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Urinary excretion of AQP2 and ENaC in autosomal dominant polycystic kidney disease during basal conditions and after a hypertonic saline infusion

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Graffe CC, Bech JN, Lauridsen TG, Pedersen EB. Urinary excretion of AQP2 and ENaC in autosomal dominant polycystic kidney disease during basal conditions and after a hypertonic saline infusion. Am J Physiol Renal Physiol 302: F917–F927, 2012. First published January 18, 2012; doi:10.1152/ajprenal.00616.2011.—Renal handling of sodium and water is abnormal in chronic kidney diseases. To study the function and regulation of the aquaporin-2 water channel (AQP2) and the epithelial sodium channel (ENaC) in autosomal dominant polycystic kidney disease (ADPKD), we measured urinary excretion of AQP2 (u-AQP2), the β-subunit of ENaC (u-ENaC), cAMP (u-cAMP), and prostaglandin E2 (u-PGE2); free water clearance (C\text{H2O}); fractional sodium excretion (FE\text{Na}); and plasma vasopressin (p-AVP), renin (p-Renin), angiotensin II (p-ANG II), aldosterone (p-Aldo), and atrial and brain natriuretic peptide (p-ANP, p-BNP) in patients with ADPKD and healthy controls during 24-hour urine collection and after hypertonic saline infusion during high sodium intake (HS; 300 mmol sodium/day) and low sodium intake (LS; 30 mmol sodium/day). No difference in u-AQP2, u-ENaC, u-cAMP, u-PGE2, C\text{H2O}, and vasoactive hormones was found between patients and controls at baseline, but during HS the patients had higher FE\text{Na}. The saline caused higher increases in FE\text{Na} in patients than controls during LS, but the changes in u-ENaC, p-Aldo, p-ANP, p-BNP, p-Renin, and p-ANG II were similar. Higher increases in u-AQP2 and p-AVP were seen in patients during both diets. In conclusion, u-AQP2 and u-ENaC were comparable in patients with ADPKD and controls at baseline. In ADPKD, the larger increase in u-AQP2 and p-AVP in response to saline could reflect an abnormal water absorption in the distal nephron. During LS, the larger increase in FE\text{Na} in response to saline could reflect a defective renal sodium retaining capacity in ADPKD, unrelated to changes in u-ENaC.

Water; salt; vasopressin; cAMP; aldosterone

DYSREGULATION OF THE expression/shuttling of the renal epithelial sodium channel (ENaC) and the aquaporin-2 water channel (AQP2) has been suggested to play a role in the pathogenesis of autosomal dominant polycystic kidney disease (4, 37, 38, 43).

ENaC is responsible for the reabsorption of sodium through the apical membrane of the connecting tubule and the collecting duct and plays a key role in controlling sodium balance, extracellular fluid volume, and blood pressure. ENaC is a heteromultimeric protein composed of three homologous subunits (α, β and γ) (5, 30). Aldosterone (Aldo) is the main hormonal regulator of ENaC (14, 20). Binding of Aldo to the intracellular mineralocorticoid receptor increases the transcription and the apical translocation of ENaC (7, 8).

Fractions of ENaC are normally excreted into the urine. Recently, our group demonstrated a significant correlation between changes in the urinary excretion of the ENaC β-subunit (u-ENaCβ) and changes in urinary sodium excretion (15). Thus u-ENaCβ has been suggested as a marker of the transport of sodium via ENaC.

AQP2 is the apical water channel of collecting duct principal cells. Vasopressin (AVP) is the main hormonal regulator of AQP2 (6, 31). Binding of AVP to V2 receptors in the basolateral membrane stimulates adenylate cyclase, producing cAMP and protein kinase A (PKA). Short-term AVP exposure results in trafficking of subapical vesicles containing AQP2 to the apical plasma membrane, whereas long-term exposure causes a marked increase in the AQP2 whole-cell abundance via regulation of AQP2 gene transcription and AQP2 protein degradation (18, 19, 31, 47). Withdrawal of AVP leads to retrieval of AQP2 from the apical plasma membrane into subapical vesicles (19). AQP2 is excreted into the urine (13, 27, 45, 45) and is used as a marker for the action of AVP on the collecting ducts.

Autosomal dominant polycystic kidney disease (ADPKD) is the most common inherited kidney disease, accounting for 6–8% of patients on dialysis. ADPKD is a multisystemic and progressive disorder characterized by the formation and gradual enlargement of cysts in the kidney, progressing to renal insufficiency. Extrarenal manifestations of ADPKD include hypertension, cysts of the liver, seminal vesicle, pancreas, spleen, arachnoid membrane, and spinal meninges and connective tissue abnormalities including mitral valve prolapse, intracranial aneurysms, and abdominal hernia.

In an orthologous animal model of ADPKD, an overexpression of V2R and AQP2 and increased cAMP level has been found in cyst epithelial cells (37), which are predominately of collecting duct origin (41). cAMP controls cystogenesis by stimulating the chloride-driven fluid secretion from the principal cells (26). Treatment with V2R antagonists reduces cAMP, normalizes the level of AQP2 and V2R, decreases cyst volume, and delays disease progression in this ADPKD animal model (37, 43). Recently, it has been shown that ADPKD cyst growth...
progresses more slowly in ADPKD patients when treated with the V2R antagonist tolvaptan for 3 yr (11). Comparable levels of cAMP and unglycosylated AQ2 have been found in random urine samples from patients with ADPKD and healthy controls (36), but urinary cAMP (u-cAMP) and AQ2 (u-AQ2) have never been examined under standardized sodium and fluid intake. The extracellular volume is increased in patients with ADPKD (42), and it probably contributes to hypertension, which is present in 50–70% of the patients with ADPKD.

In parallel with the cAMP-stimulated chloride-secretion, sodium is accumulated in the cyst lumen by electrical coupling (26). Whether this sodium secretion is transepithelial or paracellular is uncertain. ENaC can be found in the apical membrane of the cyst epithelial cells, but the expression is reduced compared with the expression in normal principal cells from healthy subjects (40).

Conflicting results exist regarding natriuresis in ADPKD, probably because of differences in the salt balance between the studies (42). Like in essential hypertension, the relationship between blood pressure and natriuresis is abnormal in ADPKD. Thus higher blood pressure is necessary to obtain the same natriuresis (33). An abnormal water and sodium transport in the collecting duct principal cells could be involved in the pathogenesis of ADPKD, but it has not been investigated under standardized conditions.

In the present study, we wanted to test the hypothesis that u-AQ2 and u-ENaC were abnormal in ADPKD during HS intake and/or during LS intake and that these variables responded abnormally to a hypertonic saline infusion.

To analyze the regulation of the expression/shuttling of AQ2 and ENaC in ADPKD, we performed a randomized, crossover trial with patients with ADPKD and healthy control subjects. We compared the absolute values of u-AQ2 and u-ENaC corrected for creatinine (u-AQ2CR and u-ENaCβ-CR), fractional sodium excretion (FENa), u-cAMP, urinary excretion of prostaglandin E2 (u-PGE2), and plasma concentrations of AVP, renin, angiotensin II (ANG II), Aldo, atrial natriuretic peptide (ANP), and brain natriuretic peptide (BNP) in patients with ADPKD and healthy controls during both HS and LS intake. Furthermore, we compared the relative changes in the above-mentioned effect parameters in response to a hypertonic saline infusion during both diets.

MATERIALS AND METHODS

Patients and Control Subjects

Patients. The inclusion criteria for patients with ADPKD were the following: 1) white men and women; 2) age between 18 and 65 yr; 3) body mass index (BMI) between 18.5 and 30 kg/m2; 4) ADPKD, diagnosed by the following findings on ultrasonography (29): patients with a negative family history for ADPKD: more than five cysts; patients with a positive family history for ADPKD: <30 yr, two cysts (unilaterally or bilaterally); 30–60 yr, two or more cysts bilaterally; >60 yr: four or more cysts bilaterally; and 5) kidney function corresponding to stages 1–4.

The exclusion criteria for patients were the following: 1) other kidney disease; 2) medical history or clinical signs of heart, lung, liver, brain, endocrine organ, or neoplastic disease; and 3) a positive family history for intracerebral aneurisms. However, patients were not excluded if they had a normal computerized tomography (CT)-angiography of the arteries of the brain from the last 10 yr and it was possible to keep their blood pressure well regulated during the study period with metoprolol and/or amlodipine; 4) drug or alcohol abuse; 5) smoking; 6) pregnancy or breast feeding; and 7) medication except antihypertensive agents and oral contraceptives.

The withdrawal criteria were as follows: 1) lack of compliance; 2) withdrawal of consent; and 3) development of one of the exclusion criteria during the study. Antihypertensive agents were discontinued 2 wk before each study day. If blood pressures rose to levels above 170 mmHg systolic or 105 mmHg diastolic, substitution treatment with metoprolol and/or amlodipin was initiated (this happened in 1 patient). The substitution treatment was stopped 3 days before the study day.

The experimental procedure on the study day was efectuated, if the clinic blood pressure on the study day was <160/100 mmHg.

Healthy control subjects. The inclusion criteria for healthy controls were as follows: 1) white men and women; 2) age between 18 and 65 yr; and 3) BMI between 18.5 and 30 kg/m2. The exclusion criteria for the healthy controls were the following: 1) arterial hypertension defined by a clinic blood pressure above 140 mmHg systolic and/or 90 mmHg diastolic; 2) medical history or clinical signs of heart, lung, liver, kidney, brain, endocrine organ, or neoplastic disease; 3) abnormal biochemical screening of the blood regarding hemoglobin, white blood cell count, platelets, sodium, potassium, creatinine, albumin, bilirubin, alanine aminotransferase, alkaline phosphatase, cholesterol, and glucose; 4) abnormal urine screening for blood, albumin, and glucose; 5) abnormal electrocardiogram; 6) drug or alcohol abuse; 7) smoking; 8) pregnancy or breast feeding; and 9) blood donation <1 mo before the examination.

The withdrawal criteria were the same as for the patients. None of the healthy controls received any medication, except oral contraceptives.

Recruitment

The patients with ADPKD were recruited from the Outpatient Nephrology Clinic of the Department of Medicine, Holstebro Hospital (Holstebro, Denmark). The control subjects were recruited by advertising in public institutions and private companies.

Ethics and Approvals

The protocol of the study was submitted to and approved by the local Medical Ethics Committee. The study was performed under the license of the committee (JRN RRS-2006-1023). The study was conducted in conformity with the principles of the Declaration of Helsinki, and written informed consent was obtained from all the subjects. The study was registered at the registration site: http://www.clinicaltrials.gov (NCT00410007).

Design

We performed two randomized, crossover studies, one with patients with ADPKD and one with healthy controls. Each subject was studied on 2 separate days at least 3 wk apart. During 4 days before the study day, the subjects consumed either a HS or LS diet in randomized order. The results of the patients are compared with the results of the healthy controls. Furthermore, in both patients and controls the results obtained during HS and LS intake are compared.

Effect Variables

The primary effect variable was u-AQ2CR, and the secondary effect variables were urinary sodium excretion rate (UNa), FENa, u-ENaCβ-CR, urine volume (V), C H20, serum osmolality (s-osm), u-osm, u-cAMP and u-PGE2, plasma concentrations of AVP (p-AVP), renin (p-Renin), ANG II (p-ANG II), Aldo (p-Aldo), ANP (p-ANP), BNP (p-BNP), systolic and diastolic blood pressure, heart rate, body weight, and glomerular filtration rate (GFR).
Number of Subjects

A difference in u-AQP2_{CR} of 40 ng/mmol was considered the minimal relevant difference. A sample size of 10 subjects who could be evaluated had 80% power to detect this difference assuming a level of significance of 5% and an SD of 30 ng/mmol. Because a few subjects were expected to drop out, 14–15 subjects were included in each group.

Experimental Procedure Before the Study Day

Five days before the study day, the subjects collected a standardized, HS (~300 mmol sodium/day/17.5 g salt/day) or LS (~30 mmol sodium/day/1.8 g salt/day), 4-day diet from the hospital kitchen. Depending on the individually estimated energy requirement, the participants were given either a diet of 8,000 or 11,000 kJ/day. The energy distribution was 55% carbohydrates, 15% proteins, and 30% lipids. The 4-day diet was started the following morning.

The fluid intake was also standardized during the 4 days. The subjects were asked to drink exactly 250 ml·1,000 kJ⁻¹·day⁻¹ and to abstain from coffee, tea, and alcoholic beverages.

The subjects were instructed to keep their physical activity unchanged during the two experiments and to abstain from hard training.

The subjects collected their urine for 24 h the day before the study day.

Experimental Procedure on the Study Day

On the study day, the subjects were asked to drink 175 ml of water every 30 min from 7:00 AM. The subjects arrived at the department at 8:00 AM. Peripheral iv lines were inserted into the antecubital veins of both forearms, one for infusion of 51Cr-EDTA and hypertonic saline, and one for withdrawal of blood samples. The subjects were kept in the supine position from 8:00 AM to 1:30 PM except during voiding, which took place in the sitting or standing position.

At 8:30 AM, a priming dose of 51Cr-EDTA was administered, followed by sustained infusion. After 60 min of equilibration, the study continued with five clearance periods, the first two of 30-min duration (P₁–P₂), the last three of 60-min duration (P₃–P₅). The first two clearance periods were baseline periods.

At 10:30 AM, 7 ml/kg of 3% saline were given over 30 min.

Blood pressure and heart rate were measured every 30 min from 9:30 AM to 1:30 PM.

Urine was collected in each clearance period and analyzed for sodium, osmolality, u-AQP2, u-ENaC_{β}, u-cAMP, u-PGE₂, and 51Cr-EDTA.

Blood samples were drawn every 30 min from 9:30 AM to 10:30 AM and every hour from 11:30 AM to 1:30 PM, and were analyzed for sodium, osmolality, and 51Cr-EDTA. In addition, analysis of p-AVP, p-Renin, p-ANG II, p-Aldo, p-ANP, and p-BNP were performed from blood samples drawn at 10:30 AM, 11:30 AM, 12:30 PM, and 1:30 PM.

Methods

All blood samples were centrifuged for 15 min at 3,000 rpm at 4°C. Plasma was separated from blood cells and kept frozen at −20°C until assayed. p-ANP was determined by RIA, as previously described (23). ANP was extracted from plasma with a C₁₈ September-Pack (Water Associates, Milford, MA) using ethanol, acetic acid, and water. For RIA, rabbit anti-ANP antibody was obtained from the Department of Clinical Chemistry, Bispebjerg Hospital (Copenhagen, Denmark). The minimal detection concentration was 0.5 pmol/l. The coefficients of variation were 12% (interassay) and 10% (intra-assay). p-BNP in plasma was determined by RIA as previously described (12). Immunoreactive BNP was extracted from plasma with a C₁₈ September-Pack (Water Associates) eluted by 80% ethanol in a 4% acetic acid solution. A rabbit anti-BNP antibody without cross-reactivity with urodilatin or α-ANP was developed in our laboratory. The minimal detection concentration was 0.5 pmol/l. The coefficients of variation were 11% (interassay) and 6% (intra-assay). p-AVP in plasma was measured by RIA using a modification of the method described previously (21). AVP was extracted from plasma with a C₁₈ September-Pack (Water Associates). The antibody against AVP was a gift from Dr. Jacques Dür (Miami, FL). The minimal detection concentration was 0.5 pmol/l. The coefficients of variation were 13% (interassay) and 9% (intra-assay). p-ANG II in plasma was determined by RIA using a modification (22) of the method originally described (17). ANG II was extracted from plasma with a C₁₈ September-Pack (Water Associates). The antibody against ANG II was obtained from the Department of Clinical Physiology, Glostrup Hospital (Glostrup, Denmark). The minimal detection concentration was 2 pmol/l. The coefficients of variation were 8.2% (interassay) and 3.9% (intra-assay). p-Aldo was determined by RIA using a commercial kit (Diagnostic Systems Laboratories, Webster, TX). The minimal detection concentration was 22 pmol/l. The coefficients of variation were 8.2% (interassay) and 3.9% (intra-assay). p-Renin was also determined by a commercial RIA (CIS Bio International, Gif-sur-Yvette Cedex, France). The minimal detection concentration was 0.5 pg/ml. The coefficients of variation were 14.5% (interassay) and 4.5% (intra-assay).

Urine samples were centrifuged for 5 min at 3,000 rpm, and 125–1,000 μl of the supernatant was freeze dried and kept frozen at −20°C for 2–8 mo until assayed. We have done pilot experiments to ensure that the concentration of the effect variables in the urine does not decrease over time. The experiments showed that the effect variables are stable at −20°C for 2 yr. u-AQP2 was measured by a RIA as previously described (24). The anti-AQP2 antibody for RIA was obtained from Sören Nielsen (The Water and Salt Research Center, Institute of Anatomy, Aarhus University, Aarhus, Denmark). The antibody was raised in rabbits against the 15 C-terminal amino acids of human AQP2. The minimal detection concentration was 32 pg/tube. The coefficients of variation were 11.7% (interassay) and 5.9% (intra-assay). u-ENaC_{β} was measured by a newly developed RIA (15, 16). ENaC_{β} was synthesized and purchased by Lofstrand Labs (Gaithersburg, MD). The β-ENaC antibody was raised against a synthetic peptide in rabbits. The lower detectable limit of the assay was 34 pg/tube. The interassay variation was 12% at a mean level of 78 pg/tube and 10% at a mean level of 155 pg/tube. The intra-assay variation was 6.4 and 9.0% at a mean level of 180 pg/tube and 406 pg/tube, respectively. u-cAMP was measured by RIA using a commercial kit (Biomedical Technologies, Stoughton, MA). The minimal detection concentration was 0.05 pmol/l. The coefficients of variation were 8% (interassay) and 3% (intra-assay). u-PGE₂ was measured by RIA using a commercial kit (Assay Designs, Ann Arbor, MI). The minimal detection concentration was 8.26 pg/ml. The coefficients of variation were 10.9% (interassay) and 6.3% (intra-assay).

u-osm and u-osm were measured by freezing-point depression (Advanced Model 3900 multisampling osmometer). C_{H₂O} was determined according to the formula C_{H₂O} = V – C_{osm}, where V is the urine output, and C_{osm} is the osmolality clearance.

GFR was measured using the constant infusion clearance technique with 51Cr-EDTA as a reference.

Blood pressure was measured with a UA-743 digital blood pressure meter (A&D, Tokyo, Japan).

Urine was screened for blood, albumin, and glucose with standard urine test strips. Plasma and urinary concentrations of sodium and creatinine were determined at the Department of Clinical Biochemistry, Holstebro Hospital (Holstebro, Denmark) using conventional methods. All clearances were standardized to a body surface area of 1.73 m².

Statistics

Statistical analyses were performed using SPSS version 15 (SPSS, Chicago, IL).
Table 1. Clinical and laboratory characteristics of 11 patients with ADPKD and 11 healthy controls

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>Controls</th>
<th>t-Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men/women</td>
<td>3/8</td>
<td>3/8</td>
<td></td>
</tr>
<tr>
<td>Age, yr</td>
<td>44 (13)</td>
<td>43 (10)</td>
<td>0.941</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25.1 (1.7)</td>
<td>23.8 (2.8)</td>
<td>0.222</td>
</tr>
<tr>
<td>p-Na, mmol/l</td>
<td>139.5 (1.8)</td>
<td>140.2 (3.0)</td>
<td>0.546</td>
</tr>
<tr>
<td>p-K, mmol/l</td>
<td>4.0 (0.4)</td>
<td>3.9 (0.3)</td>
<td>0.311</td>
</tr>
<tr>
<td>p-Cr, μmol/l</td>
<td>108 (40)</td>
<td>72 (14)</td>
<td>0.012</td>
</tr>
<tr>
<td>24-h Ambulatory</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>139 (9)</td>
<td>115 (7)</td>
<td>0.001</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>93 (9)</td>
<td>70 (4)</td>
<td>0.001</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>74 (5)</td>
<td>71 (6)</td>
<td>0.282</td>
</tr>
<tr>
<td>Daytime ambulatory</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>143 (9)</td>
<td>120 (7)</td>
<td>0.001</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>94 (12)</td>
<td>74 (4)</td>
<td>0.001</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>77 (6)</td>
<td>74 (5)</td>
<td>0.274</td>
</tr>
<tr>
<td>Nighttime ambulatory</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>125 (9)</td>
<td>103 (9)</td>
<td>0.001</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>78 (9)</td>
<td>60 (4)</td>
<td>0.001</td>
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<tr>
<td>HR, beats/min</td>
<td>64 (6)</td>
<td>61 (11)</td>
<td>0.476</td>
</tr>
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<td>Systolic clinic blood pressure</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>at baseline</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP, HS, mmHg</td>
<td>137 (14)</td>
<td>111 (11)</td>
<td>0.001</td>
</tr>
<tr>
<td>SBP, LS, mmHg</td>
<td>132 (12)</td>
<td>112 (13)</td>
<td>0.001</td>
</tr>
<tr>
<td>Diastolic clinic blood pressure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>at baseline</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DBP, HS, mmHg</td>
<td>86 (11)</td>
<td>67 (6)</td>
<td>0.001</td>
</tr>
<tr>
<td>DBP, LS, mmHg</td>
<td>86 (9)</td>
<td>66 (5)</td>
<td>0.001</td>
</tr>
<tr>
<td>Heart rate at baseline</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR, HS, beats/min</td>
<td>56 (9)</td>
<td>56 (12)</td>
<td>0.644</td>
</tr>
<tr>
<td>HR, LS, beats/min</td>
<td>62 (8)</td>
<td>59 (9)</td>
<td>0.220</td>
</tr>
<tr>
<td>GFR at baseline</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GFR, HS, ml/min</td>
<td>69 (33)</td>
<td>97 (11)*</td>
<td>0.016</td>
</tr>
<tr>
<td>GFR, LS, ml/min</td>
<td>67 (33)</td>
<td>90 (11)</td>
<td>0.035</td>
</tr>
</tbody>
</table>

Values are means (SD). ADPKD, autosomal dominant polycystic kidney disease; BMI, body mass index; p-Na, plasma sodium concentration; p-K, plasma potassium concentration; p-Cr, plasma creatinine concentration; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; GFR, glomerular filtration rate; HS, high sodium intake; LS, low sodium intake; *P < 0.05, HS vs. LS, paired-samples t-test.

Single baseline values were obtained by taking the weighted average of the measurements from the two baseline periods. The baseline values of the two groups were compared by Student’s t-test. The baseline values during HS and LS intake were compared by paired samples t-tests.

We used the “General Linear Model Repeated Measures” procedure in SPSS with time as the within-subject factor and group as the between-subject factor to compare the effect variables in patients and controls.

The changes in response to the hypertonic saline infusion in each group were analyzed with the General Linear Model Repeated Measures procedure with time as the within-subject factor and paired samples t-tests with Bonferroni correction as post hoc tests.

P values < 0.05 were considered significant. Variables are normally distributed and presented as means with standard deviations (SD) or 95% confidence intervals, if not otherwise stated.

RESULTS

Demographics

Fifteen patients with ADPKD and 14 healthy controls were enrolled in the study. Four patients were withdrawn from the study because they withdrew their consent to participate. Three healthy controls were withdrawn from the study, two because of failure to obtain intravenous access and one because of withdrawal of consent to participate.

Since this study was designed, new criteria for ultrasonographic diagnosis of ADPKD of unknown genotype have been proposed (2, 25). More than 10 cysts in each kidney are now needed to diagnose ADPKD in individuals without a family history of the disease and >3 renal cysts in individuals at risk, aged 15–39 yr. All the patients in our study had a positive family history of the disease and two or more cysts bilaterally.

Table 1 shows the clinical and laboratory data of the 11 patients with ADPKD and the 11 healthy controls completing the study. As expected, the plasma creatinine level was significantly higher in the patients with ADPKD than in the healthy controls. The patients with ADPKD had significantly higher systolic and diastolic blood pressure during both day and night compared with the healthy controls. Of the 11 patients, 4 had a positive family history of intracerebral aneurysms.

24-h Urine Collection

Table 2 shows the results of the 24-h urine collection in the patients with ADPKD and the healthy controls during HS and LS intake. No difference was found between patients and healthy controls.

Table 2. 24-h Urine collection

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>Controls</th>
<th>t-Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>UNa, μmol/min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HS</td>
<td>175 (112; 238)*</td>
<td>203 (162; 243)*</td>
<td>0.410</td>
</tr>
<tr>
<td>LS</td>
<td>42 (23; 62)</td>
<td>40 (23; 56)</td>
<td>0.816</td>
</tr>
<tr>
<td>u-ENaC&lt;sub&gt;3&lt;/sub&gt;-CR, pg/μmol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HS</td>
<td>15.26 (11.12; 19.39)</td>
<td>14.24 (11.35; 17.13)</td>
<td>0.659</td>
</tr>
<tr>
<td>LS</td>
<td>12.78 (10.65; 14.91)</td>
<td>14.49 (11.16; 17.82)</td>
<td>0.347</td>
</tr>
<tr>
<td>V, ml/24 h</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HS</td>
<td>2,548 (1,939; 3,158)</td>
<td>2,491 (1,965; 3,017)</td>
<td>0.873</td>
</tr>
<tr>
<td>LS</td>
<td>2,225 (1,779; 2,671)</td>
<td>2,656 (2,107; 3,205)</td>
<td>0.190</td>
</tr>
<tr>
<td>u-AQP2&lt;sub&gt;α&lt;/sub&gt;, ng/mol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HS</td>
<td>165 (116; 215)*</td>
<td>148 (122; 174)*</td>
<td>0.475</td>
</tr>
<tr>
<td>LS</td>
<td>118 (104; 133)</td>
<td>109 (92; 125)</td>
<td>0.339</td>
</tr>
<tr>
<td>u-osm, mosmol/kgH₂O</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HS</td>
<td>377 (299; 454)*</td>
<td>472 (340; 605)*</td>
<td>0.180</td>
</tr>
<tr>
<td>LS</td>
<td>231 (206; 258)</td>
<td>255 (221; 388)</td>
<td>0.239</td>
</tr>
</tbody>
</table>

Values are means with 95% confidence intervals in parentheses. UNa<sub>α</sub>, urinary sodium excretion rate; u-ENaC<sub>3</sub>-CR, urinary epithelial sodium channel-3 (ENaC<sub>3</sub>) excretion corrected for creatinine; V, urinary flow; u-AQP2<sub>α</sub>, urinary aquaporin-2 (AQP2) excretion corrected for creatinine; u-osm, urinary osmolality; t-test: patients compared with controls, Student’s t-test. *P < 0.001, HS vs. LS intake, paired samples t-test.
controls in u-AQP2_{CR} and u-ENaC_{β-CR} in the 24-h urine. u-AQP2_{CR} was significantly lower during LS intake than HS intake in both patients and controls, whereas u-ENaC_{β-CR} was the same during LS and HS. UNa was significantly lower in both patients and controls during LS intake compared with during HS intake, indicating that both groups had kept the supplied diets.

**Hypertonic Saline Infusion**

The effect variables are shown in Tables 3 (UNa, FE_{Na} and u-ENaC_{β-CR}), 4 (V, CH2O, u-AQP2, u-osm, u-cAMP, u-PGE2, and s-osm), and 5 (p-Renin, p-Ang II, p-Aldo, p-ANP, p-BNP and p-AVP) before (baseline) and after the hypertonic saline infusions in the patients with ADPKD and the healthy controls during HS and LS intake, respectively.

**Baseline results.** We found no difference in the above-mentioned effect variables between the patients with ADPKD and the healthy controls at baseline except a significantly mentioned effect variables between the patients with ADPKD and the healthy controls during HS intake (Table 3), and as expected a significantly lower GFR and a significantly higher blood pressure in the patients compared with the controls during both groups [120 min: UNa, patients (LS vs. HS): 467% (76; 860) vs. 75% (-9; 159), P = 0.010; UNa controls (LS vs. HS): 263% (68; 459) vs. 41% (-37; 118), P = 0.0497; FE_{Na} patients (LS vs. HS): 578% (174; 982) vs. 75% (13; 137), P = 0.007; FE_{Na} controls (LS vs. HS): 178% (11; 345) vs. 67% (-1; 136), P = 0.007).

During HS intake the hypertonic saline infusion did not affect u-ENaC_{β-CR}, but during LS intake a significant increase in u-ENaC_{β-CR} was seen in both patients and controls 120 min after the saline infusion start (Table 3). There was no difference in the relative increases between patients and controls.

**Water excretion, u-AQP2, u-osm, u-cAMP, u-PGE2, and s-osm.** In both the patients with ADPKD and the healthy controls, the hypertonic saline infusion induced a significant and sustained decrease in V (Table 4). Initially, the decrease in V was larger in the controls than in the patients during HS intake [−58% (−71; −3) vs. −36% (−55; −16), P = 0.026] and larger during LS intake than HS intake in the controls [−70% (−92; −49) vs. −58% (−71; −3), P = 0.013], but after 2 h there was no difference in the decrease in V between patients and controls or between the two diets.

CH2O decreased significantly after the hypertonic saline infusion in both groups and during both diets with a maximum after 120 min (Table 4). CH2O changed from positive values at

Table 3. Effects of hypertonic saline infusion (3%, 7 ml/kg) on UNa, FE_{Na}, and u-ENaC_{β-CR} in 11 patients with ADPKD and 11 healthy controls during high and low sodium intake

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>60 Min</th>
<th>120 Min</th>
<th>180 Min</th>
<th>P_{GLM RM}</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>UNa, μmol/min</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>HS Patients</strong></td>
<td>296 (239; 353)*</td>
<td>495 (328; 662)‡</td>
<td>514 (307; 720)‡</td>
<td>466 (360; 572)‡</td>
<td>P = 0.157</td>
</tr>
<tr>
<td><strong>Controls</strong></td>
<td>273 (218; 328)*</td>
<td>384 (257; 501)‡</td>
<td>393 (318; 467)‡</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>LS Patients</strong></td>
<td>51 (26; 75)</td>
<td>225 (110; 339)‡</td>
<td>209 (130; 288)‡</td>
<td>202 (139; 264)‡</td>
<td>P = 0.062</td>
</tr>
<tr>
<td><strong>Controls</strong></td>
<td>49 (31; 67)</td>
<td>142 (93; 192)‡</td>
<td>141 (101; 181)‡</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>FE_{Na}, %</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>HS Patients</strong></td>
<td>3.79 (2.59; 4.98)*†</td>
<td>5.84 (4.34; 7.34)‡</td>
<td>5.60 (4.26; 6.94)‡</td>
<td>5.33 (4.24; 6.62)‡</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td><strong>Controls</strong></td>
<td>2.06 (1.63; 2.50)*</td>
<td>3.00 (2.41; 3.60)‡</td>
<td>2.94 (2.47; 3.41)‡</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>LS Patients</strong></td>
<td>0.72 (0.25; 1.19)</td>
<td>2.77 (1.54; 4.00)‡</td>
<td>2.78 (1.80; 3.75)‡</td>
<td>2.61 (1.81; 3.41)‡</td>
<td>P = 0.007</td>
</tr>
<tr>
<td><strong>Controls</strong></td>
<td>0.39 (0.26; 0.53)</td>
<td>1.08 (0.75; 1.41)‡</td>
<td>1.25 (0.95; 1.55)‡</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>u-ENaC_{β-CR}, pg/μmol</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>HS Patients</strong></td>
<td>11.7 (9.0; 14.5)</td>
<td>10.9 (8.5; 13.4)</td>
<td>12.1 (9.6; 14.6)</td>
<td>12.2 (10.2; 14.3)</td>
<td>P = 0.949</td>
</tr>
<tr>
<td><strong>Controls</strong></td>
<td>11.1 (9.7; 12.6)</td>
<td>11.4 (9.5; 13.4)</td>
<td>12.2 (10.2; 14.3)</td>
<td>11.8 (8.9; 14.7)</td>
<td></td>
</tr>
<tr>
<td><strong>LS Patients</strong></td>
<td>11.4 (8.5; 14.3)</td>
<td>11.6 (8.8; 14.4)</td>
<td>14.0 (8.8; 19.2)‡</td>
<td>11.6 (9.2; 13.9)</td>
<td>P = 0.499</td>
</tr>
<tr>
<td><strong>Controls</strong></td>
<td>9.7 (7.4; 12.0)</td>
<td>10.6 (7.8; 12.2)</td>
<td>12.0 (8.6; 15.4)‡</td>
<td>10.9 (8.0; 13.9)</td>
<td></td>
</tr>
</tbody>
</table>

Values are means with 95% confidence intervals in parentheses. FE_{Na}, fractional sodium excretion; P_{GLM RM}, patients compared with controls; GLM, repeated measures with time as within-subject factor and blood pressure as between-subject factor. *P < 0.05, baseline HS vs. LS, paired samples t-test. †P < 0.05, baseline patients vs. controls, Student’s t-test. ‡P < 0.05, compared with baseline, paired-samples t-test.
baseline to negative values after infusion, indicating a change from water excretion to water reabsorption. The relative decreases in CH2O did not differ between patients and controls or between HS and LS intake.

u-AQP2CR increased significantly in response to the hypertonic saline infusion in the patients with ADPKD during both diets (Table 4). The increase reached a maximum at 120 min after the infusion started. In the healthy controls, the increase in u-AQP2CR did not reach significance. Accordingly, the relative increases were significantly higher in the patients with ADPKD than in the healthy controls throughout the experiment [HS (patients vs. controls): 46% (29; 64) vs. 16% (−7;
Patients and controls during both diets (Table 4), with a nadir intake $[333\% \ (158; \ 508) \ vs. \ 215\% \ (121; \ 309), P < 0.005]$; LS (patients vs. controls): $50\% \ (23; \ 77) \ vs. \ 9\% \ (18; \ 35), P = 0.007$.

u-osm increased significantly in response to the hypertonic saline infusion in both patients and controls during both diets (Table 4). The increases lasted throughout the experiment with a maximum 120 min after infusion started. During LS intake, baseline levels were reached 180 min after the infusion started during LS intake, where the decreases were lower in the patients $[-47\% \ (-66; \ -27) \ vs. \ -22\% \ (-35; \ -8), P = 0.007]$

The hypertonic saline infusion did not affect u-cAMP (Table 4).

u-PGE$_2$ decreased in response to the saline infusion in both patients and controls during both diets (Table 4), with a nadir 180 min after infusion start. The relative decrease in u-PGE$_2$ did not differ between patients and controls except from 180 min after the infusion started during LS intake, where the decreases were lower in the patients $[-47\% \ (-66; \ -27) \ vs. \ -22\% \ (-35; \ -8), P = 0.007]$.

s-osm increased significantly in both patients and controls during both diets, with a maximum 60 min after the infusion started. During HS intake, baseline levels were reached 180 min after the infusion started (Table 4). In the healthy controls, the relative increases were larger during LS intake than during HS intake $[60 \ min: \ 3.1\% \ (2.5; \ 3.6) \ vs. \ 2.1\% \ (1.7; \ 2.5), P = 0.001]$.

Vasoactive hormones. The hypertonic saline infusion did not affect p-Renin and p-ANG II in patients with ADPKD. In the
healthy controls, p-Renin and p-ANG II decreased 60 min after the infusion started during LS intake (Table 5). The relative changes in p-Renin and p-ANG II did not differ between patients and controls or between HS and LS intake.

p-Aldo decreased significantly in response to the hypertonic saline infusion in both groups during both diets. The decrease had a maximum 120 min after the saline infusion started and lasted throughout the study day (Table 5). During HS intake, the relative decreases were significantly larger in patients than controls [−34% (−44; −23) vs. −13% (−29; 3), P = 0.024]. Moreover, the relative decreases were larger during LS intake than HS intake in both groups [patients (LS vs. HS): −47% (−56; −38) vs. −34% (−44; −23), P = 0.030; controls (HS vs. LS): −39% (−50; −29) vs. −13% (−29; 3), P = 0.003].

The hypertonic saline infusion induced a significant increase in p-ANP in both groups on both study days (Table 5). The increase peaked 60 min after the infusion started. The p-ANP increase in response to the hypertonic saline lasted longer during LS intake than during HS intake in the healthy controls. Accordingly, the relative increase in p-ANP did not differ between HS and LS intake 60 min after the saline infusion, but was significantly higher during LS intake than during HS intake 120–180 min after the saline infusion in the healthy controls [120 min: 56% (35; 76) vs. 30% (1; 59), P = 0.038; 180 min: 38% (16; 61) vs. 9% (18; 35), P = 0.044].

In the patients with ADPKD, the hypertonic saline did not affect p-BNP. In the healthy controls, the hypertonic saline infusion caused a significant increase in p-BNP after 120 min. The increase lasted throughout the study day.

p-AVP increased significantly after the saline infusion in both patients and controls during both diets (Table 5). The increases followed the same pattern in the patients with ADPKD and the healthy controls, with a maximum after 60 min (Table 5). The relative increases in p-AVP 120 and 180 min after the saline infusion started were significantly higher in patients than controls during both diets [120 min: HS (patients vs. controls): 28% (16; 41) vs. 19% (7; 31), P = 0.002; LS (patients vs. controls): 36% (21; 52) vs. 21% (8; 33), P = 0.003].

Blood pressure, heart rate, and body weight. The hypertonic saline did not affect the systolic or diastolic blood pressure in any of the groups.

The heart rate was also unaffected by the saline infusion in both groups during both diets.

The body weight of the patients with ADPKD increased significantly during both diets [HS: from 76.1 (SD 9.5) to 76.5 kg (SD 9.3), P = 0.035; LS: from 75.0 (SD 9.5) to 75.7 kg (SD 9.3), P < 0.001]. The relative increase was higher during LS intake than during HS intake [1.1% (SD 0.5) vs. 0.6% (SD 0.8), P = 0.023].

The body weight of the control subjects also increased significantly during both diets [HS: from 71.0 (SD 13.9) to 71.5 kg (SD 14.1), P = 0.002; LS: from 70.8 (SD 14.8) to 71.6 kg (SD 15.0), P < 0.001]. The relative increase was higher during LS intake than during HS intake [1.1% (SD 0.5) vs. 0.7% (SD 0.5), P = 0.044].

GFR. GFR was unaffected by the hypertonic saline in both groups.

**DISCUSSION**

In the present study, we compared u-AQP2CR and u-ENaCp-CR in patients with ADPKD and healthy control subjects during HS and LS intake. Furthermore, we compared the relative change in u-AQP2CR and u-ENaCp-CR in response to a hypertonic saline infusion in patients and controls. u-AQP2CR and u-ENaCp-CR were normal in ADPKD at baseline. However, in response to the hypertonic saline infusion, u-AQP2CR was abnormally increased in ADPKD, whereas the response in u-ENaCp-CR was normal.

**u-AQP2CR is not Increased in ADPKD at Baseline**

We found that u-AQP2CR was not increased in the patients with ADPKD. This is in agreement with the results of a previous study, which compared u-AQP2 in random urine samples from patients with ADPKD and healthy controls. The study has unfortunately only been published as an abstract (36). The normal u-AQP2CR in patients with ADPKD does not necessarily mean that the expression of AQP2 mRNA or protein is not increased in ADPKD. There is evidence of a defective AQP2 sorting or insertion mechanism in ADPKD (9). An increase in the expression of AQP2 in ADPKD could be a compensatory mechanism for a defect in sorting or insertion. Thus u-AQP2CR was normal in the patients with ADPKD.

u-cAMP was not increased in the patients with ADPKD compared with the healthy controls during any of the diets. This is in agreement with a previous study in patients with ADPKD (36).

An increased renal cAMP level has been reported in several animal models of PKD (34, 35, 44, 46). Recently, Raphael and coworkers (28) reported no change in the cAMP level and AQP2 expression in two mouse models of ADPKD. However, the study by Raphael et al. was performed in a rapidly developing and severe PKD model, and the cAMP levels reported by them in their control mice were very high (125 pmol/mg of protein) compared with those of control mice (usually 5–10 pmol/mg of protein).

In 1979, Bia et al. (3) showed that in vivo administration of vasopressin was best demonstrated by measurement of in situ cAMP content of the renal papilla, whereas total urinary cAMP and nephrogenous cAMP are not useful indices of tubular sensitivity to this hormone (3). The use of u-cAMP as a marker of AVP activity is still controversial (1). Thus, despite the fact that we find normal u-cAMP in the patients with ADPKD, we cannot exclude that the intrarenal level of cAMP is increased in patients with ADPKD.

AVP does not differ between patients and controls, but factors other than AVP can produce cAMP in the kidneys, e.g., parathyroide hormone, catecholamines, caffeine, and a forskolin-like molecule found in ADPKD cyst fluid. Persons suffering form endocrine disorders were not included in the study, and coffee drinking was not allowed during the study. u-cAMP is also derived by glomerular filtration. The design (ADPKD patients with renal insufficiency and hypertension, compared with healthy controls) of the present study has some limitations, which do not allow for a full understanding of the aquaporin-vasopressin-cAMP and ENaC axis in ADPKD.
u-ENaC_{β-CR} is not Increased in ADPKD at Baseline

We found similar u-ENaC_{β-CR} and U_{Na} in the patients with ADPKD and the healthy controls during both diets, but higher F_{ENa} in the patients than in the controls during HS intake. These results seem conflicting, but could be explained by the lower GFR in ADPKD. Veizis et al. (40) suggested that the open probability of ENaC may be reduced in ADPKD. Additional studies are required to determine whether an abnormal regulation of ENaC exists in ADPKD.

No difference was found in p-Renin, p-ANG II, and p-Aldo between the patients with ADPKD and the healthy controls in the present study, in agreement with a previous study (38). It has been argued that it is not appropriate to use healthy volunteers as control subjects in studies with hypertensive ADPKD patients, since the raised blood pressure itself and the expansion of the extracellular fluid volume will tend to suppress the renin-angiotensin system.

u-AQP2_{CR} is Decreased During LS Intake at Baseline in Both Groups

We found significantly lower u-AQP2_{CR} and u-osm during LS intake than during HS intake in both groups. To our knowledge, u-AQP2 has not previously been studied in patients with ADPKD during HS and LS intake in a crossover study. Recently, our group reported lower AQP2 during LS intake compared with during HS intake in young healthy subjects, in agreement with the present study (10).

Thus u-AQP2_{CR} and u-osm were significantly lower during LS intake than during HS intake in both patients with ADPKD and healthy controls.

The diuresis was comparable during HS and LS intake at baseline in both groups. We have recently reported similar results in young healthy humans (10). The underlying mechanism for the comparable diureses during HS and LS intake is not clear, but reduced water permeability of the principal cells during LS intake could play a role.

Both the systolic and the diastolic blood pressures were significantly higher in the patients than in the healthy controls during both diets, as expected. The subjects were studied in the supine position, which explains the rather low blood pressure levels in both groups throughout the study days.

Comparable u-ENaC_{β-CR} During HS and LS Intake in ADPKD at Baseline

We found comparable ENaC_{β-CR} during HS and LS intake in both patients and controls in the baseline period before the infusion of hypertonic saline and also during the 24-h urine sampling.

Abnormally Higher Increase in uAQP2_{CR} in Response to Hypertonic Saline in ADPKD

u-AQP2_{CR} increased significantly more in response to the hypertonic saline infusion in patients with ADPKD than in healthy controls, which suggests that the patients reabsorb a larger proportion of the infused water. Thus somehow the antidiuretic effect of the hypertonic saline is increased in ADPKD. The underlying regulatory mechanism seems to be AVP, which increased more in patients than controls during both diets.

In both groups u-osm increased significantly, but the increase was not higher in patients than in controls. The higher increase in F_{ENa} in response to the hypertonic saline in the patients probably explains the lack of difference in the u-osm increase.

No difference in an AQP2 increase in response to the hypertonic saline infusion was found in patients between HS and LS intake.

It should be emphasized that the results of the present study are obtained during water diuresis and not during a normal physiological diuresis. The low urine osmalities and the positive C_{H2O} values at baseline reflect this. u-AQP2 is known to be reduced during water diuresis, with a nadir after 60–90 min (32). Our subjects received a continuous water load throughout the study day to avoid fluctuations in the waterload-induced reduction in u-AQP2. However, we cannot exclude that the response to the water load differs between patients with ADPKD and controls. A third study day with the water loading but without the acute sodium loading could have clarified this. However, it has been reported that acute water loading reduced u-cAMP to the same degree in healthy control subjects and patients with ADPKD (1).

To our knowledge, this is the first time that the change in u-AQP2_{CR} in response to a hypertonic saline infusion has been measured in patients with ADPKD. The increase in u-AQP2_{CR} and p-AVP was significantly higher in patients with ADPKD than in healthy controls.

Normal Response in u-ENaC_{β-CR} to Hypertonic Saline in ADPKD

u-ENaC_{β-CR} did not change in response to the hypertonic saline infusion during HS intake either in patients or in controls. However, during LS intake a significant increase was seen in u-ENaC_{β-CR} in both groups 120 min after infusion was started. The increases were comparable in patients and controls.

One would have expected a decrease in u-ENaC_{β-CR} in response to the hypertonic saline infusion, since p-Aldo decreased and U_{Na} and F_{ENa} increased in both patients and controls. We have previously reported a similar increase in u-ENaC in response to a hypertonic saline infusion in healthy humans (10, 15). The explanation of this phenomenon is not clear for the time being. A considerable decrease in the renal sodium absorption more proximally in the nephron might be compensated for by an increase in absorption in the distal part of the nephron.

We found a significantly higher increase in F_{ENa} in response to the hypertonic saline in the patients with ADPKD than in the controls during LS intake, in agreement with previous results (39). Thus our results showed that patients with ADPKD have a deficient renal ability to absorb sodium.

p-Aldo decreased more in patients compared with controls during HS intake and tended to decrease more in patients than in controls during LS intake (P = 0.063).

Since the response in u-ENaC_{β-CR} to hypertonic saline infusion was the same in patients with ADPKD and controls, an abnormal expression of ENaC cannot explain the phenomenon of exaggerated natriuresis in ADPKD. However, we cannot exclude an abnormal ENaC gating in ADPKD.
Potential Mechanisms Underlying the Increased Natriuretic Response to Hypertonic Saline Infusion During LS Intake

The relative increases in USNa and FESNa in response to the hypertonic saline infusion were higher during LS intake than during HS intake in both the patients with ADPKD and the controls (Table 4).

The larger decrease in p-Aldo after the hypertonic saline infusion during LS intake compared with that during HS intake in both groups may at least in part explain the larger increase in natriuresis in response to the hypertonic saline during LS intake.

In conclusion, no difference was found in u-AQP2CR and u-ENaCβ-CR between patients with ADPKD and controls at baseline. In ADPKD, the increased u-AQP2CR and p-AVP in response to the hypertonic saline infusion can be attributed to an abnormal water absorption in the distal part of the nephron. During LS intake, the larger increase in FENa in response to the hypertonic saline most likely reflects a deficiency in renal sodium retaining capacity in ADPKD, which was unrelated to changes in u-ENaCβ-CR.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: C.C.G., J.N.B., and E.B.P. provided conception and design of research; C.C.G. and T.G.L. performed experiments; C.C.G. and E.B.P. analyzed data; C.C.G. and E.B.P. interpreted results of experiments; C.C.G. drafted manuscript; C.C.G. and E.B.P. edited and revised manuscript; C.C.G., J.N.B., T.G.L., and E.B.P. approved final version of manuscript.

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causes a more severe cystic kidney disease than in intercalated cells. 


