Increased Water Intake Decreases Progression of Polycystic Kidney Disease in the PCK Rat

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Renal enlargement in polycystic kidney disease (PKD) is caused by the proliferation of mural epithelial cells and transepithelial fluid secretion into the cavities of innumerable cysts. Arginine vasopressin (AVP) stimulates the proliferation of human PKD cells in vitro via cAMP-dependent activation of the B-Raf/MEK (MAPK/ERK kinase/extracellular signal–regulated kinase (ERK) pathway. ERK activity is elevated in cells that line the cysts in animals with PKD, and AVP receptor antagonists reduce ERK activity and halt disease progression. For suppression of the effect of AVP physiologically, water intake was increased in PCK rats, a model of PKD, and the effect on renal morphology, cellular mechanism, and function was determined. The addition of 5% glucose in the drinking water increased fluid intake approximately 3.5-fold compared with rats that received tap water. In PCK rats, increased water intake for 10 wk reduced urinary AVP excretion (68.3%), and urine osmolality fell below 290 mOsmol/kg. High water intake was associated with reduced renal expression of AVP V2 receptors (41.0%), B-Raf (15.4%), phosphorylated ERK (38.1%), and proliferating cell nuclear antigen–positive renal cells (61.7%). High water intake reduced the kidney/body weight ratio 28.0% and improved renal function. Taken together, these data demonstrate that water intake that is sufficient to cause persistent water diuresis suppresses B-Raf/MEK/ERK activity and decreases cyst and renal volumes in PCK rats. It is suggested that limiting serum AVP levels by increased water intake may be beneficial to some patients with PKD.

Mutilations in the polycystic kidney disease (PKD) genes (PKD1, PKD2, and PKHD1) (1–4) disrupt intracellular Ca\(^{2+}\) regulation and, thereby, transform renal epithelial cells into hyperplastic fluid-filled cysts. Epithelial cells that are isolated from cysts of human autosomal dominant (ADPKD) and autosomal recessive (ARPKD) kidneys have a lower basal intracellular Ca\(^{2+}\) level compared with cells that are derived from normal renal tissue (5). cAMP accelerates the proliferation of ADPKD and ARPKD cells but has no mitogenic effect on normal renal tubule cells (6–8). The proliferative effect of cAMP in PKD cells is mediated by protein kinase A (PKA) stimulation of the MAPK/ERK kinase (MEK)/extracellular signal–regulated kinase (ERK) pathway through the intermediacy of B-Raf, a kinase that phosphorylates and activates MEK. By contrast, B-Raf is normally repressed in normal human kidney cells, and cAMP inhibits ERK activity and cell proliferation (9). Sustained reduction of intracellular Ca\(^{2+}\) predisposes normal renal cells to cAMP-dependent stimulation of the B-Raf/MEK/ERK pathway and cell proliferation (6). Conversely, restoration of intracellular Ca\(^{2+}\) concentration in cystic cells that are derived from human ADPKD and ARPKD kidneys rescues the normal antimitogenic response of cAMP (5).

Arginine vasopressin (AVP) is an important antidiuretic hormone that mediates its effect through the activation of vasopressin V2 receptors (AVPV2R) and the subsequent stimulation of adenyllyl cyclase and synthesis of cAMP (10). Normally, urine is concentrated to an osmolality that is greater than plasma. Day-to-day maintenance of urine output depends on appropriate plasma AVP levels to regulate osmotic water reabsorption by distal tubules and collecting ducts. Relatively normal plasma levels of AVP may be sufficient to stimulate cyst epithelial cell growth and renal enlargement in patients with PKD. Some patients have an intrinsic defect in the capacity to concentrate urine maximally, potentially leading to even greater levels of plasma AVP than normal (11–13).

In this study, we determined whether suppression of plasma AVP levels by increased water intake would be sufficient to slow PKD progression in PCK rats. In this model of PKD, orthologous to human ARPKD, mutations in Pkhd1 cause cysts to form in the collecting ducts (14). Many features of the disease in PCK rats resemble human ADPKD, such as focal development of cysts, although the pattern of inheritance is autosomal recessive. The results demonstrate that increased water intake...
that was sufficient to suppress the renal effects of AVP decreased MEK/ERK activity in the kidneys and slowed the progression of cystic disease in PCK rats.

Materials and Methods

PCK Rat Model

PCK rats that originally were derived from a strain of the Sprague-Dawley rats are maintained at the Education and Research Center of Animal Models for Human Diseases of Fujita Health University. These animals have been characterized previously (15). PCK rats and normal Sprague-Dawley (+/+; Charles River Japan Inc., Kanagawa, Japan) were allowed free access to water and food throughout the study. PCK and +/+ rats were randomly assigned to either the control group (normal tap water) or high water intake (HWI) group (water that contained 5% glucose) and treated as such from 4 to 14 wk of age. Twenty-four-hour samples were collected in metabolic cages after 13.5 wk to determine urine volume, water intake volume, and food consumption. At 14 wk of age, rats were anesthetized with pentobarbital sodium (Schering-Plough Corp., Kenilworth, NJ), and both kidneys were removed rapidly, causing exsanguination. Total kidney weight was measured, and then the left kidney was homogenized in lysis buffer to extract proteins and the right kidney was sectioned and immersed in 4% paraformaldehyde.

Western Blot Analysis

Kidney lysate samples were prepared for immunoblot analysis (16,17). Contents of lysis buffer (TLB buffer) were 20 mmol/L Tris (pH 7.4), 137 mmol/L NaCl, 25 mmol/L β-glycerophosphate, 2 mmol/L EDTA, 1 mmol/L sodium orthovanadate, 2 mmol/L dithiothreitol, 1 mmol/L PMSF, 5 μg/ml aprotinin, and 5 μg/ml leupeptin with 1% Triton X-100. Membrane-blotted lysates (20 μg protein/lane) were separated by 10% SDS-PAGE and transferred to nitrocellulose membrane. Membranes were blocked with 5% milk in TBS-T (20 mmol/L Tris-HCl [pH 8.0], 137 mmol/L NaCl, and 0.05% Tween 20) for 1 h at room temperature and then incubated overnight at 4°C in primary antibody diluted 1:2000 to 1:5000 in 5% milk in TBS-T. The membranes were washed three times with TBS-T and incubated with secondary antibody conjugated to horseradish peroxidase diluted 1:2000 to 1:5000 in 5% milk in TBS-T for 1 h at room temperature. The membranes were washed three times with TBS-T, and specific antibody signals were detected using an enhanced chemiluminescence system (ECL or ECL Advance Western Blotting Detection System; Amersham Life Sciences, Arlington Heights, IL). Images of the blots were captured, and the intensity of the protein bands was quantified using a CS Analyzer 2.0 with a CCD camera (ATTO Corp., Tokyo, Japan). Relative band intensity was compared with gender-matched +/+ kidneys from rats that received tap water (set to 1.0).

Immunohistochemistry

Sections that were obtained from paraffin blocks were heated to 100°C for 15 min in 10 mM sodium citrate buffer (pH 6.0) to unmask antigens. Endogenous peroxidase activity was destroyed by incubating sections in 0.3% H2O2/methanol for 30 min, and then the sections were incubated with primary antibody (1:3000) for phosphorylated ERK (P-ERK) or proliferating cell nuclear antigen (PCNA) overnight at 4°C. Sections were rinsed with PBS, incubated with biotinylated anti-mouse secondary antibody, and then incubated with streptavidin conjugated to peroxidase (Histofine; Nichirei Biosciences, Tokyo, Japan). Immunoreaction products were developed using 3,3′-diaminobenzidine. Cystic surface area was measured from 10 random fields (×100 magnification) of hematoxylin-eosin–stained sections of renal cortex (n = 3 PCK rats). Cyst area (μ²/field) was measured by a naive observer using LUZEX-FS software (Kideko Co. Ltd., Tokyo, Japan). ApopTag Peroxidase In Situ Apoptosis Detection Kit (Chemicon International, Temecula, CA) was used to detect apoptotic cells. Apoptotic cells and P-ERK or PCNA-positive nuclei were counted from 500 to 600 cells (×400 magnification) per three thin sections from either PCK or +/+ rat kidneys.

Antibodies

Primary antibodies to B-Raf (F-7, SC-5284), ERK1,2 (K-23, SC-94), P-ERK (E-4, SC-7383), and Raf-1 (C-12, SC-133) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA) for immunoblot analysis.

Table 1. Effect of HWI on urine volume and osmolality and urinary AVP

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Treatment</th>
<th>n</th>
<th>Water Intake (mL)</th>
<th>Urine Volume (mL)</th>
<th>Urine Osmolarity (mOsmol/kg H2O)</th>
<th>Urinary AVP (pg/mg creatinine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>PCK</td>
<td>8</td>
<td>18 ± 1</td>
<td>18 ± 2</td>
<td>1088 ± 96</td>
<td>257 ± 22</td>
</tr>
<tr>
<td></td>
<td>PCK</td>
<td>10</td>
<td>68 ± 5b</td>
<td>58 ± 5b</td>
<td>232 ± 47b</td>
<td>114 ± 46b</td>
</tr>
<tr>
<td></td>
<td>+/+</td>
<td>8</td>
<td>10 ± 5</td>
<td>9 ± 1</td>
<td>1498 ± 130</td>
<td>160 ± 19</td>
</tr>
<tr>
<td></td>
<td>+/+</td>
<td>8</td>
<td>94 ± 26c</td>
<td>91 ± 10b</td>
<td>197 ± 73b</td>
<td>88 ± 38</td>
</tr>
<tr>
<td>Female</td>
<td>PCK</td>
<td>10</td>
<td>18 ± 3</td>
<td>16 ± 2</td>
<td>982 ± 74</td>
<td>435 ± 83</td>
</tr>
<tr>
<td></td>
<td>PCK</td>
<td>8</td>
<td>63 ± 9b</td>
<td>49 ± 8b</td>
<td>284 ± 52b</td>
<td>111 ± 59b</td>
</tr>
<tr>
<td></td>
<td>+/+</td>
<td>7</td>
<td>13 ± 5</td>
<td>12 ± 1</td>
<td>1559 ± 211</td>
<td>335 ± 49</td>
</tr>
<tr>
<td></td>
<td>+/+</td>
<td>10</td>
<td>107 ± 10b</td>
<td>80 ± 11b</td>
<td>131 ± 25b</td>
<td>31 ± 4b</td>
</tr>
</tbody>
</table>

Addition of 5% glucose to drinking water increased water intake 3.5-fold in PCK rats and 8.2-fold in normal Sprague-Dawley (+/+ rats) (composites of male and female rats). Rats drink more water that contained glucose because they have a preference for sweet fluid (33). Urine volume increased accordingly. High water intake (HWI) decreased urine osmolalities below 290 mOsmol/kg H2O. Urinary arginine vasopressin (AVP), an indicator of plasma AVP levels, decreased significantly in both PCK and +/+ rats with HWI. Statistical differences between HWI and control (CONT) rats (either +/+ or PCK) were determined by one-way ANOVA.

*p < 0.01.

**p < 0.05.
Secondary antibodies conjugated to horseradish peroxidase were goat anti-rabbit IgG (SC-2054) and rabbit anti-mouse IgG (SC-2055) from Santa Cruz Biotechnology. Primary antibodies to AVPV2R (AB1797P; Chemicon International), PCNA (P8825; Sigma Chemical, St. Louis, MO) and P-ERK (M5670; Sigma) were used for immunohistochemistry. Secondary antibody, conjugated to biotin, for immunohistochemistry studies was rabbit anti-mouse IgG (HISTOFINE 426032) obtained from Nichirei (Tokyo, Japan).

Measurement of Urine AVP, Creatinine and Osmolality, and Serum Urea Nitrogen

Urine AVP levels were measured with a Correlate-EIA™ arg8–Vasopressin Enzyme Immunoassay Kit (cat. no. 900-017; Assay Designs, Inc., Ann Arbor, MI). Urine creatinine measurements were determined by a colorimetric microplate assay kit (Kit CR01; Oxford Biomedical Research, Inc., Oxford, MI). For an AVP concentration below the level of detection (<3.39 pg/ml), a value of 3.38 pg/ml was used for the calculation of AVP per creatinine concentration (pg

Figure 1. Effect of high water intake (HWI) on kidney weight of PCK and normal rats. (A) The addition of 5% glucose to the drinking water did not affect body weight of either the PCK or normal Sprague-Dawley (+/+) rats. (B) HWI caused a significant decrease in total kidney weight, represented as percentage of body weight in both male and female PCK rats. By contrast, HWI had no effect on total kidney weight of +/+ rats. Statistical differences between HWI and control (CONT) rats (either +/+ or PCK) were determined by one-way ANOVA; **P < 0.01.

Figure 2. Effect of HWI on serum urea nitrogen (SUN) in PCK and normal rats. Male PCK rats at 14 wk of age had an elevated SUN compared with age-matched +/+ rats. HWI decreased SUN to control levels in PCK rats. The small but significant decrease in SUN in +/+ rats with HWI is consistent with increased urea excretion as a result of elevated fluid flow in the distal nephron. Statistical differences between HWI and control (CONT) rats (either +/+ or PCK) were determined by one-way ANOVA; *P < 0.05, **P < 0.01.

Figure 3. Effect of HWI on renal cyst development in male and female PCK rats. Micrographs of representative kidney sections, stained with hematoxylin-eosin, were taken at the same magnification from a PCK male rat with normal water intake (A), a PCK male rat with HWI (B), a PCK female rat with normal water intake (C), and a PCK female rat with HWI (D). (E) Surface area of cysts (mean ± SE) from representative sections of male and female PCK kidneys was measured by morphometric analysis. Increased water intake decreased cyst area 60 and 50% in male and female PCK kidneys, respectively. Comparison between HWI and CONT rats (male or female), **P < 0.01.
Table 2. Effect of HWI on male Han SPRD (Cy/+) rats

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Treatment</th>
<th>n</th>
<th>Body Weight (g)</th>
<th>Total Kidney Weight (g)</th>
<th>Kidney Weight/Body Weight (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cy/+</td>
<td>CONT</td>
<td>10</td>
<td>337 ± 17</td>
<td>7.15 ± 0.27b</td>
<td>2.17 ± 0.16b</td>
</tr>
<tr>
<td>Cy/+</td>
<td>HWI</td>
<td>11</td>
<td>327 ± 7</td>
<td>7.53 ± 0.40</td>
<td>2.31 ± 0.13b</td>
</tr>
<tr>
<td>+/-</td>
<td>CONT</td>
<td>5</td>
<td>370 ± 11</td>
<td>2.88 ± 0.05</td>
<td>0.78 ± 0.03</td>
</tr>
<tr>
<td>+/-</td>
<td>HWI</td>
<td>4</td>
<td>339 ± 26</td>
<td>2.44 ± 0.20</td>
<td>0.72 ± 0.03</td>
</tr>
</tbody>
</table>

*Total kidney weight and kidney weight/body weight were increased in Cy/+ rats compared with normal Sprague-Dawley rats. Cy/+ male rats that were offered water that contained 5% sucrose increased fluid intake from 64 ± 5 ml (n = 6) to 155 ± 8 ml (n = 15). There was no effect of HWI on body weight, total kidney weight, or kidney weight/body weight.
*Comparison between Cy/+ and control (+/-) rats, P < 0.001, determined by unpaired t test. The lack of an effect of HWI on kidney weight in Cy/+ suggests that vasopressin may not contribute significantly to the progression of the proximal tubule cysts in kidneys of Cy/+ rats.

AVP/mg creatinine). Osmolality was determined with a freezing-point osmometer (Osmostat OM-6040; Kyoto Dioichi Kagaku, Kyoto, Japan). Serum urea nitrogen (SUN) determinations were performed using a colorimetric assay kit using a Urease-Indophenol method (Wako Pure Chemical Industries, Ltd., Osaka, Japan).

Statistical Analyses

Data represent mean and SE. Statistical significance was determined by one-way ANOVAs and Student-Newman-Keuls post test for multiple comparisons or unpaired t test for comparison between control and treated animals.

Results

In PCK rats and normal Sprague-Dawley (+/-) rats that were offered water that contained 5% glucose, fluid intake increased approximately 3.5- and 8.2-fold, respectively, in comparison with rats that were allowed free access to tap water (Table 1). The rate of urine AVP excretion, an indicator of plasma AVP levels (18), was decreased 68.3%. Suppression of the renal effects of AVP decreases intracellular cAMP levels and reduces the water permeability of collecting ducts. We found that HWI increased urine volume and decreased urine osmolality to <290 mOsmol/kg H2O in both normal and cystic animals.

Body weight was unaffected by water intake in either the +/- or the PCK rats (Figure 1A); however, kidney weight (body weight) decreased 29.8 and 27.0% in PCK male and female rats, respectively (Figure 1B). In normal rats, changes in body weight had no effect on kidney weight. A slight elevation in SUN has been reported in PCK rats at 70 and 128 d of age (14). In this study, we found that the PCK male rats had a significant elevation in SUN at 14 wk (98 d) of age (Figure 2); in contrast, PCK female rats had normal SUN at this age (data not shown). Increased water intake for 10 wk decreased SUN from 38.7 to 26.3 mg/dl in the PCK male rats, a level that was similar to that of normal rats that drank increased water. HWI also caused a small but significant decrease in SUN in +/- rats. Elevation in tubule fluid flow increases both facilitated and active urea secretion by inner medullary collecting ducts (19), thereby removing urea from the medullary interstitium and ultimately decreasing plasma urea levels. Hence, the reduction in SUN in the PCK rats with HWI was possibly due to a combination of increased urine flow as well as reduced renal disease (Figure 1B).

Representative histologic sections revealed that HWI treatment diminished cystic area 54% in kidneys of PCK rats (59% in male and 49% in female rats; Figure 3), compared with rats that drank tap water. Therefore, increased fluid intake that was sufficient to cause water diuresis and reduce urinary excretion of AVP, ostensibly as a result of reduced plasma AVP levels, slowed the progression of cystic disease in PCK rats. By contrast, in Hans SPRD (Cy/+) rats, which develop cysts in the proximal tubules (a segment that lacks AVPVR2R), HWI caused polyuria and decreased urine osmolality but failed to reduce the severity of cystic disease (Table 2). Therefore, polyuria and decreased urine osmolality per se do not account for the striking reduction in kidney size that is caused by HWI.

Previously, Gattone et al. (20) reported overexpression of mRNA for AVPVR2R in kidneys of PCK rats and C57BL/6j-cp/k/
Table 3. Effects of HWI on AVPV2 expression and B-Raf/MEK/ERK activity in PCK and +/+ kidneys

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Treatment</th>
<th>AVPV2R</th>
<th>B-Raf</th>
<th>P-ERK</th>
<th>ERK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCK</td>
<td>CONT</td>
<td>1.48 ± 0.14&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.47 ± 0.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.70 ± 0.06&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.13 ± 0.03</td>
</tr>
<tr>
<td>PCK</td>
<td>HWI</td>
<td>0.79 ± 0.09</td>
<td>1.18 ± 0.1&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.80 ± 0.42&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.07 ± 0.04</td>
</tr>
<tr>
<td>+/+</td>
<td>CONT</td>
<td>1.00 ± 0.00</td>
<td>1.00 ± 0.0</td>
<td>1.00 ± 0.00</td>
<td>1.00 ± 0.00</td>
</tr>
<tr>
<td>+/+</td>
<td>HWI</td>
<td>0.65 ± 0.07&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.00 ± 0.0</td>
<td>1.14 ± 0.11</td>
<td>1.09 ± 0.03</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCK</td>
<td>CONT</td>
<td>2.11 ± 0.25</td>
<td>1.44 ± 0.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.47 ± 0.53&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.99 ± 0.07</td>
</tr>
<tr>
<td>PCK</td>
<td>HWI</td>
<td>1.33 ± 0.04&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.28 ± 0.1</td>
<td>2.65 ± 0.17&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.99 ± 0.05</td>
</tr>
<tr>
<td>+/+</td>
<td>CONT</td>
<td>1.00 ± 0.00</td>
<td>1.00 ± 0.0</td>
<td>1.20 ± 0.05</td>
<td>1.00 ± 0.00</td>
</tr>
<tr>
<td>+/+</td>
<td>HWI</td>
<td>0.90 ± 0.20</td>
<td>1.01 ± 0.1</td>
<td>0.94 ± 0.01</td>
<td>0.99 ± 0.03</td>
</tr>
</tbody>
</table>

<sup>a</sup>Summary of the fold changes in renal expression of AVPV2R, 95-kD isoform of B-Raf, P-ERK, and total ERK in PCK and +/+ rats (male and female) treated with normal water intake (CONT) or HWI. These data were obtained from animals listed in Table 1. MEK, MAPK/ERK kinase; AVPV2R, vasopressin V2 receptor; P-ERK, phosphorylated extracellular signal-regulated kinase.

<sup>b</sup>Comparisons between PCK and +/+ rats, <sup>c</sup>P < 0.05, <sup>d</sup>P < 0.01; comparison between HWI and control (CONT) rats (either +/+ or PCK), <sup>e</sup>P < 0.01, <sup>f</sup>P < 0.05.
basis for the cAMP-mitogenic phenotype in PKD seems to be
due to dysregulation of intracellular Ca²⁺ secondary to mutations
in the PKD genes (5,6). ARPKD and ADPKD cyst-derived
cells have [Ca²⁺], that is lower than tubule cells derived from
noncystic regions of ADPKD kidneys or normal human kid-
neys. Therefore, cyst-lining cells are associated with lower basal
[Ca²⁺], and cAMP-dependent activation of B-Raf/MEK/ERK
and cell proliferation. The cellular pathway for Ca²⁺ regulation
of the cAMP-mitogenic phenotype remains to be established,
but the phosphatidylinositol 3-kinase/Akt pathway seems to
be involved (5,6). Basal Akt activity was found to be reduced in
ADPKD cells compared with normal human kidney cells, and
agents that increased [Ca²⁺] also stimulated Akt and blocked
B-Raf/MEK/ERK pathway.

Normal plasma AVP levels which respond to fluctuations in
extracellular fluid osmolality are likely to maintain renal intracel-
ular cAMP at levels that activate the MEK/ERK pathway
and proliferation of PKD cystic epithelial cells, promoting cyst

Figure 5. Effect of HWI on cell proliferation in PCK and +/+ kidney.

Figure 6. Effect of HWI on P-ERK–positive cells in PCK and +/+ kidneys.

Figure 7. Effect of HWI on cellular apoptosis in PCK and +/+ kidneys.
and kidney enlargement. Several rodent models of PKD, including the PCK rat, have been reported to have elevated renal cAMP levels (12,21).Gattone and Torres (reviewed by Torres [13]) found that novel AVP2R antagonists OPC-31261 and OPC-41061 (Tolvaptan; Otsuka Pharmaceutical, Tokyo, Japan) reduced renal cAMP levels and halted PKD progression in four different animal models, including PKD2<sup>SWSS/-</sup> mice, as well as PCK rats (13,20,28). Treatment with OPC-41061 caused a concomitant reduction in renal levels of B-Raf and P-ERK (22). On the basis of these studies, the capacity for OPC-41061 to reduce PKD progression is being examined in clinical trials (30).

As with other models of PKD, PCK rats are characterized by tubular epithelial cell hyperplasia and increased rates of apoptosis, hallmarks of human PKD (31). We used immunohistochemistry to evaluate the effect of HWI on the proliferation and apoptosis of cyst-lining cells in PCK rat kidneys. The number of cells that stained positive for PCNA, a mitotic indicator, was measured in thin kidney sections from PCK rats. Consistent with a reduction in cyst area and kidney weight, HWI decreased cell proliferation of cyst epithelial cells (PCNA-positive cells decreased approximately 60%; Figure 5E). Inhibition of cell proliferation was associated with decreased levels of B-Raf and P-ERK, suggesting that increased water intake diminished the renal activity of the MEK/ERK pathway. Moreover, the number of apoptotic cells in the cyst epithelium of water-loaded PCK rats was significantly reduced compared with control-treated PCK rats (Figure 7). Male PCK rats at 14 wk of age were found to have increased SUN. Elevated SUN was corrected by increased water intake, demonstrating that suppression of plasma AVP decreased renal cyst formation and improved renal function.

In a retrospective analysis of patients with chronic renal insufficiency, Hebert <i>et al.</i> (32) found that high urine volume was associated with a faster decline of GFR. The authors suggested that high urine volume and low urine osmolality may be risk factors for the progression of renal disease, including PKD. Conversely, an inability to concentrate urine as kidney function declines could account for the high urine volume in their study. Therefore, it is impossible to establish a cause–effect relationship between water intake and the decline in GFR on the basis of their results. In this study, we found that high fluid intake slowed the progression of PKD and significantly improved renal function in the PCK rats. On the basis of these results, we suggest that increased water intake may be beneficial to patients with PKD.

**Conclusion**

This study provides evidence for a central role of AVP in cAMP-dependent activation of the B-Raf/MEK/ERK pathway in cyst-lining cells and renal cyst enlargement. Increased water intake that was sufficient to cause a sustained reduction in plasma AVP levels decreased AVP2R expression, reduced ERK activity and renal cell proliferation, and slowed PKD progression in PCK rats. We propose that sustained hydration by increased water intake may be beneficial to some patients with PKD by limiting the detrimental effects of AVP on renal cyst growth.

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**References**


