A Pilot Clinical Study to Evaluate Changes in Urine Osmolality and Urine cAMP in Response to Acute and Chronic Water Loading in Autosomal Dominant Polycystic Kidney Disease

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Background and objectives: Autosomal dominant polycystic kidney disease (ADPKD) leads to kidney failure in half of those affected. Increased levels of adenosine 3′:5′-cyclic monophosphate (cAMP) play a critical role in disease progression in animal models. Water loading, by suppressing arginine vasopressin (AVP)-stimulated cAMP production, is a proposed therapy for ADPKD.

Design, setting, participants, & measurements: The effects of acute and sustained water loading on levels of urine osmolality (Uosm) and cAMP in 13 subjects with ADPKD and 10 healthy controls were studied. Uosm and cAMP concentrations were measured before and after water loading.

Results: Urine [cAMP] indexed to Uosm significantly decreased with acute water loading in both groups (58% in controls and 35% in ADPKD). Chronic water loading resulted in a nonsignificant 13% decrease in 24-hour urine cAMP excretion in ADPKD participants, despite an increase in 24-hour urine volume by 64% to 3.14 ± 0.32 L and decrease in mean Uosm by 46%, to below that of plasma (270 ± 21 mOsm/L).

Conclusions: Increased water intake of 3 L per day decreased Uosm in most ADPKD subjects. While urine [cAMP] accurately reflects changes in Uosm during acute water loading in ADPKD subjects, chronic water loading did not lower 24-hour urine cAMP excretion, although subjects with higher baseline [cAMP] (>2 nmol/mg Cr) responded best. Decreases in urine [cAMP] and osmolality are consistent with decreased AVP activity. These results support the need for a larger study to evaluate the effect of chronic water loading on ADPKD progression.

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Autosomal dominant polycystic kidney disease (ADPKD) is the most common, lethal, human genetic disease inherited as a dominant trait as a result of mutations in a single gene. With an estimated prevalence of 1:750, it affects nearly 600,000 individuals in the United States (1). In recent years, substantial progress has been made toward understanding the molecular genetics and biology of this disease that now makes it possible to design translational studies. Although rates of progression vary, 50% of ADPKD patients will develop ESRD.

ADPKD is caused by mutations in *PKD1* in 85% of patients or in *PKD2* in 15% of patients. The polycystins are membrane proteins that interact with other membrane proteins, the cytoskeleton, and each other to transduce extracellular stimuli into intracellular signaling cascades (2,3). Disrupting the integrity of this system results in the pathologic sequelae of cyst formation. Cyst generation and growth depend on epithelial proliferation and fluid secretion. The molecular events leading to these changes have been partially identified. Earlier studies have demonstrated the important role of adenosine 3′:5′-cyclic monophosphate (cAMP) in promoting ADPKD renal tubule epithelial proliferation and cyst growth *via* B-Raf and extracellular-regulated kinase activation (4). cAMP also drives ADPKD renal epithelial fluid secretion into the cyst by regulation of the cystic fibrosis transmembrane conductance regulator chloride channel (1).

Arginine vasopressin (AVP) is the major stimulus that leads to increased levels of cAMP in renal distal tubule epithelial cells. AVP is an antidiuretic hormone that stimulates cAMP production by activating G-protein-coupled vasopressin 2 receptors (V2Rs). A critical role for AVP and cAMP in the pathogenesis of ADPKD has been demonstrated by the finding that V2R antagonism or genetic loss of AVP markedly slows progression of disease in a number of animal models of ADPKD

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(5–9). Water loading, by suppressing plasma AVP levels, is another potential way to inhibit V2R signaling. In fact, previous studies in murine models of PKD have demonstrated that water loading also slows disease progression by inhibiting V2R-stimulated production of cAMP (10).

As an autosomal dominant trait, single germline mutations in either *PKD1* or *PKD2* are sufficient to cause disease. Although cystogenesis is stochastic on a tissue level, on a molecular level this is the result of a second somatic "hit" inactivating the second *PKD* allele (11). Other variables are known to modulate disease progression, such as hypertension, male sex, and environment. Most recently, a "third-hit" hypothesis has been tested in an animal model of PKD, where ischemia-reperfusion injury accelerated cyst formation (12,13).

One possible environmental factor that may affect disease progression is water intake. Increased water intake, by suppressing plasma AVP levels, may have a beneficial effect in patients with ADPKD. Human studies of high water intake to modulate renal cAMP levels and as therapy for ADPKD have not been reported. In this study, we sought to evaluate changes in urine osmolality and urine cAMP in human ADPKD and healthy subjects with acute water loading and also to assess the efficacy of sustained high water intake in decreasing urine osmolality and urine cAMP levels in ADPKD patients.

Materials and Methods

Subjects

13 ADPKD patients and 10 healthy controls were recruited. Included participants were older than 18 years, had no history of syndrome of antidiuresis, were not on vasopressin agonist or antagonist therapy, had baseline serum sodium concentrations of >135 mEq/L, and had preserved kidney function with an estimated GFR (eGFR) >60 ml/min per 1.73 m^2 using the Modified Diet in Renal Disease equation. Parathyroid hormone (PTH) levels were measured because PTH can elevate renal cAMP levels (14). ADPKD patients had undergone abdominal noncontrast magnetic resonance imaging (MRI) to assess kidney volumes, which have been shown to correlate well with ADPKD progression (15). The diagnosis of ADPKD was based on a positive family history and ultrasonographic diagnostic criteria (16). Informed consent was obtained from all subjects after approval by the New York University Langone Medical Center Institutional Review Board.

Acute Water Loading

Participants were asked not to ingest food or drink for 12 hours before the first study visit to achieve maximal AVP secretion and urine concentration (10). Subjects were instructed to not consume any caffeine during the period of the study because caffeine is known to increase cAMP levels (18). A urine sample was obtained at the end of the thirsting period. After this collection, participants began drinking 200 ml of bottled water every 15 minutes for 2.5 hours, for a total of 2 L. They were able to void freely. One hour after completing the water loading, a "post-water loading" urine sample was collected. All samples were aliquoted and frozen at −80°C within an hour of collection.

Chronic Water Loading

Participants collected a baseline 24-hour urine on their usual fluid intake. The urine was kept at 4°C by the participants throughout the collection period. Subjects were then instructed to drink at least 3 L of water daily for 7 days. On days 6 and 7 of sustained water loading, participants collected two consecutive 24-hour urines, which were stored at 4°C. Aliquots of the 24-hour collections were frozen at −80°C until further analysis.

Biochemical Analysis

Urine osmolality was determined by measuring freezing point depression, and urine creatinine was measured by the enzymatic Jaffe assay in the New York University Tisch Hospital clinical laboratory. For urine cAMP measurements, aliquots of the appropriate samples were thawed, and particulate matter was removed by centrifugation. Urine cAMP concentration was measured using the cAMP Biotrak enzyme immunoassay (GE Healthcare, Amersham) as per the manufacturer's instructions. All measurements were made in duplicate.

Statistical Analyses

Changes in urine cAMP concentration were indexed to urine osmolality in the acute water loading part of the study to account for effects of dilution. In the chronic water loading phase, 24-hour total urine cAMP amount was indexed to 24-hour total urine creatinine. Within-group changes in urine cAMP were analyzed using paired *t* tests (two-tailed, $\alpha = 0.05$). Comparisons between ADPKD and control subjects were made using the nonparametric Wilcoxon rank sum test. Values are reported as mean ± SEM, unless otherwise noted.

Results

Baseline Characteristics

All participants had normal serum creatinine and PTH levels. Despite preserved eGFR, the total kidney volumes as measured by MRI in ADPKD subjects ranged from 372 ml to 2153 ml (Table 1), with an average of 991 ml, representing a broad range of disease severity.

Table 1. Baseline characteristics of the two comparison groups

	ADPKD	Controls
Age, yr, mean ± SEM	38 ± 4	35 ± 4
Women, %	69	40
Race, %		
white	75	90
Asian	15	10
Hispanic or Latino ethnicity, %	31	10
Serum creatinine, mg/dl, mean ± SEM	0.82 ± 0.04	0.91 ± 0.06
eGFR, ml/min per 1.73 m^2, mean ± SEM	94 ± 5	92 ± 4
Total kidney volume by MRI, ml, mean ± SEM	991 ± 202	Not measured

Acute Water Loading

After a 12-hour thirsting period, control subjects concentrated their urine to mean 901 ± 54 mOsm/L, whereas ADPKD participants' mean maximal urine osmolality was 628 ± 57 mOsm/L ($P = 0.003$). This is consistent with the well-described impaired urine-concentrating ability found early in ADPKD (9). Both groups were able to maximally dilute their urine to a similar extent: controls to 86 ± 8 mOsm/L and ADPKD subjects to 99 ± 9 mOsm/L ($P = 0.34$). Both groups were able to significantly decrease their cAMP levels with an acute water load (Figure 1). The controls decreased their urine cAMP levels, indexed to urine osmolality, from 7.1 ± 1.2 μmol/Osm to 3.0 ± 0.4 μmol/Osm ($P = 0.007$). ADPKD participants decreased their urine cAMP levels from 6.3 ± 0.7 μmol/Osm to 4.1 ± 0.6 μmol/Osm with acute water loading ($P = 0.03$). ADPKD participants were able to decrease urine cAMP to the same degree as controls: 2.0 ± 0.1-fold (ADPKD) *versus* 2.6 ± 0.2-fold (controls) ($P = 0.38$). Thus, these findings demonstrate that urine cAMP levels in patients with ADPKD can be decreased by acute water loading.

To explore whether urine osmolality may be a surrogate marker for cAMP concentration, and thus more germane to a clinical setting, we investigated the relationship of urine cAMP concentrations and osmolality throughout the acute water loading experiment. Urine cAMP concentrations correlated well with osmolality in thirsting and acute water-loaded states for both groups ($r = 0.9$, $P < 0.0001$; Figure 2).

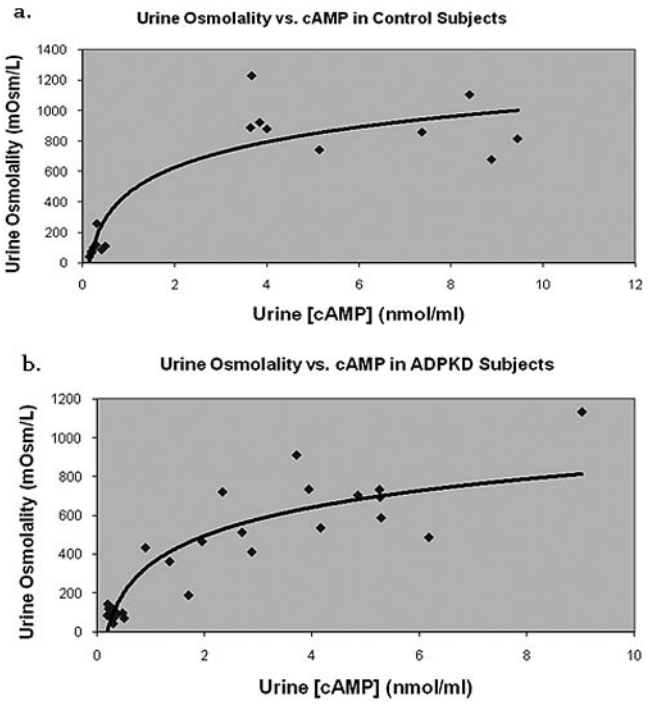

Figure 2. The overall correlation between all (thirsting and water-loaded) urine cAMP concentrations and urine osmolality was determined in the (a) control and (b) ADPKD groups. Both groups had a strong correlation between urine osmolality and cAMP concentrations ($r > 0.9$, $P < 0.0001$ for both).

Chronic Water Loading

At baseline, the average urine osmolality in a 24-hour collection for ADPKD subjects was 501 ± 55 mOsm/L, and the average urine volume was 1.9 ± 0.3 L. ADPKD subjects were able to significantly increase their urine volume with 1 week of daily water loading (3 L) by 64% to a mean of 3.1 ± 0.3 L ($P < 0.001$). Mean urine osmolality fell by 46% with chronic water loading, to 270 ± 21 mOsm/L ($P = 0.04$), in ADPKD participants. Although we did not directly measure plasma AVP levels, it is reasonable to infer that vasopressin was suppressed, because most participants were able to achieve a urine osmolality below that of plasma. Twenty-four-hour cAMP excretion, indexed to 24-hour urine creatinine excretion, did not change with chronic water loading (2.1 ± 0.4 nmol/mg Cr *versus* 1.8 ± 0.3 nmol/mg Cr, $P = 0.53$; Figure 3). Of potential interest, we found that five of seven ADPKD patients with the highest urine cAMP levels (>2 nmol/mg Cr) decreased their urine cAMP with sustained water intake.

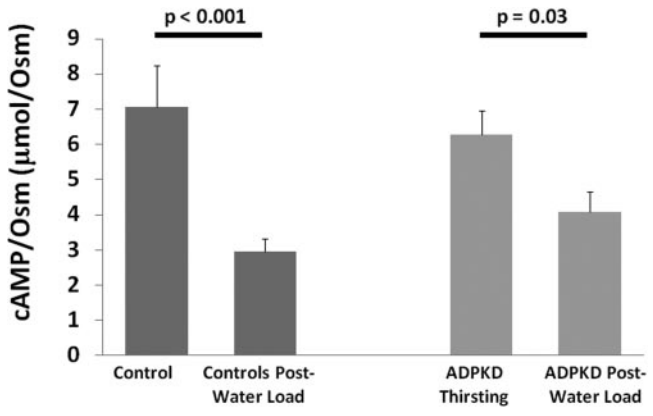

Figure 1. Changes in urine cAMP concentration indexed to urine osmolality in μmol/Osm to adjust for dilution with acute water loading (2 L over 2.5 hours) in the control and ADPKD groups. Within-group comparison showed that both the controls and ADPKD subjects significantly decreased urine cAMP with water loading (controls $P = 0.007$, ADPKD $P = 0.03$). Controls suppressed cAMP by 2.6 ± 0.2-fold and ADPKD subjects by 2.0 ± 0.1-fold ($P = 0.38$ for between-group comparison).

We also examined whether a particular threshold of volume increase correlated with decrease in cAMP/Cr after water loading. There was no correlation, and some ADPKD subjects experienced an increase in their cAMP/Cr despite a significant increase in their 24-hour urine volume after water loading (Figure 4).

As with the acute water loading data, we evaluated whether urine osmolality of the 24-hour collection may serve as a surrogate marker of urine cAMP concentration. Contrary to the acute water loading experiment, the 24-hour cAMP/Cr correlated poorly with the 24-hour urine osmolality ($r = 0.25$, $P = 0.21$; Figure 5), suggesting that urine [cAMP] may not be an adequate surrogate marker of osmolality or AVP activity on a time-averaged basis.

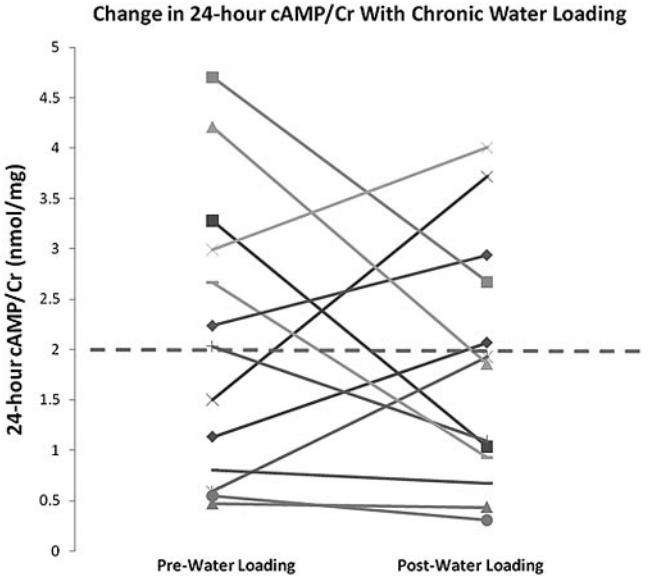

Figure 3. Individual trendlines of change in 24-hour cAMP/Cr before and after 1 week of water loading (3 L per day) for each ADPKD participant. Mean pre-water loading cAMP/Cr was 2.1 ± 0.4 nmol/mg Cr, and mean post-chronic water loading cAMP/Cr was 1.8 ± 0.3 nmol/mg Cr. The overall trend was 13% lower cAMP/Cr after water loading, but nonsignificant ($P = 0.53$). The dashed line delineates those with cAMP/Cr above 2.0, who were for the most part able to effectively decrease their cAMP/Cr (five of seven participants with the higher cAMP levels).

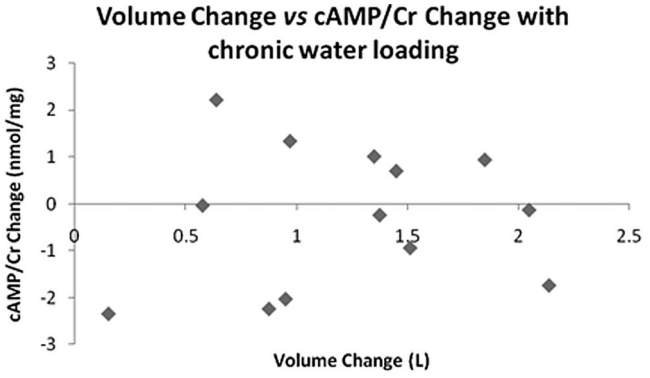

Figure 4. Scatter plot of the change in cAMP/Cr compared with the change in 24-hour urine volume with chronic water loading. The mean cAMP/Cr change with water loading was −0.27 ± 0.4 nmol/mg Cr, and the mean change in 24-hour urine volume was 1.2 ± 0.2 L. No correlation was found between the change in 24-hour urine volume and the change in cAMP/Cr with water loading.

Discussion

Patients with ADPKD are now being advised to increase their water intake (20). The hope is that this therapeutic intervention will result in lower serum AVP levels, lower cAMP levels in renal tubular epithelia, and hence slower cyst growth. Although this strategy has shown promise in animals, there is a pressing need to evaluate this therapy in people. To our knowledge, this study is the first in humans to explore the effects of water loading as a potential therapy to lower urine osmolality and cAMP in patients with ADPKD. Our ADPKD subjects spanned a broad range of disease as measured by MRI total kidney volume, yet all had preserved kidney function as measured by eGFR. We found that most participants were able to suppress their urine osmolality, and by extrapolation their serum AVP levels, by following a protocol to increase their water intake to 3 L per day. These results, coupled with our experience that most patients found it unacceptable to consume more than 3 L of water per day, suggest that advising patients to increase their water intake to 3 L throughout the day is a realistic therapeutic target, although a higher water prescription may be more potent for suppressing time-averaged urine cAMP levels.

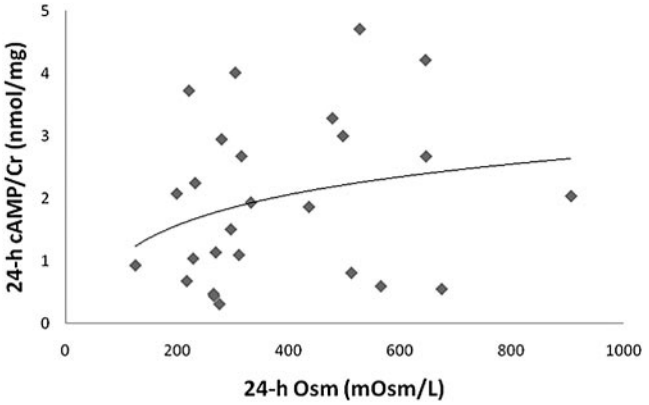

Figure 5. The relationship between all 24-hour cAMP/Cr and urine osmolality measurements (pre-water and post-water loading) showing nonsignificant correlation ($r = 0.25$; $P = 0.21$).

Because urine osmolality is the gold standard for assessing AVP activity in the distal nephron, a second goal of this study was to determine whether urine cAMP can be complementary to urine osmolality in assessing AVP activity during water loading in patients with ADPKD. For example, there are theoretical limitations to using urine osmolality as the only endpoint because of the interindividual variability of daily osmolar intake (20) and, more importantly, the presence of a urine-concentrating defect found in ADPKD. We have confirmed that ADPKD patients reach an average ceiling urine osmolality around 600 to 700 mOsm/L. Because this limit is not due to diminished vasopressin activity but rather to cell polarity and signaling aberrancies in ADPKD, urine osmolality is at least partially uncoupled from AVP-driven increases in intracellular cAMP levels, which is important in ADPKD pathophysiology. Although acute water loading, which has been shown to suppress plasma AVP levels (17), led to a significant decrease in urine cAMP levels, we found that a sustained increase in water intake for 1 week resulted in a nonsignificant 13% decrease in urine cAMP.

There are a number of possibilities that could account for the inability to detect predictable changes in urine cAMP during chronic water loading. It is possible that 24-hour urine cAMP is predominantly dependent on plasma AVP levels in only a subgroup of ADPKD patients. For example, it is well known

that other agonists can generate cAMP in the kidney, including PTH, catecholamines, caffeine, and a forskolin-like molecule that has been found in ADPKD cyst fluid. We tried to account for as many of these confounding variables as possible by including only study participants with preserved kidney function and normal screening levels of intact PTH. Furthermore, all participants were instructed to abstain from caffeine through study duration. Nevertheless, differences in non-AVP-driven cAMP among ADPKD patients could explain why only some ADPKD patients suppressed urine cAMP during the chronic phase of the study. Another variable may be insufficient water intake to suppress AVP levels. We restricted our intervention to a single water intake prescription of 3 L per day on the basis of the safety of experience of water loading in kidney stone formers. One question that needs to be addressed is whether some patients may benefit from greater water intake, although, as discussed above, this will likely not be tolerated by most patients. Finally, urine cAMP may not closely reflect kidney cAMP levels in some patients, as has been demonstrated in some animal models (21). The mechanisms of intracellular cAMP efflux into the urine and metabolism are not well understood.

One potential application of urine cAMP measurements that would complement urine osmolality is to predict responders to water therapy. For example, our finding that 24-hour urine cAMP was suppressed in five of seven patients with highest urine cAMP levels (>2 nmol/mg Cr) suggests that stratification by baseline urine cAMP levels may identify a subgroup of ADPKD patients who stand to benefit the most from chronic water therapy.

In summary, we have shown that changes in urine osmolality and cAMP can be detected with acute water loading in ADPKD patients. Our findings raise the possibility that urine cAMP may complement urine osmolality and be used to predict therapeutic effect and to monitor response to sustained water loading in ADPKD. A larger longitudinal study will be needed to both test this hypothesis as well as determine the potential of water as therapy for ADPKD using hard endpoints, such as changes in MRI total kidney volume and estimated GFR.

Disclosures

None.

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