LOW OSMOLAR DIET AND ADJUSTED WATER INTAKE FOR
VASOPRESSIN SUPPRESSION IN ADPKD

A thesis
submitted by
Osama W. Amro, MD

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ADVISORS:
Thesis Committee Chair: Ronald Perrone, MD
Project Mentor: Mark Sarnak, MD
Project Mentor: Jessica Paulus, ScD
Statistical Mentor: Farzad Noubary, PhD
ABSTRACT

Autosomal dominant polycystic kidney disease (ADPKD) accounts for 10% of patients with end-stage renal disease in the United States. Vasopressin is a detrimental factor in disease progression. This randomized trial examined the effect of a novel approach of combining low osmolar diet and adjusted water intake on vasopressin as measured by change in plasma copeptin, and urinary osmolality in 34 patients with ADPKD.

Participants were randomized to receive a low osmolar diet (low sodium (1500 mg/day), low protein (0.8 gram/kg body weight) diet) followed by adjusted water intake to achieve a urine osmolality of ≤280 mOsm/L versus no intervention for two weeks duration, with equal (1:1) allocation. Permuted block randomization was performed within strata of age and sex.

Baseline characteristics of the two groups were similar. At 2 weeks, fasting plasma copeptin (primary outcome) declined from 6.2 ±3.05 to 5.3 ± 2.5 pmol/L (p=0.3) in the low osmolar diet group compared to a non-significant increase from 4.7±3.6 to 5.08±4 in the control group; the change in mean copeptin level from baseline was statistically significant between groups (p=0.009). At 2 weeks, there was a significant decline in urine osmolality from 426 ±193 to 258 ±117 mOsm/L in the low osmolar diet group compared to a non-significant increase from 329 ±159 to 349 ±139 in the control group. The change in mean urine osmolality level from baseline was statistically significant between groups (p=0.007). Total urinary solute decreased only in the low osmolar diet group and significantly differed between groups at 2 weeks (p=0.03). The adherence rate to diet and adjusted water intake was 70% with a mean water prescription of 2.6 liters/daily.
In conclusion, we identified a step wise dietary intervention that led to significant reduction in plasma vasopressin levels as measured by reduction in fasting plasma copeptin and 24 hour urine osmolality in patients with ADPKD. Furthermore, this dietary intervention led to reduction in water required for vasopressin suppression. Long-term studies are needed to evaluate the ability of patients to adhere to a reduced solute diet and adjusted water intake, and to determine if the reduction in vasopressin slows ADPKD progression.
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<table>
<thead>
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<th>Abbreviation</th>
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<tr>
<td>AVP</td>
<td>Arginine Vasopressin</td>
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<td>ADPKD</td>
<td>Autosomal Dominant Polycystic Kidney Disease</td>
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<td>BMI</td>
<td>Body Mass Index</td>
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<td>Cr</td>
<td>Creatinine</td>
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<td>CTRC</td>
<td>Clinical and Translation Research Center</td>
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<td>EDTA</td>
<td>Ethylenediaminetetraacetic Acid</td>
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<td>eGFR</td>
<td>Estimated Glomerular Filtration Rate</td>
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<td>ESRD</td>
<td>End Stage Renal Disease</td>
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<td>Gm</td>
<td>Gram</td>
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<td>IRB</td>
<td>Institutional Review Board</td>
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<td>Liter</td>
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<td>Milliequivalent</td>
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<td>ml</td>
<td>Milliliter</td>
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<td>mOsm</td>
<td>Milli-Osmoles</td>
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<tr>
<td>Na</td>
<td>Sodium</td>
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<tr>
<td>Osm</td>
<td>Osmole</td>
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<tr>
<td>PCK</td>
<td>Polycystic Kidney Rat</td>
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<tr>
<td>Pmol</td>
<td>Picomole</td>
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<tr>
<td>SSRI</td>
<td>Selective Serotonin Reuptake Inhibitor</td>
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<tr>
<td>TCA</td>
<td>Tricyclic Antidepressant</td>
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<td>TUHS</td>
<td>Tufts University Health Sciences Campus</td>
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INTRODUCTION

Autosomal dominant polycystic kidney disease (ADPKD) affects an estimated 600,000 persons in the United States and 12.5 million persons worldwide (1), and accounts for 8-10% of patients with end-stage renal disease (ESRD) in the United States and Europe (2). ADPKD affects many aspects of a patient’s physical and social life. The disease involves multiple organ systems in addition to major manifestations due to chronic kidney disease, and complications related to kidney and liver enlargement. ADPKD is a source of constant psychological and emotional stress for patients and families. In spite of promising therapies, treatments that prevent development of ESRD in ADPKD do not currently exist (3)

Patients with ADPKD have impaired urine concentrating ability and higher levels of arginine vasopressin (AVP) as compared to healthy controls (4). AVP is a potent activator of adenylyl cyclase in collecting duct cells and has an important role in the progression of ADPKD (5, 6). AVP-V2 receptor inhibition controls disease progression in both animal models and humans, as does genetic elimination of vasopressin in the Polycystic Kidney (PCK) rat; thereby providing further support for a detrimental role of vasopressin (6-8) and indicates a promising target for therapeutic intervention. Tolvaptan is the only clinically tested medication that blocks AVP-V2 receptors and it is associated with side effects including frequent urination, nocturia, hypernatremia, hyperuricemia and elevated liver enzymes.

One treatment that has been shown to suppress plasma levels of AVP and slow cyst progression in an animal model of polycystic kidney disease is high fluid intake (9). However, adherence to a high fluid intake diet is difficult to maintain in clinical
Part of the difficulty in sustaining a low AVP level with daily water ingestion is the consumption of a high osmolar diet (diet high in protein and salt) which stimulates AVP secretion to maintain water homeostasis (12). To address the adherence challenges associated with a fluid-based treatment, we have developed a novel stepwise approach of combining a low osmolar diet with adjusted water intake, with the goal of lowering the amount of water intake needed to suppress AVP secretion.

We conducted the first investigation of the effect of a low osmolar diet and adjusted water intake on AVP secretion, as measured by change in copeptin levels and urine osmolality, in subjects with ADPKD. Copeptin has been shown to be a reliable marker of AVP secretion (13, 14). The change in urine osmolality is used to assess the efficacy of vasopressin suppression (15). The long-term goal of this study is to develop a safe, easily tolerated and affordable intervention that can be adopted early in the ADPKD process to prevent permanent kidney damage and slow cyst progression. Given the large numbers of people affected by ADPKD and the substantial impact of the disease on mortality, morbidity, dialysis or transplant, and societal costs of caring for those patients, developing such a therapeutic approach has tremendous public health relevance and is relevant to clinical care.
MATERIALS AND METHODS

The major objective of this trial is to evaluate whether a stepwise approach of combining a low osmolar diet and adjusted water intake can suppress vasopressin secretion in patients with early ADPKD. Vasopressin suppression is assessed by measuring copeptin levels and changes in urine osmolality. Copeptin has been shown to be a reliable surrogate marker for the circulating AVP concentration.

2.1 Study Design

Randomized controlled trial in which 2 groups of patients with ADPKD were randomized to receive either a low osmolar diet* and adjusted water prescription versus no intervention, with equal (1:1) allocation. Permuted block randomization was performed within strata of age and sex to assure balance of these factors (16, 17).

*The low osmolar diet consisted of three components: low sodium diet (1500 mg/day), low protein (daily protein dietary allowance of 0.8 gram/kg body weight), and low urea (avoidance of preservatives, food additives, bulking agents, and chewing gum).

2.2 Study Population

Inclusion Criteria: Eligible patients were adults 18 to 60 years of age, who had ADPKD with an estimated glomerular filtration rate (eGFR) of 60 ml/min/1.73m² or above determined using the Chronic Kidney Disease Epidemiology Collaboration equation (18).
Exclusion Criteria: Patients with the following conditions or characteristics were excluded: chronic use of medications known to affect AVP secretion: (selective serotonin reuptake inhibitors (SSRI), opioids, tricyclic antidepressants (TCA) and tolvaptan), a history of conditions influencing renal concentrating capacity other than ADPKD (diabetes insipidus, adrenal or thyroid insufficiency, present or prior use of lithium), baseline hyponatremia (serum sodium below 135 mEq/l), presence of physical or cognitive impairments which prevent participation or consent, pregnancy (due to safety concerns (dilutional hyponatremia (19)), change in vasopressin level (20) and because their volume and metabolic status is markedly changed from non-gravid state (21)).

2.3 Regulatory approval

Tufts Medical Center institutional review board (IRB) approval was obtained on March 5th 2014.

The study was registered on Clinicaltrials.gov and updated throughout the study period (https://clinicaltrials.gov/show/NCT02225860).

2.4 Recruitment

Subjects were recruited by contacting patients from the Tufts Polycystic Kidney Disease Research Registry, Tufts IRB # 8891 (principal investigator: Ronald D. Perrone, MD).

The registry currently includes 356 subjects with ADPKD. All patients in the registry have previously consented to allow contact for future studies. The study was also marketed during the Polycystic Kidney Disease Walk in Boston (September 2014) and through the Tufts Medical Center Weekly Pulse newsletter (Appendix I: Marketing Materials).
Initial patient screening was done by reviewing Tufts PKD registry patients’ medical records. A letter (Appendix II: Study Letter) was sent to all potentially eligible subjects explaining the purpose of the study. The letter also indicated that the principal or co-investigator would be contacting them by phone (Appendix III: Telephone Script) to further discuss the study procedures and to determine their interest in participating in the study.

2.5 Informed Consent Process and Timing of Obtaining Consent

Subjects were consented in person for the study during initial visit, if they were deemed eligible at the time. We anticipated that most participants would decide and sign the consent forms at the time of the visit; however, participants had the opportunity to take the consent forms home and speak to study personnel or study physicians over the phone later if they requested additional time.

Legal guardian for informed consent

A legal guardian was not required during the consent process because only adult subjects cognitively capable of providing informed consent were allowed to participate in this research study.

Determination of ability to provide informed consent

Subjects with history of cognitive impairment were excluded. During the recruitment and consent process the research team member assessed the level of the potential subject’s cognitive ability. This was based on his/her ability to understand what the study would entail and exactly what would be required of them for participation; whether the
potential subject was able to answer and ask questions about the study; and whether he/she could demonstrate the ability to arrive in a timely manner and complete the health screening study without problems. Written consent was obtained from all participants at the beginning of the study.

Non-English speaking persons informed consent process

We enrolled one subject who was a non-English (Spanish) speaker. We followed the Tufts Short-Form policy for obtaining informed consent (http://viceprovost.tufts.edu/HSCIRB/short-form-policy/).

Vulnerable Populations

The study did not include vulnerable population. In particular, no children, economically or educationally disadvantaged, mentally disabled or otherwise cognitively impaired patients, wards of the state, or prisoners were included.

The study did not exclude any available patients on the basis of sex, race, or ethnicity. The Tufts Polycystic Kidney Disease Registry population, which is the source of subjects for this study, reflects patients seen at Tufts Medical Center (primarily from Massachusetts, but also from other New England states including Maine, New Hampshire, Vermont, Rhode Island, and Connecticut). Therefore, the sample population is fairly generalizable to the national ADPKD population, although there is a higher participation of Caucasians. Nonetheless, we made every effort to recruit minorities. Accordingly, we expected that the sample used for this study would include both men and women and patients from all races and ethnicities.

Justification for Exclusion of Children
Although the research topic studied is potentially relevant to children, we believe that adherence to dietary restrictions and water intake would be more challenging than with adults. Therefore, we excluded children from this initial proof of concept study. The intervention can be expanded to include younger individuals with enhanced monitoring and support in future studies.

**Study location**

Study procedures were conducted at the Tufts Clinical and Translational Research Center (CTRC) at Tufts Medical Center [mailing address: 800 Washington Street, Box 831, Boston, MA 02111; Office: 646 Farnsworth, 6th Floor, Boston, MA 02111]

**Block Randomization**

We chose a randomized block design to take into account age and sex which are known factors that affect the primary outcome (copeptin) but are not of primary interest. Using R statistical(22) package ‘blockrand’ a random assignment for the trial was created. The randomization was done within blocks so that the balance between treatments remained equal throughout the trial. In addition to stratification by sex, two equal blocks of age were used (18-<40, ≥40-60 years of age) for assignment stratification.

The software was then used to create a pdf file of randomization cards based on the output from 'blockrand'. This was then printed and the cards put into envelopes for assigning subjects to treatment.

**Transportation**
Patients were responsible for providing their own transportation.

**Registration**

Participants were registered in the study after the screening had been performed, the person was deemed eligible for participation in the study, and a study visit had been scheduled.

Subjects who agreed to participate in the trial, based on the phone conversation, were offered two options: an in-person screening clinical visit in which they could receive the container for 24 hour urine collection with instructions for appropriate collection method or, for their convenience, subjects were offered the option of receiving the container and instructions for 24 hour urine collection by mail. Subjects who elected to come for a screening visit signed the informed consent during that visit. Subjects who agreed to participate but elected to receive the container for urine collection by mail signed the consent during the baseline visit (see below). The following data were obtained and recorded from medical records for each subject who agreed to participate in the study: age, sex, race and ethnicity, serum creatinine, estimated glomerular filtration rate, serum sodium, serum potassium, list of medications, and radiology imaging studies documenting polycystic kidney disease.

**2.6 Study Timeline and Procedures**

The study duration of two weeks was chosen to ensure sustainability of a diet and water intake intervention and to account for variability of urine osmolality and plasma copeptin levels.
Three visits, each one week apart, were conducted for eligible subjects who provided informed consent (Figure 1).

At the 1st visit (baseline), subjects submitted a 24 hour urine collection and had blood collected. The intervention group received dietary counseling and was asked to adhere to a low osmolar diet. At the 2nd visit (week 1), subjects in both groups repeated a 24 hour urine collection and had their second blood draw. Patients from interventional group were given a water prescription to follow based on the results of their 2nd urine collection. At the 3rd visit (week 2), a final 24 urine collection and blood draw was performed.

- Subjects from both groups were asked to avoid caffeine intake for 24 hours prior to each blood draw as caffeine can increase vasopressin secretion (23, 24).

*Adjusted Water: Individualized water prescription based on total urine solute at visit 2

Figure 1. Study Timeline
Water Prescriptions for Intervention Group

A specific water prescription (Appendix IV: Water Prescription) was tailored for each patient in the intervention group based on the osmolar content of the 24 hour urine collection obtained after one week of the low osmolar diet; the water prescription was adjusted to produce a mean urine osmolality of 280 mOsm/L. Subjects were asked to spread water consumption throughout the day. The rationale for using 280 mOsm/L as a target is based on the knowledge that lowering urine osmolality below that of plasma will likely drive AVP secretion to lower levels. We used the following formula to calculate the prescribed daily water intake:

\[
\text{Total solute (mOsm)}
\]

\[
\text{Water Prescription (in liters):} \quad \frac{\text{Total solute (mOsm)}}{280 \text{ (mOsm/L)}}
\]

- Where: Total solute = urine volume (L) in 24 hours \times urine osmolality (mOsm/L)

An additional 500 ml water a day was added to each individual prescription to account for insensible losses.

Dietary Intervention and Control Group

The dietary intervention consisted of three elements: low sodium (1500 mg/day), low protein (daily protein dietary allowance of 0.8 gram/kg body weight), and low urea (avoidance of preservatives, food additives, bulking agents, and chewing gum).

Control subjects received no specific dietary intervention and were asked to follow their usual dietary intake of salt and water.
In order to enhance diet adherence, subjects from both study groups filled a two day food and water log before each study visit (Appendix V: Two Day Food and Water Log), the dietary recall information was used to provide individualized nutritional counseling for subjects in the intervention group. Written teaching materials were also provided (Appendix VI-VII: Dietary Counseling and Instruction for the Intervention Group).

2.7 Outcome Measures

The primary outcome measure is the difference in mean plasma copeptin level between baseline and the end of week 2, which reflects the combined effect of low osmolar diet and adjusted water intake. Copeptin level is a reflection of endogenous vasopressin production, measurement of vasopressin is problematic as more than 90% of vasopressin in the circulation is bound to platelets, AVP is unstable in isolated plasma, and most AVP assays have limited sensitivity (26).

Secondary outcome measures included total daily urinary solute reduction (a surrogate for diet adherence), total daily solute (total amount of osmoles detected in 24 hour urine collection), and the difference in mean plasma copeptin between baseline and the end of week 1 (which reflects the effect of low osmolar diet alone).

2.8 Measurements and Definitions

Participants have been diagnosed with ADPKD (Ravine(27)) and followed at the specialized PKD center at Tufts Medical Center. Complete medical records were available including imaging studies, and medication history. Patients also have previously completed a questionnaire on disease factors, family and social history, and lifestyle
including caffeine intake. At the study screening visit, the above information was verified with the patients to ensure accuracy. Height and weight were recorded and body mass index (BMI) was calculated. Measurements of sodium, potassium, urea, creatinine, and osmolality were performed in three 24-hour urine samples. Extensive verbal and written instructions were provided to patients on the collection procedure. Sodium, potassium and protein intake were estimated from the measurements of urine sodium, potassium and urea nitrogen respectively in the 24 hour urine collections (28, 29). Protein intake was factored by measured body weight.

**Laboratory Tests**

Standard biochemical evaluation was performed using fresh urine and plasma samples, using ARCHITECT ci8200 Integrated System (Abbot Diagnostics). GFR was estimated with the Chronic Kidney Disease Epidemiology Collaboration equation. Plasma and urine osmolality were measured directly via determination of freezing point depression (Advanced Instruments Osmometer 2020, Advanced Instruments, Inc).

**Copeptin Measurement**

Morning blood samples were collected during standardized hydration status (10 hours fasting) for all patients. Samples were collected into lavender vacutainer tubes (#VT-6450) which contain EDTA. Those tubes were gently rocked several times immediately after collection of blood for anti-coagulation. The blood was then transferred from the lavender vacutainer tubes to centrifuge tubes containing aprotinin (0.6TIU/ml of blood) and gently rocked several times to inhibit the activity of proteinases (we also processed a similar amount with no aprotonin). The blood then was centrifuged at 1,600 x
g for 15 minutes at 4°C and the plasma was collected and immediately frozen at -70°C for the duration of the study. Samples then were shipped in dry ice to a specialized laboratory (Groningen, Netherlands) for copeptin measurement. The lab has developed extensive experience in measuring copeptin over the years. Copeptin was measured using a sandwich immunoassay (B.R.A.H.M.S. AG/ThermoFisher), with a lower limit of detection of 0.4 pmol/L and functional assay sensitivity (defined as when the assay has a 20% interassay coefficient of variation), 1 pmol (30).

To minimize inter-assay variability samples were thawed at the same time by the same well trained lab technician with expertise in immunoassays. The technician was blinded to the groups’ assignments. To further minimize inter-assay variability, the 3 samples associated with each individual were tested using the same kit.

**Deviation from initial plan for copeptin measurement**

Our initial plan was to measure copeptin in the Clinical and Translational Research Center (CTRC) Core Laboratory using fluorescent enzyme immunoassay (Catalog # FEK-065-32, Phoenix Pharmaceuticals, INC.). However, we encountered problems with peptide extraction. We used two samples to explore the consistency and reliability of the kit, no consistent readings were obtained. We could not use the alternative well validated kit (B.R.A.H.M.S. AG/ThermoFisher) which does not require extraction as this kit is instrument specific (Kryptor Compact Plus, automated random-access immunoassay system) which we didn’t have at the CTRC core laboratory at Tufts.
2.9 Statistical Analysis

Primary analysis of the primary and secondary outcome was based on an intention to treat analysis. As a secondary approach, we conducted an as-treated and per-protocol analysis. Copeptin is measured in each subject at baseline and week 2 and the delta is calculated for all subjects. The delta copeptin between subjects in the intervention and control arms was compared with a two sample t test for independent groups. A significance level of $\alpha = 0.05$ with a two-sided alternative hypothesis was used. All analyses were performed using the R statistical package(22). We evaluated the correlations of 24 hour urine osmolality and copeptin using Pearson’s correlation coefficients.

Safety analysis: Typically, the safety analysis is based on as treated analysis since it better reflects the safety of the intervention. This was not done for this study as no adverse events including hyponatremia were recorded.

Per-protocol Analysis

Per-protocol analysis for the primary outcome (copeptin) was conducted by excluding subjects who deviated from the protocol. Specifically, we excluded 5 subjects from the intervention group who didn’t follow the water prescription given at visit 2 as evident by measured urine volume at visit 3. In this analysis we didn’t exclude subjects who didn’t follow the low osmolar diet part of the intervention but followed water prescription as the rationale for water adjustment is to compensate for incomplete adherence to low osmolar diet. Although the result of per protocol analysis provides a lower level of evidence, it better reflects the effect of low osmolar diet and adjusted water intake treatment when followed appropriately.
As Treated Analysis

Since our study is a proof of concept study, as treated analysis was conducted for the primary outcome as well. For this analysis we defined the treatment group as all subjects, regardless of their initial assignment, who adjusted their water intake based on total urine solute in visit 2 such that they achieved a urine osmolality of 280 mOsm/L or below at visit 3.

Missing Data

The magnitude of missing data is very low in this trial which is likely related to short study duration. One patient from the intervention group didn’t present for visit 2 and 3 citing job conflict. We used a conservative approach by assuming no change in urine osmolality for that patient, as this would bias our results towards the null.

Results Below the Level of Detection

Few urine sodium measurements (5 observations (4% of measured values in the study population)) were reported as below the detection level (<20 mEq/L). Those values were observed mainly (80%) in the intervention group. For analysis purposes, we took a conservative approach of assigning values of 20 mEq/L (limit of detection by Tufts laboratory) to all urinary sodium values below level of detection. As most values below the limit of detection were in the intervention group, this approach would bias the results towards the null.
Data Collection and Management

Study data were collected and managed using REDCap electronic data capture tools hosted at Tufts Clinical and Translational Science Institute (31).

Sample Size Justification

A power and sample size analysis was performed to determine the number of participants required for this study. The literature provides data on copeptin levels in ADPKD patients at an early stage of their disease compared to healthy controls (32): the median copeptin levels in the 2 groups were 14.74 pmol/ml (interquartile range (IQR): 7.47—18.96) in patients with ADPKD compared to 4.62 pmol/ml (3.42—7.84) in the control group. There are no data available comparing copeptin levels based on dietary habits and water intake, the primary parameter of interest; we therefore powered this study based on the available data comparing patients with ADPKD to healthy controls.
We use a 1:1 sample allocation ratio of patients in the intervention and control arms. Figure 2 illustrates the required sample size in each arm (the y-axis) to detect differences in mean plasma copeptin (pmol/L) between the intervention and control arms of varying magnitude at the end of the study. The differences described on the x-axis actually represent differences of differences, as we compute a within-group difference for each arm (baseline copeptin – week 2 copeptin) and then subsequently assess the between-group difference (intervention – control). If we assume that there will be no difference between baseline and week 2 in the control arm (within-group difference of 0), then the x-axis can then be simplified to the within-group difference in the intervention arm (baseline copeptin – week 2 copeptin).
Using the estimate of mean copeptin at baseline of 14.74 pmol/ml (32) and assuming that the lowest achievable mean copeptin after the intervention would be that previously observed among healthy controls (4.62 pmol/L), the mean difference (baseline copeptin – week 2 copeptin) we would expect in the intervention arm would be approximately 10.

If this were the case we would only require 9 and 11 patients in each arm to achieve 80% and 90% power, respectively. Using a more conservative approach, for an assumed mean difference in copeptin value of 6 in the intervention arm, we would require 20 and 26 patients in each arm to achieve 80% and 90% power, respectively. Assuming a 10% dropout rate, we planned to recruit 22 patients in each arm.

**Deviation from original plan for sample size**

17 patients were recruited in each arm instead of 22 due to time restraints. However, we noticed the dropout rate to be much lower than previously anticipated (2% compared to 10%). Enhanced adherence to the study protocol is possibly related to high patient motivation because of the familial nature of ADPKD(2).
2.10 Personnel Who Conducted the Study

Present during study procedure(s):

1- Osama Amro

2- Ronald Perrone

3- Nursing staff from Clinical and Translational Research Center (CTRC).

Roles in accomplishing the study:

**Ronald Perrone, MD:** Dr. Perrone is a world renowned expert in ADPKD, he has extensive experience in managing and conducting clinical trials and research related to ADPKD. Dr. Perrone met in person weekly with the research team to ensure that the research process was being productive, to provide mentoring and advice, and to address questions related to study conduct. Dr. Perrone also provided advice on technical aspects of the study and had the overall responsibility for the project.

**Osama Amro, MD:** Dr. Amro is a nephrology clinical research fellow. The research idea was developed jointly by Dr. Amro and Dr. Perrone. Drs. Amro and Perrone have continued to refine the proposal in subsequent discussions, formalized the research plan, and obtained regulatory approval. Dr. Amro performed the patient recruitment, and data examination, as well as the subsequent statistical analysis with the assistance of his statistical mentor Farzad Noubary, PhD. Dr. Amro designed detailed subject instructions for the low osmolar diet and water prescription.
2.11 Human Subjects Protection

Risk/benefit assessment

Patients are theoretically at risk of developing hyponatremia (serum Sodium (Na) below 135 mEq/l). This can happen if the kidneys diluting ability is overwhelmed due to combination of low osmolar intake along with high water ingestion. However, previous studies conducted on patients with ADPKD showed no dilution defect and safe administration of high water prescription was demonstrated (25).

To ensure patient safety, we monitored serum Na level weekly during the study period. The prescribed water intake is similar to those commonly used for prevention of nephrolithiasis.

Physical risk:

Blood collection: Risks associated with blood collections may include minor, temporary discomfort or pain directly associated with the needle stick itself (venipuncture), lightheadedness, bruising during or after the needles are placed, and (rarely) infection or inflammation of the vein. During each study visit, we collected 25 mL of blood. The risk due to the loss of this amount of blood is minimal.

Psychological, Social, and Economic Risks:

As with all research, there is a potential risk of loss of confidentiality due to participation in this study. Mishandling of interview data may lead to loss of confidentiality with regard to medical history and demographic information. As described below, specific protocols were developed for the handling, storage, and
transmission of subject information to ensure that privacy and confidentiality are maintained. There are no economic risks or costs to the subject. The subjects did not receive any stipend for completion of the study visits. The subject had to pay for any costs associated with traveling to the site of the study to participate in a study visit. All tests and procedures that were specified as part of this study were provided free of charge to the subject. Participation in the study or lack of it did not and will not affect the subject’s medical care.

**Benefit of participating in the study:**

There was no direct benefit to participating in this research study; however, subjects enrolled in this study received information about their disease and healthy diets. General information regarding a healthy balanced diet was given to both study groups, information pertinent to salt and water intake was given to intervention group only unless patients have a condition other than ADPKD that warrant salt restriction (hypertension, heart failure, etc.). If such conditions existed in subject from the control group, subject were instructed to limit sodium intake to 2 grams daily, no information regarding water intake was provided to the control group. Both groups had closer follow up for their kidney disease than usually provided to patients with a similar stage of kidney disease.

**Potential Benefit to the Population from which the Subject is Drawn:**

As discussed in the introduction, the prevalence of ADPKD is very high. Measures that can provide definitive data regarding dietary and water intake in relationship to ADPKD can set the floor for future treatment plan that eventually can alter the course of the disease.

**Potential Benefit to Science, Society and Humanity:**
This study will provide valuable information on copeptin levels with different dietary and water ingestion habits. This understanding may help to identify modifiable risk factors which are important to the treatment of ADPKD and other kidney diseases that are related to vasopressin secretion.

Costs

Subjects were not required to pay for any part of the procedures performed as part of our study. The only way in which payment might have been required is if subjects were to be injured during the course of the study and require medical care, which would have been the responsibility of the subject and/or their insurance carrier.

Payment for Participation in Research:

Subjects did not receive financial compensation for participation in the research.

Payment for a research-related injury: The subject’s insurance carrier was responsible to pay for any costs associated with such medical care. Any needed medical care was available at the usual cost. All needed facilities, emergency treatment, and professional services were available, just as they are to the general public. There were no plans to pay for treatment if someone gets hurt or sick as part of this study. The likelihood of harm to subjects was thought to be extremely low.

Outcome: The primary outcome of the study will be the acquired information on the level of copeptin in relation to dietary and water intake. This information will be relevant for clinical practice and therefore the primary measure of success is the ability to complete recruitment.

Alternatives: Participation is voluntary. An alternative would be for a subject not to participate.
Plan to convey information to the study subject

By direct communication with the patient through contact info that is obtained and updated through Polycystic Kidney Disease (PKD) Research Registry.

Withdrawal/Termination criteria

Subjects were allowed to withdraw at any point during the study. The principal investigator also had the option to withdraw the subject from the study for the following reasons (which include but are not limited to):

- The principal investigator feels it is not safe for the subject to continue in the study.
- The subject fails to follow the study instructions.
- The subject experiences an adverse reaction that requires other medical treatment such as but not limited to development of hyponatremia (Na <135) during the study period.

No additional safety precautions are required if a subject withdraws/is withdrawn from the study.

Assessment of Subject Safety and Development of a Data and Safety Monitoring Plan

Adverse Event Monitoring

Study subjects were monitored for the occurrence of events defined as any undesirable experience over the course of the study visit. The main potential risk posed to study subjects was the possibility of hyponatremia. Symptoms of hyponatremia are most likely to be experienced after administration of water prescription. Patients were educated about the symptoms of hyponatremia and were asked to report any unusual
symptoms to the primary investigator, as a safety measure, baseline and 2 weeks serum sodium were obtained during the study. An adverse event ascertainment form (Appendix VIII: Adverse Events Ascertainment Form) was administered to each participant at the beginning of the study. The questionnaire contains questions related to specific symptoms as well as time of occurrence, severity, required interventions, and subject’s condition after the event.

Classification of adverse events

**Not related:** The event is clearly related to factors such as the subject’s clinical state, not to interventions associated with the study protocol.

**Remote:** The event is most likely related to factors such as the subject’s clinical state, not to interventions associated with the study protocol.

**Possible:** The event follows a reasonable temporal sequence from interventions associated with the study protocol but is possibly related to factors such as the subject’s clinical state.

**Probable:** The event follows a reasonable temporal sequence from interventions associated with the study protocol and cannot be reasonably explained by factors such as the subject’s clinical state.

**Highly Probable:** The event follows a reasonable temporal sequence from interventions associated with the study protocol and cannot be reasonably explained by factors such as the subject’s clinical state.

The severity of an adverse event is defined as a qualitative assessment of the degree of intensity of an adverse event. The principal investigator will also determine the severity as follows:
Mild: Does not impact (in any way) the subject’s life.

Moderate: Impacts the subject’s life but is not life-threatening or incapacitating.

Severe: Fatal, life threatening, permanently disabling; severely incapacitating; requires/prolongs inpatient hospitalization.

Unanticipated Problem Definition: An Unanticipated Problem is an incident, experience, or outcome that meets all of the following criteria:

1. The nature, severity, or frequency is unexpected for the subject population or research activities as described in the current IRB approved protocol.

2. It is related or possibly related to participation in the research.

3. It suggests the research may place the subject or others at a greater risk of harm then was previously recognized.

All three of the criteria in the definition above must be met to be an Unanticipated Problem.

Reporting Adverse Events Plan

A. All Unanticipated Problems were to be promptly reported to the IRB. Reports, when required, would have been submitted to the IRB for each event occurring for each subject individually using the Tuft Medical Center (MC)/Tufts University Health Sciences Campus (TUHS) IRB Event Reporting Form. All supporting documentation would have been attached to the Event Reporting Form.

Action Plan for Each Unanticipated Problem:

1. Immediate corrective action would have been taken to eliminate or minimize risk to enrolled subjects. This could necessitate a voluntary hold on further enrollment and/or research activities for already enrolled subjects. If subjects were at immediate risk,
these corrective actions would have been initiated immediately, and if necessary for subject safety, simultaneous with completion of reporting requirements. In such an instance, the PI would have immediately called the IRB office.

2. Enrollment of new subjects would have been voluntarily stopped until a revised protocol and/or Informed Consent Form (ICF) are reviewed and approved by the Tufts Medical Center/TUHS IRB.

3. The problem would have promptly reported to the Tufts Medical Center/TUHS IRB, the study sponsor, and all data monitoring entities involved with the study.

An initial report to the Tufts MC/TUHS IRB would have been submitted in writing no later than two business days after the PI/study team become aware of the problem. This report is to briefly summarize the nature of the event, summarize the corrective action plan as developed and initiated at that time, and clarify whether subject enrollment is continuing. In the rare circumstance where an original written report could not be submitted directly to the IRB office, it would have been faxed within 2 business days (617-636-8394). The IRB office also would have been contacted by phone at 617-636-7512 for necessary guidance.

An Event Reporting Form would have been completed with accompanying documentation addressing each item in this list and submitted to the Tufts MC/TUHS IRB no later than five business days after the PI/study team became aware of the problem.

1. A severe adverse effect not meeting criteria for an Unanticipated Problem would have been reported to the Tufts Medical Center/TUHS IRB within fifteen business days of the PI/research team learning of the event.
If changes are required to the protocol and/or ICF, subject enrollment and study activities related to the adverse event, and not necessary for subject safety, would not have continued until the changes have been reviewed and approved by the Tufts Medical Center/TUHS IRB.

2. Mild and Moderate Adverse Events not meeting criteria of Unanticipated Problems would have been summarized and submitted to the Tufts Medical Center/TUHS IRB at the time of the continuing review, or when the PI terminates the study if this were to occur before the date of the next continuing review.

Procedures to protect subject confidentiality

Participants in the study are assigned a unique ID number; thereafter, the subject’s ID number is used as a coded identifier for the purpose of the study. Whenever possible, subject ID numbers were used in place of names and contact information on hard-copy forms. Any information linking the ID number and personal identifiers are kept in a password-protected computer file in the Nephrology division at Tufts Medical Center. The principal investigator, Dr. Ronald Perrone, have overall responsibility for the security and accessibility of the databases. All participant documents or samples are identified with a study ID. The code is kept in a password-protected file.

Analyses are performed at Tufts Medical Center. An electronic copy of all data is kept in a password protected file on the computer database in the nephrology division at Tufts Medical Center during analysis.

Confidentiality

Individual subject data was de-identified.
The key to the de-identified data is maintained by the principal investigators, Ronald Perrone and Osama Amro, they will not provide the key to any Tufts Medical Center researchers and the Tufts Medical Center researchers will not request access to the key. Therefore, the risk associated with identification of individual persons is low. All hard copies of data collected during the study visits are stored in a locked file cabinet at the Nephrology division to which access is limited to specific individuals (principal investigator, co-investigator, study coordinator, biostatistician, and their delegates).

**Data coding, recording, and storage:**

Information is stored in REDCap and one of the division office computers which is protected by password known to the primary investigators only, the door to the division is access restricted. REDCap is a secure web application for building and managing online surveys and databases.

**Parties who have access to the data, including the key to the identity code:**

Ronald Perrone, Osama Amro.

**Parties who have access to research records:**

Ronald Perrone, Osama Amro.

**Certificate of Confidentiality:**

Not required for this study

**Tissue banking considerations:**

Not applicable
RESULTS

Of 356 patients screened from the Tufts PKD registry, 272 did not meet inclusion criteria due to age (n=61), low eGFR (n=172), SSRI/opioid use (n=20), or other reasons (n=15). 84 patients were approached of which 40 declined to participate and 10 could not be reached. Thus, 34 patients (28 women) were randomized from May 2014 to April 2015 (Figure 3): with 17 patients randomized to low osmolar diet followed by adjusted water intake and 17 patients randomized to receive no dietary intervention. All patients completed the study protocol except one patient from the low osmolar diet group who withdrew citing job conflict.
Figure 3. Consolidated Standards of Reporting Trials diagram. Of the 34 patients who were randomized, 97% (n=33/34) completed the study and were included for analysis. Reason for one patient withdrawal was conflict with job.
3.1 Baseline Characteristics

Baseline characteristics of the two groups were similar (Table 1) except for mean 24 hour urine volume which was statistically significantly higher in the control group (2726 ml) compared to low osmolar diet group (1903 ml).
Table 1. Baseline clinical and laboratory characteristics of study participants (n=34)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Randomization Assignment</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low osmolar diet (n = 17)</td>
<td>Control (n = 17)</td>
</tr>
<tr>
<td><strong>Socio-demographic characteristics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, (years)</td>
<td>43.1 ±13</td>
<td>44.4±9.8</td>
</tr>
<tr>
<td>Male gender</td>
<td>3(17)</td>
<td>3(17)</td>
</tr>
<tr>
<td>Race/Ethnicity*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>12(70)</td>
<td>14(80)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>4 (23)</td>
<td>2(13)</td>
</tr>
<tr>
<td>Asian/Pacific Islander</td>
<td>1(5)</td>
<td>1(5)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>68.5±15</td>
<td>68.9±14.6</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.4(5.6)</td>
<td>24.1(4.0)</td>
</tr>
<tr>
<td><strong>Comorbidities</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>7 (41)</td>
<td>6(35)</td>
</tr>
<tr>
<td>Diuretic use</td>
<td>1(5)</td>
<td>1(5)</td>
</tr>
<tr>
<td><strong>Laboratory Data</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>0.86±0.14</td>
<td>0.81±0.12</td>
</tr>
<tr>
<td>eGFR (ml/min/1.73m²)†</td>
<td>89.3±19</td>
<td>91.8 ± 16</td>
</tr>
<tr>
<td>Serum sodium (mEq/L)</td>
<td>141.9±2</td>
<td>141.8±1.6</td>
</tr>
<tr>
<td>Plasma osmolality (mOsm/L)</td>
<td>295±2.7</td>
<td>293.5 ± 3.5</td>
</tr>
<tr>
<td>Urine volume (ml)</td>
<td>1903 ±723</td>
<td>2726 ± 995</td>
</tr>
<tr>
<td>Total solute intake (mOsm/day) ‡</td>
<td>722 ±254</td>
<td>856±410</td>
</tr>
<tr>
<td>Urine osmolality</td>
<td>426 ±193</td>
<td>329 ±151</td>
</tr>
<tr>
<td>Sodium intake (mg/day)</td>
<td>2909 ±1300</td>
<td>3591±2645</td>
</tr>
<tr>
<td>Protein intake (gm/kg/day) §</td>
<td>1.00 ±0.24</td>
<td>1.12 ±0.34</td>
</tr>
<tr>
<td>Fasting copeptin (pmol/L )</td>
<td>6.2 ±3.05</td>
<td>4.7±3.6</td>
</tr>
</tbody>
</table>

Data are presented as n (%), or mean ± SD.
*Race and ethnicity are self-reported
† GFR was estimated using the Chronic Kidney Disease Epidemiology Collaboration equation
‡ Total solute = urine volume (L) in 24 hours X urine osmolality (mOsm/L)
§ Sodium, and protein intake were estimated from measurements of urine sodium, and urea nitrogen respectively in 24 hour urine collection
3.2 Diet and Water Intake Adherence

Estimation of dietary sodium and protein intake as measured in 24 hour urine collections indicated good adherence to sodium and protein targets. Compared with baseline, the mean daily sodium and protein intake declined at week 1 from 2909 (±1300) to 1937 (±1044) mg and from 1.0 (±0.24) to 0.9 (±0.24) gm/kg body weight, respectively. Sodium and protein intake remained stable in the control group (3591±2645 mg to 3546±2453 and from 1.1 (±0.34) to 1.19 (±0.45) respectively). Achievement of target urine osmolality (≤280 mOsm/L) as the result of combined low salt, low protein diet and adjusted water intake adherence at week 2, with 70% of patients from low osmolar diet group achieving target urine osmolality compared to 30% in the control group (Figure 4).

![Achieved Target Urine Osmolality](image)

Figure 4. Diet and water adherence as measured by achievement of target urine osmolality (≤280mOsm/L) at week 2 (binary outcome).
3.3 Effect of Low Osmolar Diet on Fasting Copeptin and Other Outcomes

Fasting plasma copeptin and urine osmolality are shown in Figure 5. Baseline plasma copeptin was not significantly different between the low osmolar diet and the control group (6.2 ±3.05 and 4.7 ±3.6 pmol/L respectively, p = 0.9). At visit 2, there was a non-significant increase in plasma copeptin to 7.1 ±5.6 in the low osmolar diet group and to 6.1 ±5.5 in the control group. The change in mean plasma copeptin level between baseline and visit 2 was not statistically significant between groups. At visit 3, copeptin decreased to 5.3± 2.5 (p=0.3) in the low osmolar diet group compared to non-significant increase to 5.07 ±4 in the control group, the change in mean plasma copeptin level between baseline and visit 3 was statistically significant between groups (0.86 pmole/L in the low osmolar diet group compared to -0.39 in the control group, p=0.009).

A similar trend was observed with urine osmolality. Baseline urine osmolality was not significantly different between the low osmolar diet and control group (426 ±193 and 329 ±151 respectively, p = 0.11). At visit 2, there was a non-significant decrease in urine osmolality from 426 ±193 to 353 ±117 mOsm/L in the low osmolar diet group compared to the observed change in the control group (from 329 ±159 to 365 ±159) (p=0.06). At visit 3, there was a significant decline in urine osmolality from 426 ±193 to 258 ±117 (p=0.003) in the low osmolar diet group compared to a non-significant increase in the control group from 329 ±159 to 349 ±139. The change in mean urine osmolality level between baseline and visit 3 was statistically significant between groups (167mOsm/L in the low osmolar diet group compared to -20 in the control group=0.007).
Figure 5.

Panel A: Urine osmolality and plasma copeptin compared to baseline in the two study groups. In the low osmolar diet group, plasma copeptin declined by 15 % by visit 3 compared to a 7% increase in the control group reflecting the effect of combined low osmolar diet and adjusted water. In the low osmolar diet group, the 24 hour mean urine osmolality dropped by 17% at visit 2 reflecting the effect of diet alone and by 40% on visit 3 reflecting the combined effect of low osmolar diet and adjusted water intake. Urine osmolality stayed relatively stable in the control group during the study period.

Panel B: Low osmolar diet decreased plasma copeptin and urine osmolality at 2 weeks. The mean 24 hour urine osmolality and plasma copeptin in each individual patient in control (left) and low osmolar diet (right) groups before (visit 1) and after 2 weeks of assignment (visit 3). The purple line represents the mean. V, visit; Uosm, urine osmolality.
Urinary solute (measured in 24 hour urine collection) decreased only in the low osmolar diet group (Figure 6). A significant difference between the two groups was noted at visit 2 (p = 0.03). The decline in total urinary solute (as a proxy for dietary solute intake) led to a significant change in water requirement in order to achieve the target urine osmolality for the intervention group. Mean water prescription for the intervention group at visit 2 was 2.6 L/daily (compared to 3.2 required at baseline). In the control group, urinary solute was not statistically different between the three visits. Table 2 shows individual total solute intake and water requirement in the low osmolar diet group.
Figure 6. Change in total solute* and corresponding water requirement† at visit 2 needed to reach a target urine osmolality of 280 mOsm/L

* Total solute = urine volume (L) in 24 hours X urine osmolality (mOsm/L).

† Including 0.5 liter for each individual to account for insensible loss. Urine osmolality (Uosm)
Table 2. *Individual Total Solute Intake and Water Requirement in the Low Osmolar Diet Group*

<table>
<thead>
<tr>
<th>Subject (n=16)</th>
<th>Baseline Total Solute* (Liters/Day)</th>
<th>Water Requirement† (Liters/Day)</th>
<th>Total Solute Following Low Osmolar Diet‡ (mOsm)</th>
<th>Water Requirement (Liters/Day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>1140</td>
<td>4.57</td>
<td>546</td>
<td>2.45</td>
</tr>
<tr>
<td>4</td>
<td>661</td>
<td>2.86</td>
<td>477</td>
<td>2.2</td>
</tr>
<tr>
<td>5</td>
<td>981</td>
<td>4.0</td>
<td>451</td>
<td>2.11</td>
</tr>
<tr>
<td>8</td>
<td>681</td>
<td>2.93</td>
<td>546</td>
<td>2.45</td>
</tr>
<tr>
<td>10</td>
<td>713</td>
<td>3.04</td>
<td>468</td>
<td>2.17</td>
</tr>
<tr>
<td>13</td>
<td>561</td>
<td>2.5</td>
<td>494</td>
<td>2.26</td>
</tr>
<tr>
<td>15</td>
<td>444</td>
<td>2.08</td>
<td>496</td>
<td>2.26</td>
</tr>
<tr>
<td>16</td>
<td>1266</td>
<td>5.02</td>
<td>871</td>
<td>3.61</td>
</tr>
<tr>
<td>17</td>
<td>379</td>
<td>1.85</td>
<td>463</td>
<td>2.15</td>
</tr>
<tr>
<td>18</td>
<td>550</td>
<td>2.46</td>
<td>299</td>
<td>1.56</td>
</tr>
<tr>
<td>20</td>
<td>724</td>
<td>3.09</td>
<td>282</td>
<td>1.5</td>
</tr>
<tr>
<td>22</td>
<td>542</td>
<td>2.43</td>
<td>696</td>
<td>3.05</td>
</tr>
<tr>
<td>23</td>
<td>1131</td>
<td>4.54</td>
<td>901</td>
<td>3.71</td>
</tr>
<tr>
<td>29</td>
<td>601</td>
<td>2.65</td>
<td>524</td>
<td>2.37</td>
</tr>
<tr>
<td>31</td>
<td>655</td>
<td>2.84</td>
<td>514</td>
<td>2.33</td>
</tr>
<tr>
<td>32</td>
<td>683</td>
<td>2.94</td>
<td>512</td>
<td>2.33</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>732 ±260</td>
<td>3.2 ±0.9</td>
<td>534 ±166</td>
<td>2.4 ±0.6</td>
</tr>
</tbody>
</table>

* Total solute = urine volume (L) in 24 hours X urine osmolality (mOsm/L)

† Water needed to achieve a 24 hour urine osmolality of ≤280 mOsm/L

‡ The lowest total solute achieved during the 2 week study period
3.4. Plasma Copeptin and Urine Osmolality Correlation

Plasma copeptin levels were directly correlated with 24 hour urine osmolality at baseline and throughout the study (Pearson’s r = 0.4, p<0.001) (Figure 7).

![Figure 7. Correlation between 24 hour urine osmolality and fasting plasma copeptin. Plasma copeptin levels were directly correlated with 24 hour urine osmolality at baseline and throughout the study (Pearson’s r = 0.4, p<0.001).](image)

3.5 As Treated and Per-protocol Analysis

The effect of the low osmolar diet on plasma copeptin was magnified when an as treated analysis was performed. Patients who achieved target urine osmolality of ≤280 at visit 3
had a mean copeptin level of 3.9 ±1.4 compared to 6.3 ±4.1 for those who didn’t achieve
the target urine osmolality (p=0.001). After excluding 5 patients from the intervention
group who didn’t follow water prescription protocol, mean plasma copeptin at week 2
decreased significantly from 5.4±2.5 to 4.5±1.9 in the low osmolar diet group compared
to an no significant change (4.7±3.6 to 5.08±4) in the control group (p=0.004).

3.6 Special case (Monozygotic Twins)
Two of the randomized patients were identical (monozygotic) twins who shared a
residence. By virtue of randomization, each twin was assigned to a different study group.
Baseline urine osmolality for each individual was similar (649 and 616 mOsm/L),
reflecting similarity of total daily dietary and fluid intake. Following low osmolar diet
and water prescription, the twin who was randomized to the intervention group had a
reduction in urine osmolality and plasma copeptin from 649 to 120 mOsm/L and from
11.17 to 8.0 pmol/L respectively at week 2 while no change was observed with the twin
randomized to the control group (616 to 627 mOsm/L and 17.4 to 19 pmol/L)

DISCUSSION AND FUTURE DIRECTIONS
In this randomized, controlled trial, we identified a novel dietary approach that
achieved a significant reduction in vasopressin secretion as measured by reduction in
plasma copeptin and urine osmolality after two weeks in patients with early ADPKD.

The idea of the trial originated from animal and human studies that showed
detrimental effects of vasopressin in ADPKD progression. To date, the only therapeutic
approach that is being evaluated to target vasopressin in ADPKD is vasopressin blockage
with medication (tolvaptan). In a large randomized placebo controlled trial (TEMPO 3:4),
tolvaptan reduced the rates of total kidney volume increase and the decline in kidney
function over a 3-year period in ADPKD patients, but was associated with a high (23% in the
tolvapan group, vs. 14% in the placebo group) discontinuation rate, due to
aquaresis (excretion of electrolyte-free water) related side effects including polyuria and
excessive thirst(33). Furthermore, concerns have been raised regarding the long term
safety of this medication due to reversible elevation of transaminases that were observed
in a 1.2 % of subjects. Thus, there is a need for a sustainable, safe, and well-tolerated
therapeutic intervention for individuals with ADPKD.

While it is well known that high water intake suppresses vasopressin, to date no
randomized trials have evaluated such an approach in patients with ADPKD due to the
widely held assumption that the water requirement needed to achieve significant
vasopressin suppression is prohibitively high and not realistic in clinical practice.
However, in this study, we were able to show that by targeting both diet (solute) and
water intake in a stepwise individualized manner, a significant reduction in urine
osmolality can be achieved with a reasonable amount of water intake.

Part of the difficulty in sustaining a low vasopressin level with daily water
ingestion is the consumption of a diet that generates high osmoles; high osmolar load
stimulates vasopressin secretion to maintain water homeostasis. For the most part, daily
intake of water and osmoles is not determined by physiologic requirements but is more a
function of dietary preferences and cultural influences. Healthy adults have an average
daily fluid ingestion of approximately 1 to 3 L, but with considerable individual
variation. Approximately one third of this is derived from food or the fat metabolism, and
the rest is from discretionary ingestion of fluids. Similarly, of the 1200 milliosmoles of solute typically ingested or generated by the metabolism of nutrients each day in the typical American diet, nearly 40% is intrinsic to food, another 35% is added to food in the form of salt as a preservative or flavoring, and the rest is mostly urea derived from protein metabolism(12). In this trial, data from the control arm showed a daily fluid ingestion of over 3 L per day, which is above average daily fluid ingestion of the average American (1.2 liters/day) (34). This modest difference between healthy average adults and the study’s ADPKD population may be related to dietary counseling and behavioral health practices specific to individuals with ADPKD.

To the best of our knowledge, this trial is the first to describe a dietary approach that led to significant reduction in water requirement needed to suppress vasopressin for patients with ADPKD. The stepwise approach of reducing salt and protein intake followed by adjusting water intake led to significant reduction of water requirements (2.6 L) needed to achieve target urine osmolality (≤280mOsm/L). This amount of water is reasonable in clinical practice and is likely to be well tolerated by a large number of patients.

Our approach showed a robust decrease in urine osmolality which is used to assess the efficacy of vasopressin suppression or blockage. In fact, the reduction in mean urine osmolality achieved in this trial was equivalent to the one achieved with Tolvaptan in TEMPO trial (426 to 258 compared to 472-264 mOsm/L) (15, 33). It is important to point out that low urine osmolality can be a sign of advanced ADPKD with significant urinary concentrating defect and therefore, the change in osmolality rather than the actual level is more important to assess the efficacy of therapeutic interventions targeting...
vasopressin especially over a short period of time. Participants from this trial had preserved eGFR, thus the potential effect of defective urinary concentrating ability is likely not significant.

High copeptin levels are associated with disease progression in ADPKD as shown in a large observational study (35). This finding is consistent with what is known about the pathophysiological role of vasopressin in ADPKD. Our trial identified a dietary approach that can reduce copeptin and presumably vasopressin levels over a relatively short duration. Therefore, copeptin may prove to be a valuable biomarker to predict the risk of disease progression and to monitor the effectiveness of therapies and lifestyle modifications targeting vasopression. The biomarker might be exceptionally important in patients with advanced ADPKD when the effect of defective urinary concentrating ability precludes utilization of urine osmolality as a surrogate for vasopressin suppression. This study found a direct association between plasma copeptin and 24 hour urine osmolality. This is likely related to the fact that we are comparing fasting copeptin at a single point of time to an average urine osmolality which is a reflection of overall vasopressin effect over a 24 hour duration.

The major limitations of this trial are its small sample size and short follow up duration. However, given that water homeostasis is expected to be achieved in 48 hours, we anticipate that the study duration was sufficient to reflect true change in vasopressin secretion. The relatively small sample size reflects the goal of this trial as a proof of concept. As adherence to diet and water is difficult, patients were provided with questionnaires as well as individualized counseling by the investigators to reinforce dietary adherence (48-hour dietary recall (36)). The study did not explore the long term
adherence rate to the diet and water prescription, and future studies are needed to explore
the adoption and maintenance of such approach in clinical practice. Copeptin daily
variability may have affected the study outcome; therefore copeptin level measurements
were done in the fasting state. Major strengths were the controlled randomized design
with limited potential for confounding and other biases, the novel step wise approach
with water prescription designed to compensate for the incomplete adherence of low
protein and law salt diet, and the rigorous monitoring of study participants with
objectives measurements of study outcomes.

In conclusion, we developed a novel step wise dietary intervention that led to
significant reduction in vasopressin secretion as measured by reduction in plasma
copeptin and 24 hour urine osmolality in patients with early ADPKD. Furthermore, this
dietary intervention led to significant reduction in water required for vasopressin
suppression. Long-term studies are needed to evaluate diet and adjusted water intake
adherence, and determine if the reduction in vasopressin slows ADPKD progression.

**Funding:** Dr. Amro is supported by NIH 5T32DK007777 institutional training
grant (PI Levey).

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official views of the NIH.

**Financial Disclosure:** None.
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6.1 Appendix I: Marketing Materials
Study Brochure

Facts about PKD

- PKD is one of the most common life-threatening genetic diseases
- PKD causes fluid-filled cysts to grow in the kidneys
- Normal kidneys are the size of a fist, while PKD kidneys can grow up to the size of a football and weigh over 30lbs
- Around 50% of people with PKD will develop kidney failure by the age of 55
- Common PKD symptoms:
  - High blood pressure
  - Kidney stones
  - Blood in the urine
  - Chronic pain in the back and side of the stomach
- Parents with the ADPKD have a 50% chance of passing the disease to their children
- There is currently no treatment or cure for PKD

Diet as a Potential Treatment for PKD

A Clinical Research Study to investigate
Diet and Adjusted Water intake As a Potential Treatment for Polycystic Kidney Disease

Tufts Medical Center
Division of Nephrology
PKD Center
Ronald Perrone, MD
Osama Amro, MD
800 Washington St, Box 391
Boston, MA 02111
Phone: 617.636.6424
Fax: 617.636.8429
rperonne@tuftsmedicalcenter.org
oamro@tuftsmedicalcenter.org

For more information on Polycystic Kidney Disease, please contact the
PKD FOUNDATION
1-800-PKD-CURE
Or www.pkd-cure.org
Tufts Weekly Pulse Advertisement

Nephrology Team Raises Awareness for PKD through Studies

In recognition of PKD Awareness Day (Thursday, September 4), Tufts MC’s Division of Nephrology is conducting two studies to raise awareness and find treatments for autosomal dominant polycystic kidney disease (ADPKD), a life-threatening, genetic disease.

1. Tolvaptan REPRISE Study. Tolvaptan (Samsca®), a drug approved to treat hyponatremia (low sodium in the blood), is studied as a possible treatment for ADPKD. For people with ADPKD, the kidneys respond abnormally to the hormone vasopressin which may be involved in cyst development/growth in humans. Tolvaptan interferes with the effect vasopressin has on the kidney and reduces cyst growth when given to animal models of ADPKD. Its effects will continue to be studied in humans to further test how useful Tolvaptan will be in treating ADPKD.

2. Diet in ADPKD. The purpose of this study is to learn if dietary habits can affect vasopressin secretion in patients with ADPKD. Vasopressin increases the growth of kidney cysts and accelerates disease progression. Understanding how to control secretion of this hormone based on dietary habits may help to develop treatments to control this disease.

These studies will be conducted on patients or friends who qualify. To learn more, contact Clinical Research Coordinator II Elise Hoover at ext. 6-7914 or ehoover@tuftsmedicalcenter.org or Fellow Osama Amro at ext. 6-8424 or oamro@tuftsmedicalcenter.org.
6.2 Appendix II: Study Letter

[insert date]

Dear [insert name]

You are being invited to take part in a research study for patients with autosomal dominant polycystic kidney disease. The purpose of this study is to investigate the role of diet and a hormone (Vasopressin) in relationship to polycystic kidney disease. Vasopressin is a hormone that helps to regulate excretion of water by the kidneys. When you drink large amounts of fluid, vasopressin is not produced and the kidneys make large amounts of dilute (clear) urine. When you don’t drink enough fluid, vasopressin is produced and the kidneys make small amounts of concentrated (dark yellow) urine. Understanding this hormone’s secretion based on dietary habits may help to develop treatments to control this disease.

The study will include about 60 patients from Tufts Medical Center. The study will last for 2 weeks. Blood and urine tests will be done 3 times during the study period. Your medical records will be reviewed. Your participation in this study is completely voluntary. You can decide to not be in this study, or stop being in this study at any time for any reason. If you decide not to be in this study it will not affect the care you get at Tufts Medical Center.

Included with this letter is an informed consent for participation in the study; the form includes details about the study procedures and participation requirements. Dr. Perrone or Dr. Amro will contact you by phone in few days to see if you are interested in participating in the study and to further discuss the study procedure.

Sincerely

Dr. Ronald Perrone     Dr. Osama Amro

Principal Investigator: Ronald D. Perrone, M.D.

Co-investigator: Osama W. Amro, M.D.
Office: 617-636-8424; Tufts Medical Center Page Operator: 617-636-5114 (ask for beeper 3097)
6.3 Appendix III: Telephone Script

Hello, my name is______________. I am a nephrology (fellow/doctor) and a researcher from the Nephrology Division of Tufts Medical Center Boston who works with Dr. Perrone. You are being invited to take part in a research study for patients with autosomal dominant polycystic kidney disease. Would you be interested to hear more about the study?

[IF NO] Thank you for your time. Good-bye.

[IF YES] Continue

Please feel free to ask questions about the study at any time during this call.

The study title is (Low Osmolar Diet and Adjusted Water Intake for Vasopressin Suppression in ADPKD)

The purpose of this study is to investigate the role of diet and a hormone (Vasopressin) in relationship to polycystic kidney disease. Vasopressin is a hormone that helps to regulate excretion of water by the kidneys. When you drink large amounts of fluid, vasopressin is not produced and the kidneys make large amounts of dilute (clear) urine. When you don’t drink enough fluid, vasopressin is produced and the kidneys make small amounts of concentrated (dark yellow) urine. Understanding this hormone’s secretion based on dietary habits may help to develop treatments to control this disease.

The study will include about 60 patients from Tufts Medical Center. The study will last for 2 weeks. Blood and urine tests will be done 3 times during the study period. Your medical records will be reviewed. Your participation in this study is completely voluntary. You can decide to not be in this study, or stop being in this study at any time for any reason. If you decide not to be in this study it will not affect the care you get at Tufts Medical Center.
6.4 Appendix IV: Water Prescription

Name: 
Date: 

Water Prescription

Below is the minimum amount of water that you need to drink every day.
The amount of water was determined based on the salt content of your urine.
Please try to distribute the amount of water through the day and if awake at night.
You should drink _______ Liters every day
This means you have to drink at least

- _____ 12 oz glasses of water
  Or
- _____ 330 ml (11.2 FL oz) bottled water
  Or
- _____ 500 ml (16.9 FL oz) bottled water
  Or
- _____ 1000 ml (33.8 FL oz) bottled water

Note:
Water prescription refer to water intake only (tap or bottled water) with no additions, you are allowed to drink any additional fluid if desired, this include but is not limited to vitamin water, Gatorade, tea, juice, etc.
6.5 Appendix V: Two Day Food and Water Log

Two Day Food and Water Log

Please write down everything you eat and drink for 2 typical days. Try to include at least one weekend day - Saturday or Sunday.

Record this in the columns marked Food, Water and Beverages.

Record only the amount that is actually consumed, not the amount served.

Record the brand name and method of cooking in the "Method of Preparation / Brand Name" column.

Under ‘Amount’ please record in ‘teaspoons’, ‘cups’, or fractions of these. You may use ‘slices’ or ‘pieces’ when necessary. If something eaten has a specific measurement for sodium on the label, record that amount. For example, Sodium content in 2 tablespoon of canned tomato paste is 25 mg.

It is important to remember the following while recording different types of food:

Water: This refers to water intake only (tap or bottled) with no additions, record the amount in ‘cups’, ‘bottles’ or ounces

Beverages and Liquids other than water: Record the amount of all beverages other than water in ‘cups’ or ‘ounces’. This includes but is not limited to vitamin water, Gatorade, juice, tea, etc.

Bread: Specify white, rye, whole wheat, raisin, etc.

Meats: Give the size of the portion, or its weight in ‘ounces’ after cooking.

Cereals, rice, and pasta: Record amount of cereals, rice, and pasta in ‘cups’ or fractions of cup. List anything added e.g. fruit, sugar

Fruits and Vegetables: Specify, fresh, frozen, canned, dried, or freeze dried.

Condiments: Record any jelly, butter, ketchup, mayonnaise or seasonings added.

Canned foods: Record what food is packed in – oil, water, syrup, etc.

Added salt: Record if you used a shaker to add salt to the food

If you have any questions, please feel free to email me at OAmro@tuftsmedicalcenter.org or call 617-636-8424. I will be happy to answer your questions.

Dr. Osama Amro
Dr. Ronald Perrone
Two Day Food and Water Log

Day (1) 24 Hour Diet and Water Recall

Name:

Day of the week: Monday Tuesday Wednesday Thursday Friday Saturday Sunday

Does this day represent your typical eating habits? Yes No

Have you used chewing gum? Yes No

<table>
<thead>
<tr>
<th>Time</th>
<th>Food</th>
<th>Method of Preparation - (baked, fried, boiled, canned etc.) Brand Name</th>
<th>Amount/Serving Size</th>
<th>Added Salt</th>
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# Two Day Food and Water Log

## Day (2) 24 Hour Diet and Water Recall

**Day of the week:** Monday Tuesday Wednesday Thursday Friday Saturday Sunday

Does this day represent your typical eating habits? Yes No

Have you used chewing gum? Yes No

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6.6 Appendix VI: Dietary Counseling

Dr. Amro performed the dietary counseling during the clinical encounters with the patients. In addition to his training as a clinician and long term interest in nutrition, he successfully completed an introductory course in nutrition (NUTR 202: Principles of Nutrition Science) at the Gerald J. and Dorothy R. Friedman School of Nutrition Science and Policy (September 2-December 18, 2014). The course covered the fundamental scientific principles of human nutrition.
6.7 Appendix VII: Diet Instruction for the Intervention Group

Name:  
Date:  

Low Osmolar PKD Diet Instructions

While on this diet, you will be given a diet prescription that will include limiting protein (primarily meats and dairy) and salt, and avoiding urea-(carbamide) based compounds such as preservatives, food additives, bulking agents, and sugar-free chewing gum.

1) How to reduce protein intake

Based on your weight your daily protein intake should not exceed ___ grams

This is a list of high-protein foods and amount of protein in each:

Note: 3 ounces of meat is about the same size as a deck of playing cards.

Beef

Hamburger patty, 4 oz – 28 grams protein
Steak, 6 oz – 42 grams
Most cuts of beef – 7 grams of protein per ounce

Chicken

Chicken breast, 3.5 oz - 30 grams protein
Chicken thigh – 10 grams (for average size)
Drumstick – 11 grams
Wing – 6 grams

Fish

Most fish fillets or steaks are about 22 grams of protein for 3 ½ oz (100 grams) of cooked fish, or 6 grams per ounce
Low Osmolar PKD Diet Instructions

Tuna, 6 oz can - 40 grams of protein

Pork
Pork chop, average - 22 grams protein
Pork loin or tenderloin, 4 oz – 29 grams
Ham, 3 oz serving – 19 grams
Ground pork, 1 oz raw – 5 grams; 3 oz cooked – 22 grams
Bacon, 1 slice – 3 grams

Eggs and Dairy
Egg, large - 6 grams protein
Milk, 1 cup - 8 grams
Cottage cheese, ½ cup - 15 grams
Yogurt, 1 cup – usually 8-12 grams, check label
Soft cheeses (Mozzarella, Brie, Camembert) – 6 grams per oz
Medium cheeses (Cheddar, Swiss) – 7 or 8 grams per oz
Hard cheeses (Parmesan) – 10 grams per oz

Beans (including soy)
Tofu, ½ cup 20 grams protein
Tofu, 1 oz, 2.3 grams
Soy milk, 1 cup - 6 -10 grams
**Low Osmolar PKD Diet Instructions**

Most beans (black, pinto, lentils, etc) about 7-10 grams protein per half cup of cooked beans

Soy beans, ½ cup cooked – 14 grams protein

Split peas, ½ cup cooked – 8 grams

**Nuts and Seeds**

Peanut butter, 2 Tablespoons - 8 grams protein

Almonds, ¼ cup – 8 grams

Peanuts, ¼ cup – 9 grams

Cashews, ¼ cup – 5 grams

Pecans, ¼ cup – 2.5 grams

Sunflower seeds, ¼ cup – 6 grams

Pumpkin seeds, ¼ cup – 8 grams

Flax seeds – ¼ cup – 8 grams
Low Osmolar PKD Diet Instructions

2) How to limit sodium intake
Your daily sodium intake should not exceed 1500 mg.
Ways to control your sodium intake:

- Buy fresh vegetables.
- Avoid canned or processed food.
- Know terms that commonly indicates higher sodium contents “pickled”, “cured”, “brined”, and “broth.”
- Cut back on frozen dinners, pizza, packaged mixes, and salad dressings.
- When available, buy low- or reduced-sodium, or no-salt-added versions of foods.
- Choose cereals that are lower in sodium.

Sodium Adds Up
*Sodium levels in the same food can vary widely.

1,500 mg a day; Foods that you eat several times a day can add up to a lot of sodium, even if each serving is not high in sodium.

Modified from Center for Disease Control and Prevention Website. CDC.gov/salt
Low Osmolar PKD Diet Instructions

3) How to limit urea (carbamide) intake

- Reduce protein intake as described before
- Avoid chewing gum as much as possible
- Know terms that are commonly indicate urea (carbamide) use “bulking agent”, “thickener”, “emulsifier”.
- Avoid processed food and food with synthetic additives (Artificial food colors).
6.8 Appendix VIII: Adverse Events Ascertainment Form

Adverse Event Ascertainment Form

Name:
Date:

Please indicate whether you encounter any of the following symptoms:

Nausea          Weakness          excessive urination          lightheadedness
Vomiting        Dizziness         Seizure (abnormal movement)

Other symptom or unusual feeling:

If you have any problems or questions about the study, you may contact us at the following phone numbers:

Principal Investigator: Ronald D. Perrone, M.D.

Co-investigator: Osama W. Amro, M.D.
Office: 617-636-8424; Tufts Medical Center Page Operator: 617-636-5114 (ask for beeper 3097)