated in PAS-stained glomeruli using the index of glomerular

80 sclerosis (13). The percentage of interstitial fibrosis area was estimated in MT-stained tissue, except
81 for the cyst areas, glomeruli, and blood vessels, as described previously (11, 14).

82 **Immunohistochemical analysis**

83 Deparaffinized kidney sections (5 μ m thick) were immunostained with antibodies against
84 desmin (ab8470, Abcam, Cambridge, UK), Ki-67 (#418071, Nichirei Biosciences, Tokyo, Japan),
85 p-mTOR (#293133, Santa Cruz Biotechnology, Santa Cruz, CA, USA), and p-ERK (#4376, Cell
86 Signaling Technology, Danvers, MA, USA) according to the instructions for analyzing under a light
87 microscope (Eclipse 80i microscope, Nikon, Tokyo, Japan). For each section, 30 randomly chosen
88 fields were photographed using a digital color camera (DS-Fi2-U3 color camera, Nikon). Using
89 ImageJ, the stained percentage of the target area was then estimated after selecting a glomerular
90 area with desmin staining(13). The percentage of cells positive for Ki-67, was calculated from the
91 total number of cells containing epithelial cysts and non-cystic tubules from each kidney section
92 using ImageJ, as described previously (27).

93 **Western blot analysis**

94 The frozen kidney of each rat was thawed, dissected into the cortex and medulla, and then
95 homogenized in 100 mmol/L potassium buffer (pH 7.25) containing 30% glycerol, 1 mmol/L

96 dithiothreitol, and 0.1 mmol/L phenylmethylsulfonyl fluoride (14). Protein expression and
97 phosphorylation were examined using western blot analysis, as described previously (18).
98 Antibodies against Raf-B (#5284; Santa Cruz), ERK (#4695; Cell Signaling Technology), p-ERK
99 (#4376; Cell Signaling Technology), mTOR (#2983; Cell Signaling Technology), p-mTOR (#2971;
100 Cell Signaling Technology), S6 (#2217; Cell Signaling Technology), and p-S6 (#2211; Cell
101 Signaling Technology) were used. Secondary HRP-conjugated mouse anti-rabbit (#2357; Santa
102 Cruz) and rabbit anti-mouse (#516102; Santa Cruz) antibodies were then used. Relative band
103 intensities were quantified using ImageJ and normalized using β -actin (A2228; Sigma-Aldrich, St.
104 Louis, MO, USA) as an internal standard.

105 **cAMP assay**

106 The frozen kidneys were ground to a fine powder with liquid nitrogen in a stainless-steel mortar.
107 After the liquid nitrogen had evaporated, the tissues were assayed for cAMP using an enzyme-linked
108 immunosorbent assay kit (Enzo Life Sciences Inc., Farmingdale, NY, USA) (28). Results are
109 expressed in pmol/mg of tissue protein.

110 **Statistical analysis**

111 Data are expressed as the mean \pm SEM. Statistical comparisons between the groups were
112 performed using the two-tailed unpaired *t*-test or one-way ANOVA. All analyses were carried out
113 using GraphPad Prism software (version 8.4; GraphPad Inc., La Jolla, CA, USA). *P*-values of <0.05
114 were considered statistically significant.

115

116 **Results**

117 **General parameters and urinary parameters**

118 PCK rats as a slow progression model of PKD and Sprague-Dawley (SD) rats as a control model,
119 were used to assess general parameters and urinary parameters in the kidney. Bodyweight was
120 similar between the control SD rats (Con-SD) and sedentary PCK rats (Sed-PCK) groups, but was
121 significantly lower in the exercise PCK rats (Ex-PCK) group than in the Sed-PCK group after 10
122 weeks of age ($P<0.05$) (Figure 1A). There were no differences in food or water intake among the
123 three groups (Figure 1B and 1C). Urine output was similar between the Con-SD and Sed-PCK
124 groups, but was significantly lower in the Ex-PCK group than in the Sed-PCK group at the end of
125 the experiment ($P<0.05$) (Figure 1D). Urinary protein and liver-type fatty acid-binding protein (L-
126 FABP) excretions were significantly increased in the Sed-PCK group after 14 weeks of age

127 compared with the beginning of the experiment, and were significantly higher in the Sed-PCK group
128 than in the Ex-PCK group by the end of the experiment ($P<0.01$ and $P<0.01$, respectively) (Figure
129 1E and 1F).

130 **Plasma parameters**

131 Table 1 shows the plasma parameters of the groups. Total cholesterol and creatinine were
132 significantly higher in the Sed-PCK group than in the Con-SD group, and plasma glucose was
133 significantly lower in the Sed-PCK group than in the Con-SD group. Glucose, total cholesterol,
134 triglyceride, urea nitrogen, and creatinine were significantly lower in the Ex-PCK group than in the
135 Sed-PCK group. Plasma irisin was similar between the Con-SD and Sed-PCK groups, but was
136 significantly higher in the Ex-PCK group than in the Sed-PCK or Con-SD group ($P<0.01$ and
137 $P<0.05$, respectively).

138 **Kidney weight and morphology**

139 Figure 2A shows representative images of the HE-stained kidney from the three groups. Renal
140 cysts were observed in the outer medulla of both the Sed-PCK and Ex-PCK groups, and cyst sizes
141 were smaller in the Ex-PCK group than in the Sed-PCK group. Total kidney weight was significantly
142 lower in the Ex-PCK group than in the Sed-PCK group ($P<0.01$) (Figure 2B), but the kidney-to-

143 body weight ratio was not significantly different between the two PCK groups (Figure 2C). The
144 cystic index was significantly higher in the Sed-PCK group than in the Con-SD group ($P<0.01$), and
145 significantly lower in the Ex-PCK group than in the Sed-PCK group ($P<0.05$) (Figure 2D).

146 **Glomerular damage and renal interstitial fibrosis**

147 Figure 3A shows representative images of PAS-stained and desmin-immunostained glomeruli
148 and MT-stained kidneys in each group. Glomerular sclerosis, podocyte injury, and renal interstitial
149 fibrosis were observed in the Sed-PCK group. The index of glomerular sclerosis was significantly
150 higher in the Sed-PCK group than in the Con-SD group ($P<0.01$), and significantly lower in the Ex-
151 PCK group than in the Sed-PCK group ($P<0.05$) (Figure 3B). The desmin-positive staining area in
152 the glomeruli was significantly larger in the Sed-PCK group than in the Con-SD group ($P<0.01$),
153 and significantly smaller in the Ex-PCK group than in the Sed-PCK group ($P<0.01$) (Figure 3C).
154 The renal interstitial fibrosis area was significantly higher in the Sed-PCK group than in the Con-
155 SD group ($P<0.01$), and smaller in the Ex-PCK group than in the Sed-PCK group (Figure 3D).

156 **Cell proliferation and signaling cascades**

157 Figure 4A shows representative images of the kidney immunostained for Ki-67 from the Sed-
158 PCK and Ex-PCK groups. Ki-67-positive cells were highly expressed in the cyst-lining epithelium,

159 interstitium, and non-cystic tubules of the Sed-PCK group. Chronic exercise led to fewer Ki-67-
160 positive cells. The Ki-67 labeling index was significantly lower in the cyst-lining epithelium and
161 non-cystic tubules in the Ex-PCK group compared with the Sed-PCK group ($P<0.01$ and $P<0.01$,
162 respectively) (Figure 4B and 4C).

163 Urinary AVP excretion was significantly higher in the Sed-PCK group than in the Con-SD group
164 ($P<0.05$), and was considerably higher in the Ex-PCK group than in the Sed-PCK group ($P<0.05$)
165 (Figure 5A). Renal cAMP content was significantly higher in the Sed-PCK group than in the Con-
166 SD group ($P<0.05$), but it was not significantly different between the Sed-PCK and Ex-PCK groups
167 (Figure 5B). Renal B-Raf expression was significantly higher in the Sed-PCK group than in the
168 Con-SD ($P<0.01$), and significantly lower in the Ex-PCK group than in the Sed-PCK group
169 ($P<0.01$) (Figure 5C).

170 Figure 6A and 6B show representative images of kidneys immunostained for phosphorylated
171 (p-) ERK and p-mTOR, respectively, from each group. The p-ERK and p-mTOR proteins were
172 highly expressed in the cyst-lining epithelium and non-cystic tubules in the Sed-PCK group, and
173 chronic exercise decreased their expressions (Figure 6A and 6B). Renal ERK and mTOR
174 phosphorylation were significantly higher in the Sed-PCK group than in the Con-SD group ($P<0.01$

175 and $P < 0.01$, respectively), and S6 phosphorylation also tended to be higher in the Sed-PCK group
176 compared with the Con-SD group (Figure 6C, 6D, and 6E). Renal ERK, mTOR, and S6
177 phosphorylation was significantly lower in the Ex-PCK group than in the Sed-PCK group ($P < 0.01$,
178 $P < 0.01$, and $P < 0.01$, respectively).

179

180 **Discussion**

181 Chronic exercise has renal protective effects in CKD patients and models (10-14); however, the
182 renal protective effects of chronic exercise have not yet been reported in PKD patients or models.
183 The present study revealed that chronic exercise at a moderate intensity slowed the progression of
184 renal cyst growth, glomerular damage, interstitial fibrosis, and renal dysfunction in PCK rats,
185 despite increasing AVP. Chronic exercise also inhibited excessive cell proliferation, with down-
186 regulation of the cAMP/B-Raf/ERK and mTOR/S6 pathways in renal epithelial cells. To the best of
187 our knowledge, the present study is the first to report that chronic exercise has therapeutic potential
188 against cyst growth and renal dysfunction in PKD.

189 We chose the exercise protocol in the present study based on our previous study of CKD model
190 rats with 5/6 nephrectomy (11), in which proteinuria and glomerular sclerosis were significantly

191 attenuated after 12 weeks of chronic exercise. We confirmed that when PCK rats run at a speed of
192 28 m/min on the treadmill, oxygen consumption ($\dot{V}O_2$) corresponds to approximately 65% of the
193 maximal $\dot{V}O_2$, which is assumed to be aerobic exercise at a moderate intensity (18). In contrast to
194 the present results, Darnley et al. reported that treadmill exercise (14 m/min, 30 min/day, 3
195 days/week) for 6 weeks did not lead to any changes in serum urea nitrogen or creatinine in
196 Han:SPRD-cy rats (19). Similarly, in our pilot studies, chronic exercise for 8 weeks did not
197 significantly affect renal cyst growth in PCK rats (data not shown). Thus, the intensity, time,
198 frequency, and duration of the exercise protocol may be important to obtain benefits in PKD models.
199 In agreement with our previous studies (10-13), chronic exercise lowered proteinuria and plasma
200 creatinine and attenuated glomerular sclerosis and podocyte injury in PCK rats. Chronic exercise
201 for 8 weeks significantly decreased urinary protein excretion (Figure 1E) without significant effects
202 on renal cyst growth in PCK rats (data not shown). Therefore, glomerular protection may be a
203 primary effect of chronic exercise, rather than being secondary to slowing renal cyst growth. Urinary
204 L-FABP excretion, a biomarker of proximal tubular stress and tubulointerstitial disorder, increases
205 linearly with age and reflects the progression of tubulointerstitial disorder in PCK rats (29). Chronic
206 exercise might therefore strongly attenuate proximal tubular stress and tubulointerstitial disorder in

207 PCK rats. As an indicator of cell proliferation, chronic exercise decreased the number of Ki-67-
208 positive cells in the kidneys of PCK rats, indicating the inhibition of excessive cell proliferation. As
209 well as in the kidney, chronic exercise has also been recently reported to slow the progression of
210 cyst growth and fibrosis in the liver of PCK rats (18).

211 The present study indicates that chronic exercise increases AVP in PCK rats. In agreement with
212 these results, AVP synthesis and secretion have been previously reported to increase during exercise
213 (30). Sustained moderate exercise (at an intensity threshold of 40%–65% of $\dot{V}O_{2max}$) increased
214 plasma AVP (31, 32). Furthermore, chronic exercise with a treadmill for 5 weeks increased plasma
215 AVP in Wistar rats (33). The present study also indicates that chronic exercise did not change renal
216 cAMP content and did decrease the cAMP-inducible B-Raf expression in PCK rats, despite
217 increasing AVP, suggesting that chronic exercise might inactivate adenylate cyclase via the
218 inhibitory G protein (Gi). Previous studies indicate that norepinephrine and $\alpha 2$ -adrenergic receptor
219 ($\alpha 2$ -AR) agonists inhibit the AVP-activated adenylate cyclase, cAMP content, and water transport
220 in the collecting ducts (34-36). Therefore, it is possible that chronic exercise might stimulate renal
221 sympathetic activity and activate $\alpha 2$ -AR in the collecting ducts to slow the progression of renal cyst
222 growth with reducing the renal cAMP content in PCK rats. In this regard, our preliminary study

223 indicates that chronic treatment of the α 2-AR agonist, clonidine slows the progression of renal cyst
224 growth in PCK rats (data not shown).

225 Previous studies indicate that even normal plasma AVP levels increase B-Raf expression and
226 ERK phosphorylation in the kidneys of PCK rats, and that inhibition of AVP by V2R antagonists
227 and hydration can down-regulate the B-Raf/ERK pathway (24, 37). The present study indicates that
228 chronic exercise down-regulates not only the B-Raf/ERK pathway but also the mTOR/S6 pathway
229 in the kidneys of PCK rats. Both mTOR and ERK are involved in excessive cell proliferation and
230 cyst growth in the renal tubules and cholangiocytes of PCK rats (20). However, neither tolvaptan
231 nor an ERK inhibitor, AEZ-131, affected S6 phosphorylation in the kidney of PCK rats, and the
232 suppressive effects of tolvaptan and an mTOR inhibitor, rapamycin, on renal cyst growth were
233 additive (38). The suppressive effects of chronic exercise on excessive cell proliferation and renal
234 cyst growth in the present study might therefore be mediated by down-regulation of both the B-
235 Raf/ERK and mTOR/S6 pathways in the kidneys of PCK rats. Several types of exercise affect
236 mTOR and ERK in the skeletal muscle, fat, liver and vasculature (39-41). However, the effects of
237 exercise on mTOR or ERK have not previously been reported in the kidney, especially in the renal
238 tubules. We recently reported that chronic exercise down-regulates mTOR and ERK

239 phosphorylation in the liver and cholangiocytes in PCK rats (18). In agreement with the results from
240 PCK rats, chronic exercise with a treadmill inactivated mTOR and suppressed excessive cell
241 proliferation in hepatocellular carcinoma in PTEN-deficient mice (42) and carcinoma-implanted
242 rats (43).

243 The present study also demonstrates that chronic exercise increases plasma irisin in PCK rats.
244 Irisin mediates the beneficial effects of exercise, such as by promoting the brown adipose formation
245 and improving the metabolism, and also has a beneficial role in kidney and heart diseases (15, 44-
246 46). In one study, plasma irisin levels were significantly decreased in CKD patients and were
247 inversely correlated with blood urea nitrogen and creatinine levels (47). In another study, skeletal
248 muscle-specific PGC-1 α overexpression increased irisin production and plasma irisin levels and
249 attenuated renal damage in mice with folic acid nephropathy, unilateral ureteral obstruction, and 5/6
250 nephrectomy (15). Moreover, recombinant irisin administration attenuated renal damage in the
251 mouse kidney disease models (15). Although it is unknown whether irisin can inhibit cyst growth,
252 irisin has been reported to inhibit mTOR, ERK, and cell proliferation in cultured cardiomyocytes,
253 cardiomyoblasts, and pancreatic cancer cells (48, 49). Additionally, irisin increased intracellular

254 Ca^{2+} in cultured cardiomyoblasts and endothelial cells (50). Future study is necessary to examine
255 whether irisin directly acts on renal epithelial cells and inhibits cyst growth in PCK rats.

256 In conclusion, chronic exercise slows the progression of PKD pathologies, such as renal
257 dysfunction, renal cyst growth, glomerular damage, and renal interstitial fibrosis in PCK rats.
258 Despite increasing AVP, chronic exercise also inhibits excessive cell proliferation, with down-
259 regulation of the cAMP/B-Raf/ERK and mTOR/S6 pathways in the kidney of PCK rats. Although
260 the results of the present study may not be directly applicable to humans, chronic exercise may be
261 a novel therapeutic approach against cyst growth and renal dysfunction in PKD patients.

262

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271 Medicine.

272 **Conflict of Interest**

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

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Figure legends

Figure 1. Effects of chronic exercise on general parameters and urinary parameters in PCK rats. Time courses of (A) body weight, (B) food intake, (C) water intake, (D) urine volume, (E) urinary protein excretion, and (F) urinary L-FABP excretion 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 the 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 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. (B) Total kidney weight, (C) kidney-to-body weight ratio, and (D) cystic index 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 the mean \pm SEM. ** $P<0.01$ compared with the Con-SD group; # $P<0.05$ compared with the Sed-PCK group; ns: no significant difference.

Figure 3. Effects of chronic exercise on glomerular sclerosis, podocyte injury, and renal interstitial fibrosis in PCK rats. (A) Representative images of periodic acid–Schiff (PAS)-stained, desmin-immunostained glomeruli and Masson’s trichrome stained kidneys in the Con-SD, Sed-PCK, and Ex-PCK groups. (B) Index of glomerular sclerosis, (C) desmin-positive staining area (%), and (D) interstitial fibrosis area (%) 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 the 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 non-cystic tubules of the Sed-PCK (closed dots) and

Ex-PCK (round dots) groups ($n=10$ in each group). Data are presented as the mean \pm SEM. $##P<0.01$ compared with the Sed-PCK group.

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. (B) Renal cAMP content in the Con-SD (rectangle dots), Sed-PCK (closed dots), and Ex-PCK (round dots) groups ($n=10$ 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 the 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.

Figure 6. Effects of chronic exercise on the phosphorylation of ERK, mTOR, and

S6 in PCK rats. Representative images of kidney specimens immunostained for (A) p-

ERK and (B) p-mTOR in the Con-SD, Sed-PCK, and Ex-PCK groups. Western blotting

analysis of (C) p-ERK, (D) p-mTOR, and (E) p-S6 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. 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 the 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.

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Table 1. Blood pressure and plasma parameters

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

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

Data are presented as means ± SEM. *n*=10 in each group. **P*<0.05 compared with the Con-SD group; #*P*<0.05, ##*P*<0.01 compared with the Sed-PCK group.

Figure 1

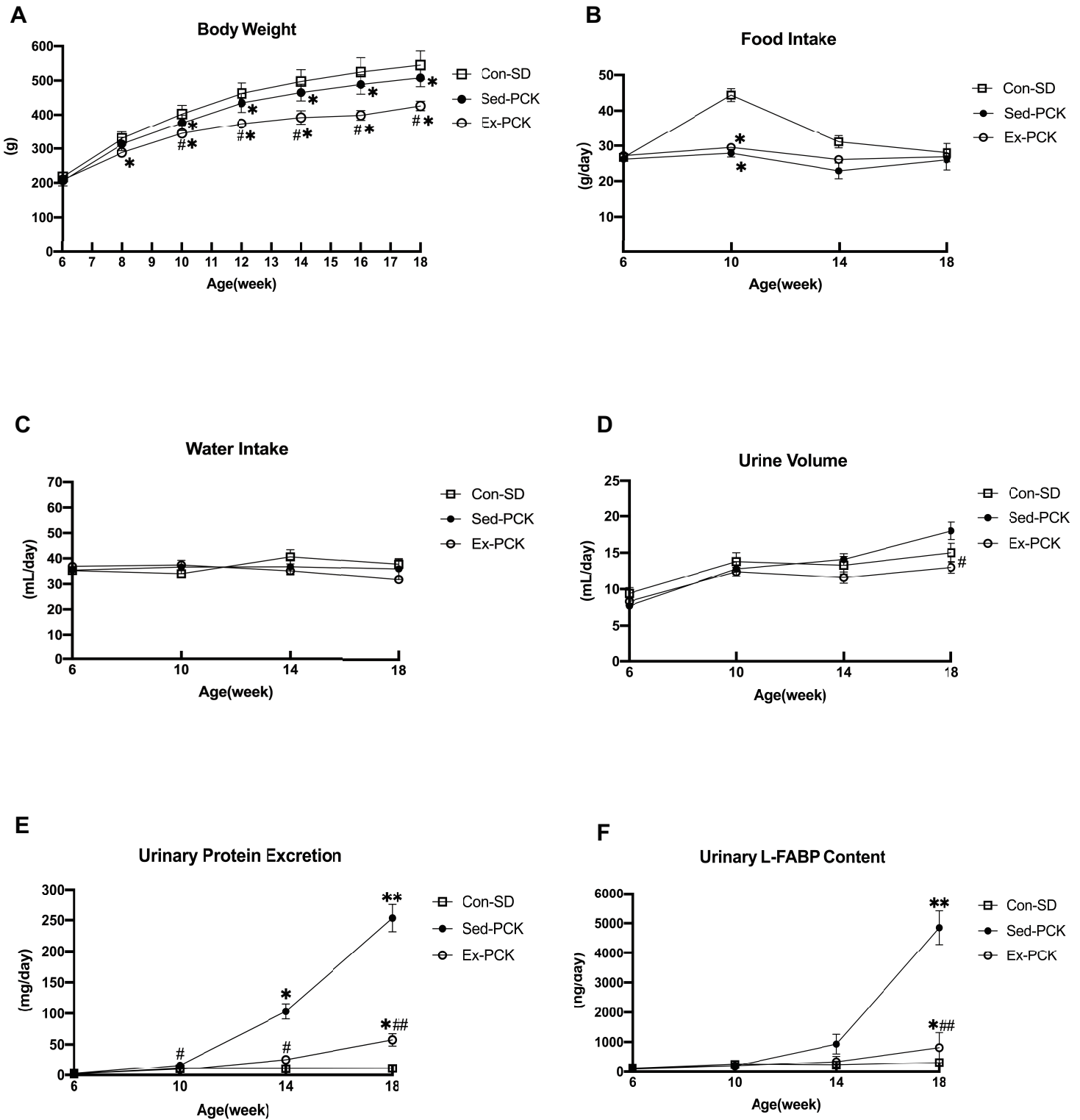
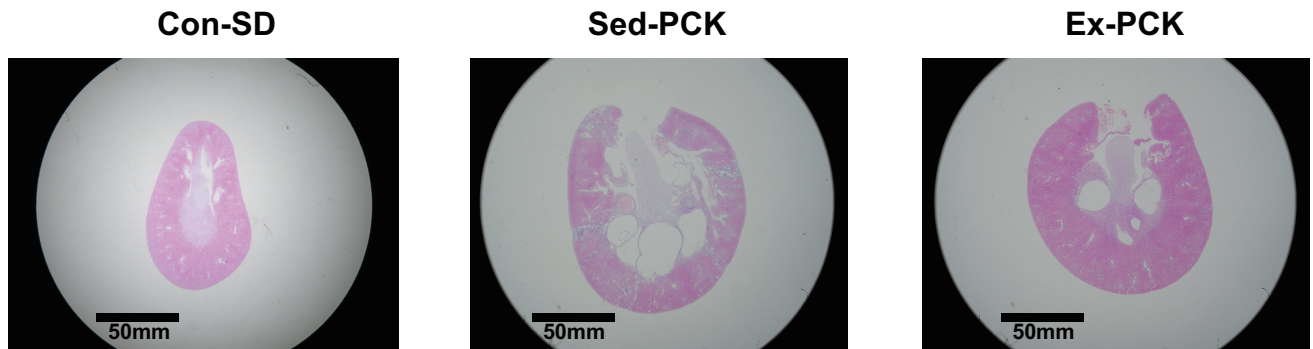
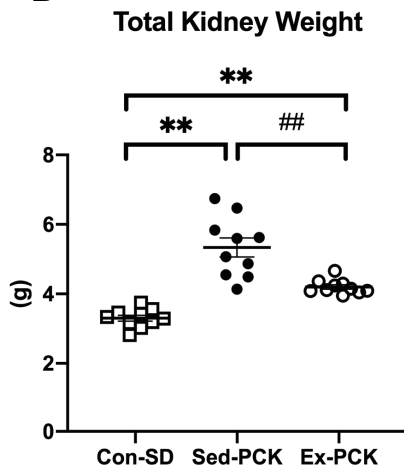


Figure 2

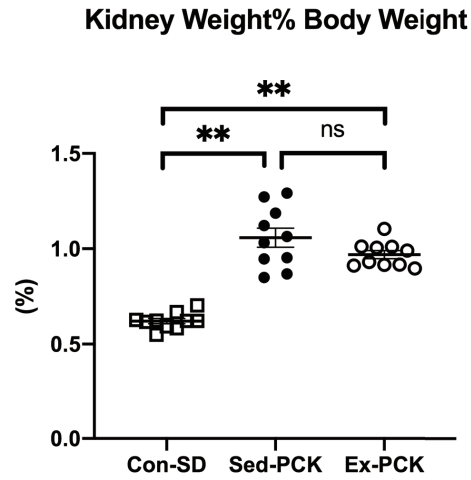
A



B



C



D

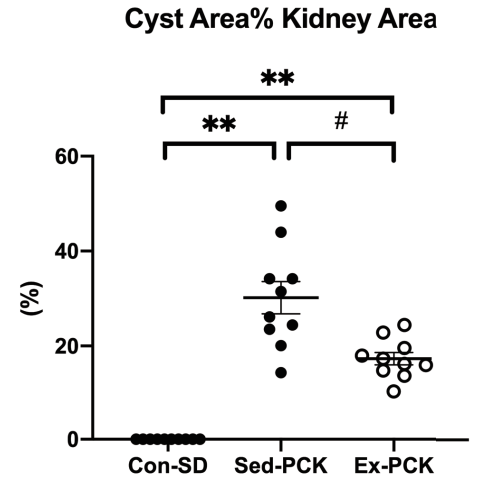


Figure 3

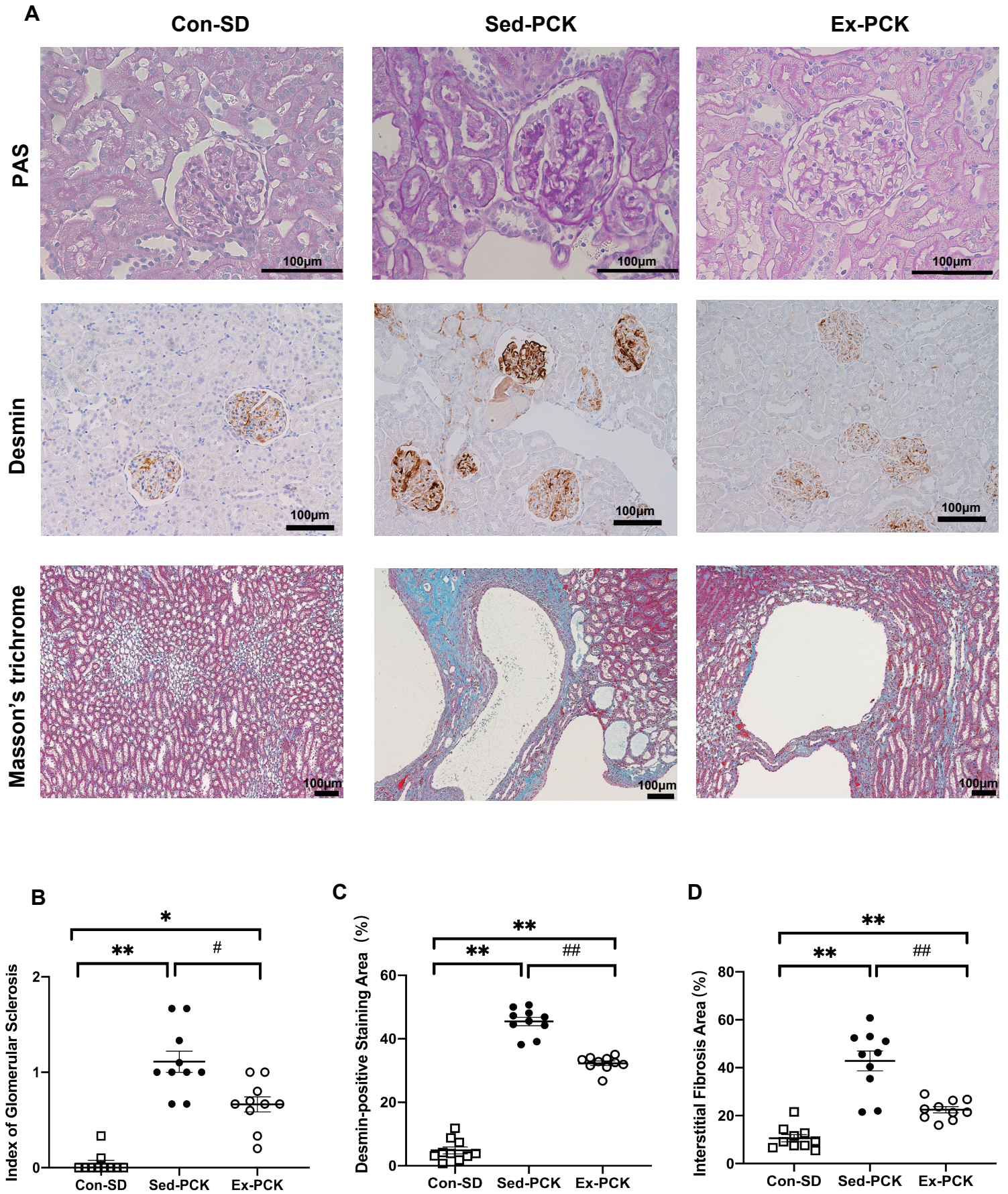
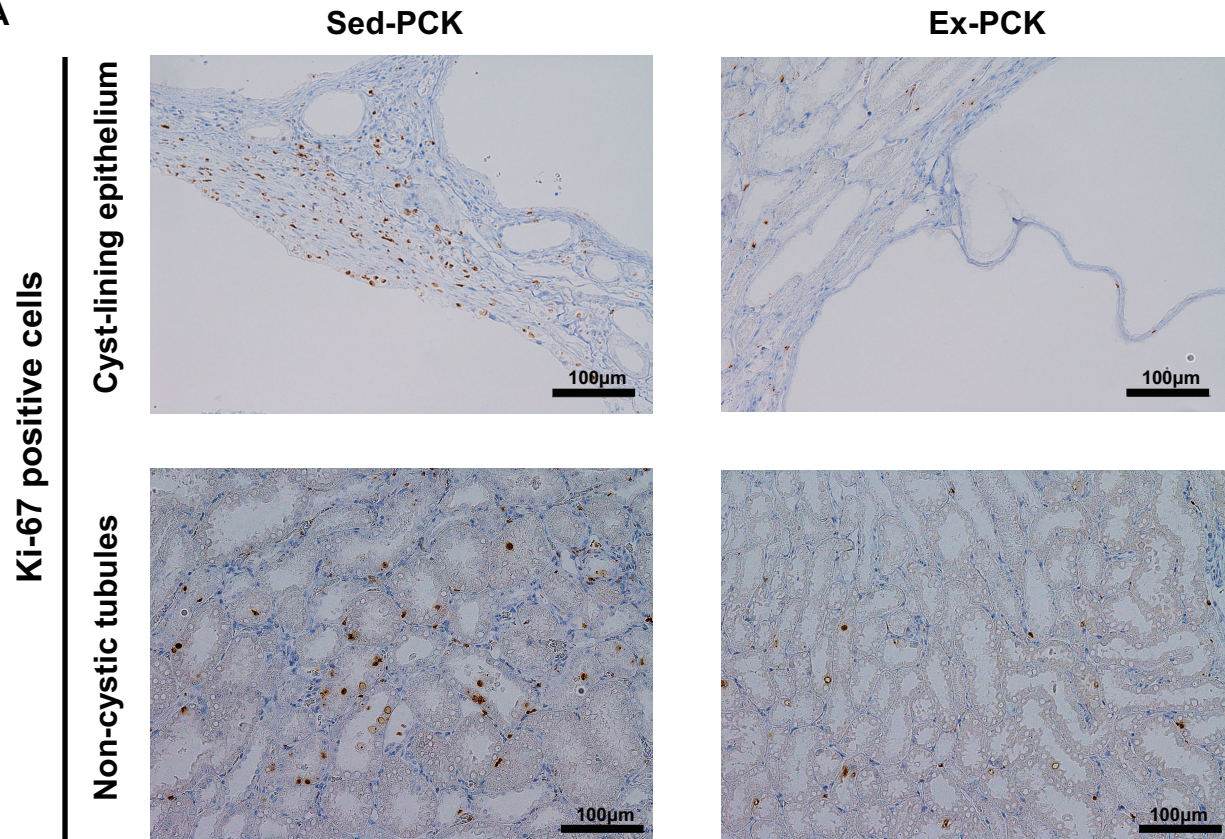
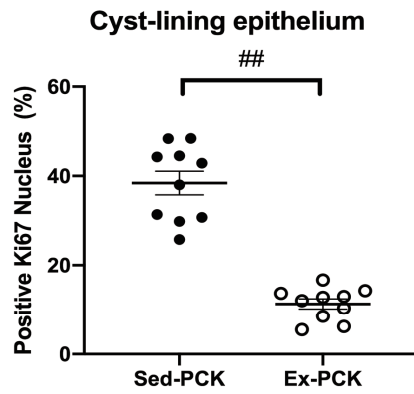


Figure 4

A



B



C

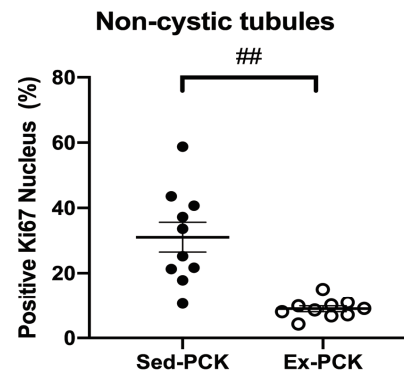
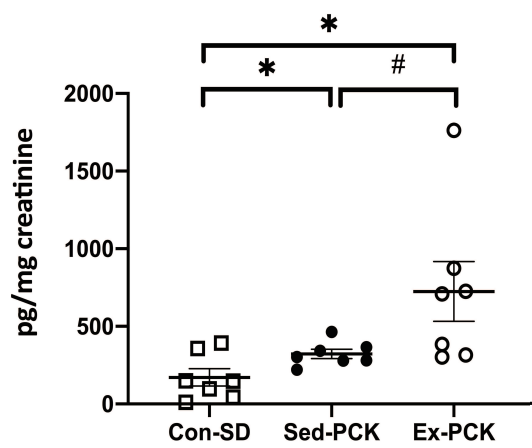


Figure 5

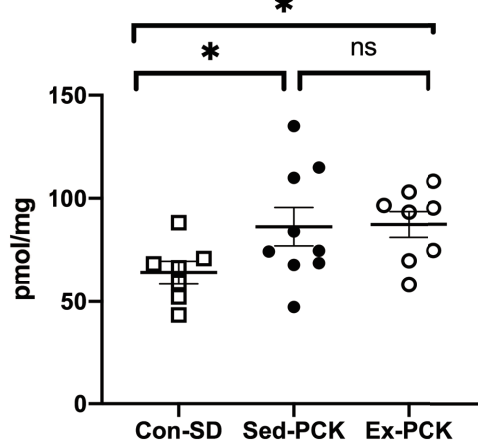
A

Urinary AVP excretion



B

Renal cAMP content



C

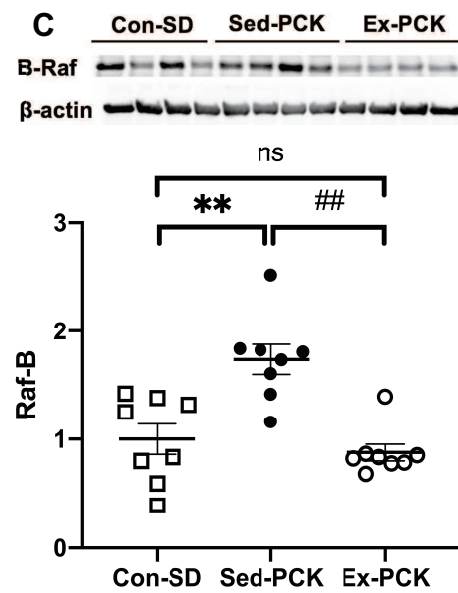


Figure 6

