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Effects of salt and protein intake on polyuria in V2RA-treated ADPKD patients

Paul Geertsema [1], Iris W. Koorevaar[1], Karin J.R. Ipema[2], Bart J. Kramers[1], Niek F. Casteleijn[3], Ron T. Gansevoort [1] and Esther Meijer[1]

[1]Departments of Nephrology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands
[2]Dietetics, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands
[3]Urology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands
Correspondence to: Esther Meijer; E-mail: Esther.Meijer@umcg.nl

ABSTRACT

Background. The only treatment proven to be renoprotective in autosomal dominant polycystic kidney disease (ADPKD) is a vasopressin V2-receptor antagonist (V2RA). However, aquaresis-associated side effects limit tolerability. We investigated whether salt and/or protein intake influences urine volume and related endpoints in V2RA-treated ADPKD patients.

Methods. In this randomized, controlled, double-blind, crossover trial, ADPKD patients treated with maximally tolerated dose of a V2RA were included. While on a low salt and low protein diet, patients were given additional salt and protein to mimic regular intake, which was subsequently replaced by placebo in random order during four 2-week periods. Primary endpoint was change in 24-h urine volume. Secondary endpoints were change in quality of life, measured glomerular filtration rate (mGFR), blood pressure and copeptin level.

Results. Twelve patients (49 ± 8 years, 25.0% male) were included. Baseline salt and protein intake were 10.8 ± 1.3 g/24-h and 1.2 ± 0.2 g/kg/24-h, respectively. During the low salt and low protein treatment periods, intake decreased to 5.8 ± 1.6 g/24-h and 0.8 ± 0.1 g/kg/24-h, respectively. Baseline 24-h urine volume (5.9 ± 1.2 L) decreased to 5.2 ± 1.1 L (–11%, $P = .004$) on low salt and low protein, and to 5.4 ± 0.9 L (–8%, $P = .04$) on low salt. Reduction in 24-h urine volume was two times greater in patients with lower urine osmolality (–16% vs –7%). Polyuria quality of life scores improved in concordance with changes in urine volume. mGFR decreased during the low salt and low protein, while mean arterial pressure did not change during study periods. Plasma copeptin decreased significantly during low salt and low protein periods.

Conclusion. Lowering dietary salt and protein intake has a minor effect on urine volume in V2RA-treated ADPKD patients. Reduced intake of osmoles decreased copeptin concentrations and might thus increase the renoprotective effect of a V2RA in ADPKD patients.

Keywords: ADPKD, protein, RCT, salt, tolvaptan

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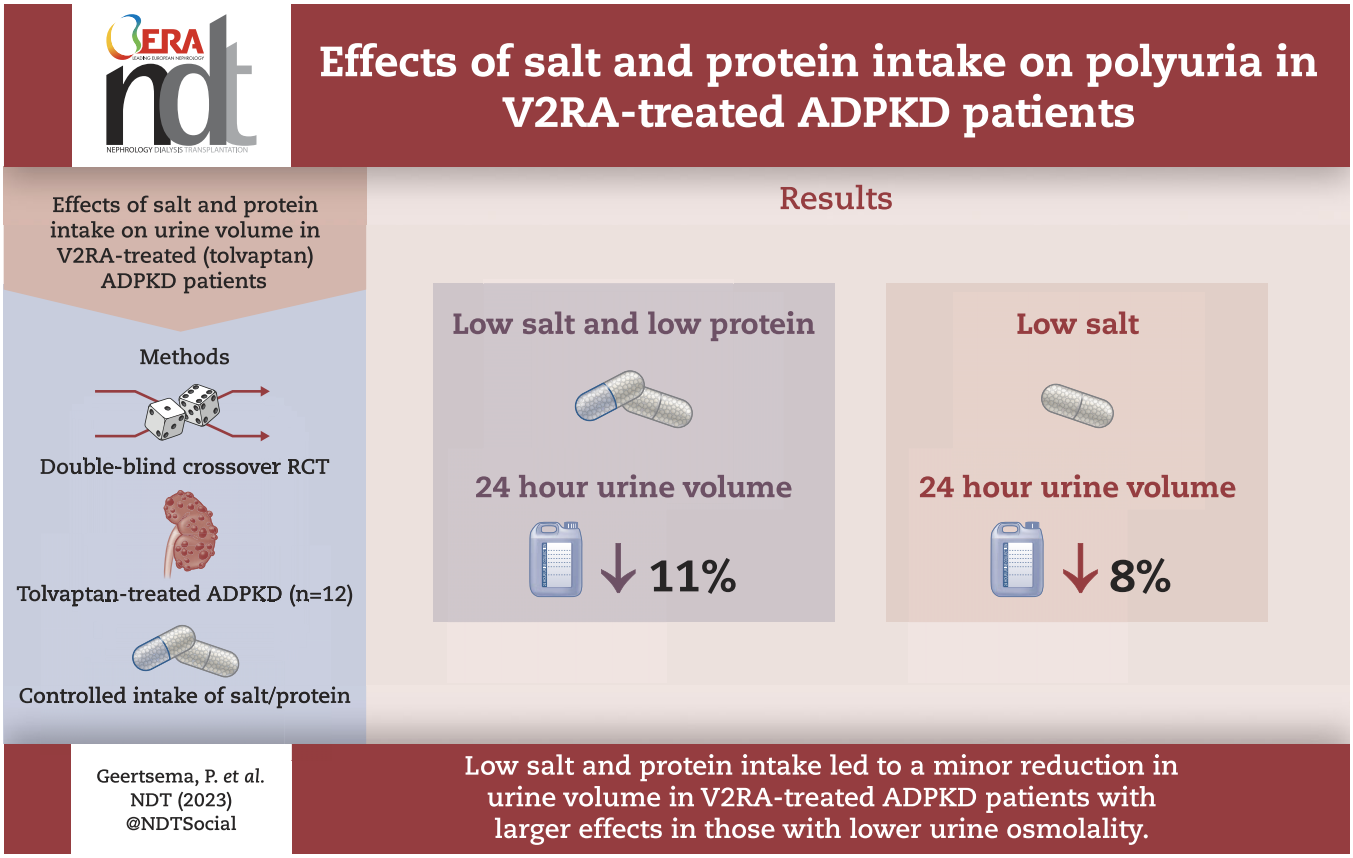
GRAPHICAL ABSTRACT



KEY LEARNING POINTS

What was known:

- Aquaresis-associated side-effects limit tolerability of vasopressin V2-receptor antagonist (V2RA) treatment in patients with autosomal dominant polycystic kidney disease (ADPKD).
- We investigated whether salt and/or protein intake influences urine volume and related endpoints in V2RA-treated ADPKD patients.

This study adds:

- Lowering dietary intake of salt and protein has a minor effect on urine volume in V2RA-treated ADPKD patients.
- In subjects with the lowest baseline urine osmolarity this antipolyuric effect was more pronounced.
- Additionally, reduction of osmotic (and especially salt) intake decreased copeptin levels, a surrogate for vasopressin.

Potential impact:

- As vasopressin is known to cause cyst growth and V2RA treatment leads only to partial blockage of vasopressin V2-receptor activity, lower dietary intake of osmoles, and specifically salt, might increase the degree of renoprotection induced by the V2RA in ADPKD patients.

INTRODUCTION

Autosomal dominant polycystic kidney disease (ADPKD) is a hereditary disease characterized by progressive cyst growth and kidney function decline [1]. The majority of patients with ADPKD reach end-stage kidney disease at a median age of 58 years, and 10% of all patients treated with kidney replacement therapy have ADPKD [2, 3]. The only treatment proven to slow kidney function decline in ADPKD patients is a vasopressin V2-receptor antagonist (V2RA) [4, 5]. While V2RA treatment reduces kidney function decline by 26%–34%, it causes aquaresis-associated side effects such as polyuria, thirst, polydipsia and nocturia [4, 5]. In the Tolvaptan Efficacy and Safety in Management of Autosomal Dominant Polycystic Kidney Disease and its Outcomes (TEMPO) 3:4 trial study, these side effects were the main reason for discontinuation or down-titration of V2RA treatment [4].

Aquaresis is the result of competitive antagonism of the vasopressin V2 receptor, thereby inhibiting migration of aquaporin-2 (AQP2) to the apical cell membrane in the principal cells of the distal collecting duct, making water reabsorption impossible [6–8]. Since every osmole excreted via the urine is passively accompanied by water, and water cannot be reabsorbed during V2RA treatment, total osmolar excretion is probably an important factor in determining total urine production during V2RA treatment. This phenomenon is also observed in subjects with

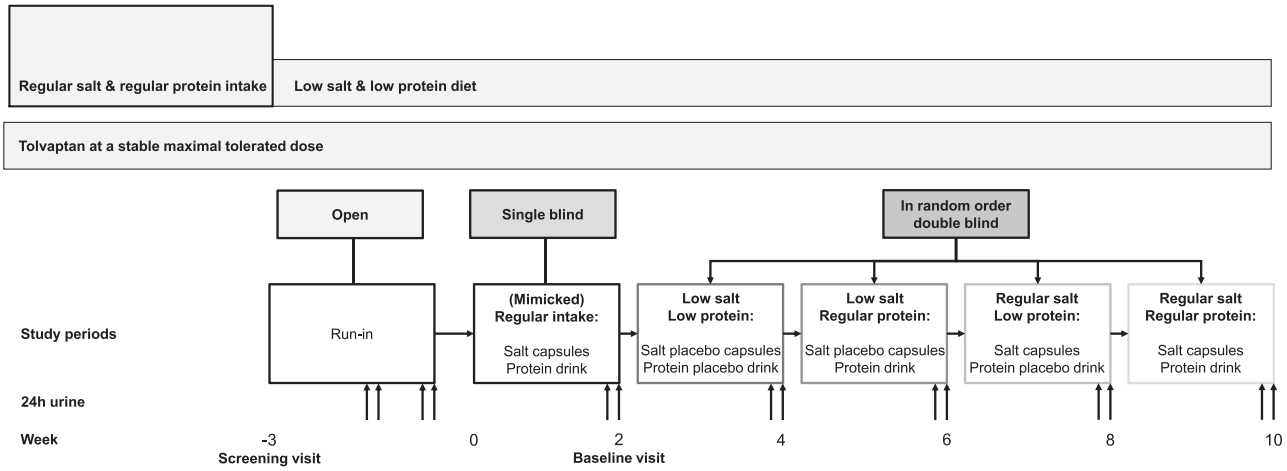


Figure 1: Flowchart study design. During the run-in period patients were prescribed a low salt (~6 g/24-h) and low protein (~0.8 g/kg bodyweight per 24-h) diet. During the regular intake period (Week 0–2), patients received, in addition to their low salt and low protein diet, single-blind salt capsules (containing 6 g of sodium chloride) and protein drink (containing 40 g of protein) to mimic regular intake of ~12 g salt and ~1.2 g/kg protein intake.

congenital or acquired central and nephrogenic diabetes insipidus (NDI) [8, 9]. In a previous observational study, we found a strong association between osmolar intake (measured as osmolar excretion) and urine volume during V2RA treatment [10]. However, whether a reduction in osmolar intake reduces 24-h urine volume in V2RA-induced polyuria has not been studied prospectively.

The main contributors of osmolar excretion are intake of proteins and salts [9]. In observational studies, sodium intake was one of the main determinants of copeptin (the stable precursor of vasopressin), whereas urea was not. It is thus of interest to investigate whether both nutrients have the same influence on urine volume and copeptin during V2R blockade [11]. Therefore, this study aimed to prospectively investigate whether a lower dietary intake of salt and/or protein influences urine volume and copeptin levels in ADPKD patients treated with a V2RA.

MATERIALS AND METHODS

Study design and participants

The WATER study (Wishing to decrease Aquaresis in ADPKD patients Treated with a V2Ra; the Effect of Regulating protein and salt) is a randomized, controlled, double-blind, crossover trial performed at the University Medical Center of Groningen (UMCG), the Netherlands. The study protocol was approved by the medical ethical committee of the UMCG (METc 2019 643) and was listed on www.ClinicalTrials.gov (NCT04310319). All patients provided written informed consent before entry into the trial.

Patients were eligible for inclusion if they were ≥18 years, had a diagnosis of ADPKD based upon the modified Pei-Ravine criteria, were on a stable, highest tolerable dose of tolvaptan, had an estimated glomerular filtration rate (eGFR) of >30 mL/min/1.73 m^2 and were able to comply with the recommended diet [12].

Exclusion criteria were conditions that, in the opinion of the investigator, may present a safety risk, patients unlikely to comply with the trial procedures, and being pregnant or breastfeeding. Further exclusion criteria were a blood pressure of >160/100 mmHg at baseline, chronic use of systemic corticosteroids, diuretics, mineralocorticoid receptor antagonists, sodium-glucose cotransporter 2 inhibitors or non-steroidal anti-inflammatory drugs, diabetes mellitus and diabetes insipidus, and other medication or diseases likely to confound endpoint assessments.

Randomization and interventions

Prior to the screening visit at the start of the study patients collected two 24-h urine samples in which urea and sodium intake were assessed as measures of daily protein and salt intake, respectively. During the run-in period of the study, personalized dietary instructions were given by an experienced renal dietician to achieve a low protein (~0.8 g protein/kg body weight per 24-h) and a low salt diet (~6 g salt per 24-h). Compliance was measured using 24-h urine urea and sodium excretion. Daily salt intake was calculated by multiplying 24-h sodium excretion by the sum of the molecular mass of sodium and chloride: salt intake (g/24-h) = sodium excretion (mmol/24-h) × (22.99 + 35.45)/1000 [13]. Daily protein intake was calculated by converting urea excretion to urea nitrogen and finally to protein (factor 6.25), taken the non-urinary nitrogen excretion into account (31 mg/kg bodyweight per 24-h): protein intake (g/24-h) = [urea excretion (mmol/24-h) × 0.4667 × 0.06 + 0.031 × bodyweight (kg)] × 6.25 [14]. Total daily dietary intake was also reported by patients using dietary questionnaires. The run-in was considered successful after two consecutive 24-h urine measurements indicated compliance. In case of non-compliance, the run-in was extended, and additional dietary advice was given. For this reason, in one patient, the run-in period was extended for 2 weeks, after which compliance was achieved. After the run-in period, patients continued on the low protein, low salt diet and started a 2-week treatment period in which they were given, in a single-blind manner, capsules containing 6 g of salt and fluid containing 40 g of protein daily to mimic regular intake at the start of the study (Fig. 1). Baseline measurements were performed at the end of this 2-week period. After baseline, patients were randomized to four double-blind periods of 2 weeks with a different treatment during each period in random order. These treatments were: salt/protein (regular diet), salt/placebo (low protein), placebo/protein (low salt), placebo/placebo (both low salt and protein). Salt was given as four capsules containing 750 mg of sodium chloride twice daily. Matching salt placebo capsules contained potato starch. Protein was given as 40 mL fluid containing 0.5 g/mL (PROSource Nocarb Orange Crème, Medtrition Inc., USA) twice daily. Matching protein placebo fluid contained sugarless fruit syrup, mimicking the protein drink in viscosity and taste. Investigational products were distributed by the hospital pharmacy, blinded for patients and study investigators. During the entire study, fluid intake was *ad libitum*, i.e. no specific instructions

were given regarding fluid intake. Patients filled in dietary questionnaires, which were used to check whether they adhered to the low protein and low salt diet throughout the study.

Outcomes and measurements

The primary outcome of this study was change in volume of 24-h urine samples, which were collected during the 2 days before every study visit. Secondary outcomes were change in copeptin (a surrogate of vasopressin), change in iohexol-measured glomerular filtration rate (mGFR), change in blood pressure and change in quality of life, assessed using two questionnaires: the validated ADPKD Impact Scale (ADPKD-IS) and a polyuria questionnaire based upon the Nagasaki Diabetes Insipidus Questionnaire [15–20]. In addition, we assessed changes in plasma osmolality, urine osmolality, free water clearance, volume status (assessed as change in renin, aldosterone and NT-proBNP), and kidney damage markers [monocyte chemotactic protein–1 (MCP-1), epidermal growth factor (EGF) and β2 microglobulin (β2MG)]. Further details of measurement procedures are given in the Supplementary methods.

Statistical analysis

A priori, we considered a decrease in urine volume of 30% after reduction of salt or protein to patients' diets to be clinically relevant. Sample size calculation indicated a minimum enrollment of 11 patients to detect a 1.8 L (30%) difference from a mean pretreatment urine volume of ~5.9 ± 1.8 L, using a paired sample T-test with a two-sided α of 0.017 (80% power and a Bonferroni correction of the α for multiple comparisons) [21]. Linear mixed-effects models were performed, with study periods as fixed effects and patients as random effects to compare the results of the various study periods. Potential carry-over effects were not tested, as a steady state of salt and protein excretion was expected after 3–8 days based on the literature [22, 23]. Skewed variables were logarithmically transformed for analysis using linear mixed-effects models. Twenty-four-hour creatinine excretion was used to compare completeness of 24-h urine collections. *A priori*, it was decided to exclude 24-h urine collections with >20% difference from the average creatinine excretion of a given subject. In total, five urine collections were excluded. Baseline measurements of one patient were replaced with the measurements of the regular diet period because tolvaptan was not taken during baseline. A subanalysis was performed, comparing the results in six patients with the lowest versus six patients with the highest urine osmolality at baseline. A two-sided *P*-value of <.05 was considered to indicate statistical significance. All statistical analyses were performed using R version 4.0.5 (Vienna, Austria).

RESULTS

Between 2020 and 2022, 30 patients were asked to participate, of which 13 patients were screened. Of these patients, 1 was excluded due to a low eGFR, and the other 12 were enrolled in the study. All 12 patients completed all treatment periods and were included in analysis. Mean age was 49 ± 8 years, 25.0% were male, and mean arterial pressure was 88 ± 7 mmHg (Table 1). Patients were treated with a stable, maximum tolerated V2RA dose of 90/30 mg ($n = 10$), 60/30 mg ($n = 1$) and 45/15 mg ($n = 1$). The reason for the non-maximum V2RA dosage in two patients was that they did not tolerate polyuria-related side effects on the highest tolvaptan dose. Tolvaptan vintage was 4.7 ± 3.7 years. Nine patients had a Mayo risk class of 1C/1D/1E. The three patients with Mayo risk classes 1A/1B were treated with a V2RA because of rapid kidney function decline prior to therapy. PKD mutation was known in 10 patients, all had a PKD1 mutation—7 (58.3%) had a PKD1 truncating and 3 (25.0%) a PKD1 non-truncating mutation. No patients were from the same family.

Table 1: Baseline characteristics.

Baseline characteristics	Total
Age (years)	49 ± 8
Gender, male, *n* (%)	3 (25.0)
Height (cm)	173 (166–179)
Weight (kg)	74 (74–101)
Mean arterial pressure (mmHg)	88 ± 7
Tolvaptan dosage, *n* (%)	
45/15 mg	1 (8.3)
60/30 mg	1 (8.3)
90/30 mg	10 (83.3)
Tolvaptan vintage (years)	4.7 ± 3.7
RAAS inhibition, *n* (%)	10 (83.3)
Mayo risk class, *n* (%)	
1A/1B (low-risk disease)	3 (25.0)
1C/1D/1E (high-risk disease)	9 (75.0)
PKD mutation, *n* (%)	
PKD1 truncating	7 (58.3)
PKD1 non-truncating	3 (25.0)
Missing	2 (16.7)
Salt intake (g/24-h)[a]	10.8 ± 1.3
Protein intake (g/kg/24-h)[a]	1.2 ± 0.2
Measured GFR (mL/min/1.73 m^2)	59 ± 23
Volume 24-h urine (L)	5.9 ± 1.2

Data are presented as mean ± standard deviation, mean (interquartile range) or *n* (%).
[a]Dietary intake of salt and protein were based upon sodium and urea excretion in 24-h urine.
RAAS, renin–angiotensin II–aldosterone system.

Interventions

Daily dietary salt and protein intake prior to the study was 9.0 ± 2.8 g/24-h and 1.0 ± 0.2 g/kg/24-h, respectively. After personal dietary advice, salt and protein intake decreased to 5.9 ± 1.6 g/24-h and 0.8 ± 0.2 g/kg/24-h, respectively, during the run-in period. After additional salt capsules and protein drinks, baseline daily salt and protein intake was 10.8 ± 1.3 g/24-h and 1.2 ± 0.2 g/kg/24-h, respectively (Fig. 2). During the low salt periods, daily salt intake was significantly lower compared with baseline (low salt and low protein: –4.9 g/24-h, $P < .001$; low salt: –5.0 g/24-h, $P < .001$), while there was no difference during other treatment periods [Table 2; Supplementary data Tables S1–S3 depict the changes during the treatment periods, both absolute (Supplementary data, Table S1) and relative (Supplementary data, Table S3)]. Similarly, daily protein intake was significantly decreased during low protein periods (low salt and low protein: –0.4 g/kg/24-h, $P < .001$; low protein: –0.4 g/kg/24-h, $P < .001$), with no differences during regular intake and low salt periods. Dietary questionnaires, evaluating only the dietary intake without double-blinded intervention, indicated no difference in salt intake between study periods, while there was a slight but significantly higher dietary protein intake during the low protein period compared with baseline (+0.1 g/kg/24-h, $P = .03$).

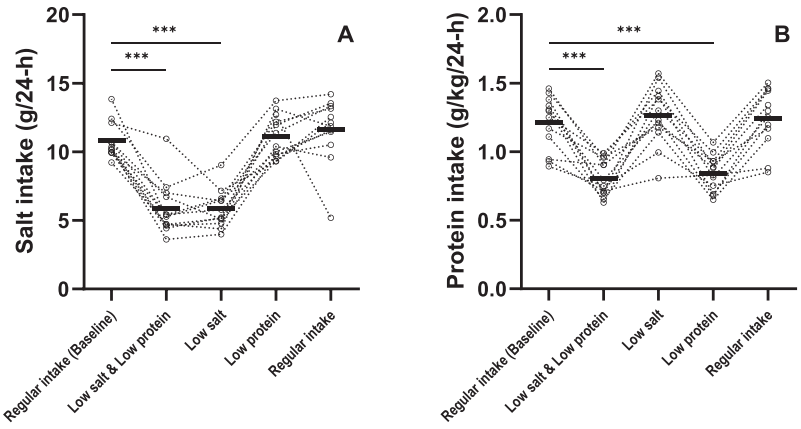


Figure 2: Dietary interventions. (**A**) Salt intake. (**B**) Protein intake. Salt and protein intake was calculated using 24-h urine sodium and urea excretion. Linear mixed effects models were used to compare treatment periods with baseline: *$P < .05$; **$P < .01$; ***$P < .001$.

Urine volume

Urine volume at baseline was 5.9 ± 1.2 L and was lowered to 5.2 ± 1.1 L ($P = .004$) during the low salt and low protein treatment period, and to 5.4 ± 0.9 L ($P = .04$) during the low salt treatment period (Fig. 3). Although 24-h urine volume decreased during the low protein period, this change did not reach statistical significance (–0.4 L, $P = .1$) (Table 2). Urine volume during the regular intake period was similar to baseline urine volume. After Bonferroni correction for multiple testing, the effect on urine volume was still significant during the low salt and low protein treatment period ($P = .02$) (Supplementary data, Table S2).

Secondary outcomes

At baseline, the mean plasma copeptin concentration was 33.2 ± 10.2 pmol/L (Fig. 3). During the low salt and low protein period, copeptin decreased significantly compared with baseline values (–13.5 pmol/L, $P < .001$), as did copeptin levels in the low salt as well as in the low protein periods, albeit to a lesser extent (low salt: –10.4 pmol/L, $P < .001$; low protein: –5.7 pmol/L, $P < .001$) (Table 2). mGFR was significantly lower during the low salt and low protein period compared with baseline (56 ± 21 vs 59 ± 23 mL/min/1.73 m^2, $P = .01$). Baseline urine volume and changes in urine volume after lowering osmolar intake were not correlated with mGFR (Pearson correlation coefficient $P = .9$ and $P = .7$). Mean arterial pressure did not change during any of the study periods. Quality of life measured by the ADPKD-IS did not differ between any of the treatment periods (Table 3; Supplementary data, Table S4 depicts the absolute change in QoL scores). The polyuria QoL score indicated fewer complaints during the low salt and low protein period and the low protein period when compared with the baseline period (22.50 ± 3.40 and 21.83 ± 4.04 vs 20.75 ± 2.86, $P = .002$ and $P = .046$, respectively).

Exploratory outcomes

Baseline plasma osmolality (298 ± 8 mOsm/kg) decreased significantly during the low salt and low protein period and the low protein period (–10 mOsm/kg, $P < .001$; –6 mOsm/kg, $P = .01$, respectively) (Supplementary data, Table S1), whereas plasma osmolality levels were similar during other treatment periods. Twenty-four-hour urine osmolality was significantly lower during the low salt and low protein (126 ± 35 mOsm/kg, $P < .001$), low protein (160 ± 31 mOsm/kg, $P = .048$) and low salt periods (159 ± 36 mOsm/kg, $P = .04$) when compared with the baseline (172 ± 41 mOsm/kg) and regular intake periods (182 ± 43 mOsm/kg).

Twenty-four-hour urine creatinine excretion and eGFR were similar during all treatment periods. Free water clearance was significantly higher during the low salt and low protein period compared with baseline (2.99 ± 1.10 vs 2.55 ± 1.24 L/24-h, $P = .049$). There were no large differences between the various treatment periods in measured kidney damage markers (Supplementary data, Table S5). Only EGF concentration was higher (+387 pg/mL, $P = .03$) during the low protein period compared with baseline, as were β2MG concentrations during the low protein (+14.52 ng/mL, $P = .01$) and the regular intake period (+2.83 ng/mL, $P = .04$).

Renin concentrations were 17 pg/mL [11–53] during baseline and increased significantly during the low salt periods [low salt and low protein: 52 pg/mL (14–68), $P = .01$; low salt: 57 pg/mL (41–127), $P < .001$]. Baseline aldosterone concentrations were 149 ± 94 pmol/L. During the periods with a low salt diet, aldosterone concentrations were significantly higher (low salt and low protein: 288 ± 186 pmol/L, $P < .001$; low salt: 311 ± 131 pmol/L, $P < .001$). NT-proBNP levels were 111 ± 79 ng/L during baseline and decreased to 66 ± 54 ng/L ($P = .01$) during the low salt period, while being similar during all other study periods.

Safety

Adverse events are summarized in Table 4. There were 48 adverse events in total, with no serious adverse events. The most frequent adverse events were headache ($n = 10$), nausea ($n = 4$) and abdominal pain ($n = 4$). The incidence of adverse events did not differ significantly between the various treatment periods.

Sub-analyses

When comparing the six patients with the lowest urine osmolality at baseline (140 ± 19 mOsm/kg) with the six patients with the highest urine osmolality (203 ± 31 mOsm/kg), all six patients with a low urine osmolality had a higher urine volume at baseline (6.9 ± 0.8 L vs 5.0 ± 0.4 L) (Supplementary data, Table S6). During the low salt and low protein treatment period, both the absolute and relative change in 24-h urine volume from baseline tended to be more substantial in the low urine osmolality group (–1.1 L vs –0.4 L and –15.8% vs –7.8%). The reduction in 24-h urine volume in the low osmolality group was statistically significant ($P = .03$), while the reduction in the high osmolality group was not ($P = .1$) (Supplementary data, Fig. S1). In this low urine

Table 2: Absolute change in main study outcomes during the various treatment phases.

		Absolute change from baseline (mean and 95% CI) and P-values							
	Baseline	**Low salt and low protein**		**Low salt**		**Low protein**		**Regular intake**	
Primary study outcomes									
Volume 24-h urine (L)	5.9 ± 1.2	−0.7 (−1.2 to −0.3)	.004	−0.5 (−1 to 0)	.04	−0.5 (−0.9 to 0)	.1	−0.1 (−0.6 to 0.4)	.6
Secondary study outcomes									
Copeptin (pmol/L)	33.2 ± 10.2	−13.5 (−17.5 to −9.5)	<.001	−10.4 (−14.5 to −6.4)	<.001	−5.7 (−9.7 to −1.7)	.01	−4.0 (−8.0 to 0.0)	.1
mGFR (mL/min/1.73 m^2)	60 ± 23	−4 (−6 to −1)	.01	−1 (−3 to 2)	.5	−1 (−3 to 1)	.4	1 (−2 to 3)	.6
Mean arterial pressure (mmHg)	88 ± 7	−1 (−5 to 2)	.5	−3 (−7 to 0)	.1	3 (−1 to 6)	.2	−1 (−5 to 3)	.6
Exploratory study outcomes									
Creatinine excretion (mmol/L/24-h)	12.7 ± 4.1	−0.4 (−1.0 to 0.3)	.3	−0.1 (−0.7 to 0.5)	.8	−0.6 (−1.2 to 0.1)	.1	0.2 (−0.4 to 0.8)	.6
Salt intake (g/24-h)[a]	10.8 ± 1.3	−4.9 (−6.1 to −3.8)	<.001	−5.0 (−6.1 to −3.8)	<.001	0.3 (−0.8 to 1.5)	.6	0.8 (−0.4 to 2.0)	.2
Protein intake (g/kg/24-h)[a]	1.2 ± 0.2	−0.4 (−0.5 to −0.3)	<.001	0.1 (−0.04 to 0.2)	.3	−0.4 (−0.5 to −0.3)	<.001	0.04 (−0.1 to 0.1)	.5
Plasma osmolality (mOsm/kg)	298 ± 8	−10 (−14 to −6)	<.001	−4 (−7 to 0)	.1	−6 (−10 to −2)	.01	−1 (−5 to 3)	.5
Urine osmolality (mOsm/kg)	172 ± 41	−46 (−57 to −34)	<.001	−13 (−25 to −1)	.04	−12 (−24 to −1)	.048	11 (−1 to 22)	.1
Free water clearance (L/24-h)	2.55 ± 1.24	0.44 (0.02 to 0.86)	.049	−0.03 (−0.44 to 0.39)	.9	−0.14 (−0.55 to 0.28)	.5	−0.23 (−0.65 to 0.18)	.3
eGFR (mL/min/1.73 m^2)	50 ± 15	−2 (−5 to 0)	.1	−0.1 (−2 to 2)	.9	−0.3 (−3 to 2)	.8	−1 (−3 to 2)	.6

Linear mixed effects models were used to compare treatment periods with baseline[a], dietary intake of salt and protein were based upon sodium and urea excretion in 24-h urine.

osmolality subgroup, the low salt treatment period also caused a greater reduction in polyuria than in the high urine osmolality subgroup (–0.8 L vs –0.2 L and –11.8% vs –3.9%). When the interaction between subgroups and intervention for 24-h urine volume was tested, this turned out to be significant for the low salt treatment period ($P = .03$) and formally not significant for the low salt and low protein diet ($P = .1$). After removal of an outlier with an unexpected increase in urine volume during this study period (Fig. 3), the difference in reduction in urine volume during the low salt and low protein period was statistically significant between the two subgroups ($P = .02$). After Bonferroni correction for multiple testing, the change in urine volume in the low urine osmolality group did not meet the threshold of formal statistical significance ($P = .1$). When comparing copeptin, mean arterial pressure and measured GFR levels between subgroups at baseline, no differences were found.

When males and females were investigated separately, baseline urine volume was not different with 5.8 vs 6.0 L in males and females, respectively. During the various study periods, urine volume decreased in both genders: low salt and low protein: –0.5 vs –0.8 L; low salt: –0.1 vs –0.8 L; low protein: –0.7 vs –0.4 L, in males and females. The differences in volume reduction between the genders were not statistically significant.

DISCUSSION

This study found that lowering dietary intake of salt and protein has a lowering effect on urine volume in V2RA-treated ADPKD patients. This effect, however, was less than expected and appeared to be dependent on baseline urine osmolality. In subjects with a low baseline urinary osmolality, changing dietary intake of salt and protein tended to have more effect. Polyuria scores improved in concordance with changes in urine volume, while other quality of life scores did not change. In addition, lowering the intake of osmoles, especially salt, decreased copeptin concentration.

Our data with respect to urine volume during the use of the V2RA as well as of salt and protein intake compare well to the literature, indicated by the mean baseline urine volume during regular salt and protein intake (5.9 ± 1.2 L) being similar to that in a previous study in 27 patients treated with tolvaptan (5.9 ± 1.8 L) [21]. In addition, the level of salt and protein intake (salt: 10.8 ± 1.3 g/24-h; protein: 1.2 ± 0.2 g/kg/24-h) of the patients in this study during the regular intake period were comparable to salt and protein intake prior to the study (salt: 9.0 ± 2.8 g/24-h; protein: 1.0 ± 0.2 g/kg/24-h), as well as to salt intake in a national observational cohort and the Halt Progression of Polycystic Kidney Disease trials studies (salt: 9.1 ± 3.6 g/24-h; and 10.4 ± 4.7 g/24-h) [13, 24].

In subjects with debilitating polyuria due to central or NDI, it is general practice to lower osmolar intake to decrease urinary volume [8, 9]. The mechanism by which a V2RA causes polyuria is very similar to the pathophysiology of NDI. In NDI, the collecting duct is (relatively) impermeable to water due to insensitivity to vasopressin, caused either by defects in the V2R or defects downstream [25, 26]. V2RAs cause a similar effect by competitively binding to the V2R, inhibiting vasopressin [27]. V2RA use is so similar to NDI that in animal experiments, treatment with tolvaptan is used to simulate NDI [28]. Because of these similarities in pathophysiology between NDI and a V2RA, we hypothesized that the influence of osmotic load on urinary volume was equal to that of NDI. In a study including patients with complete nephrogenic or central diabetes insipidus, a decrease in osmolar intake of –724.6 mOsm/24-h caused a decrease in urine

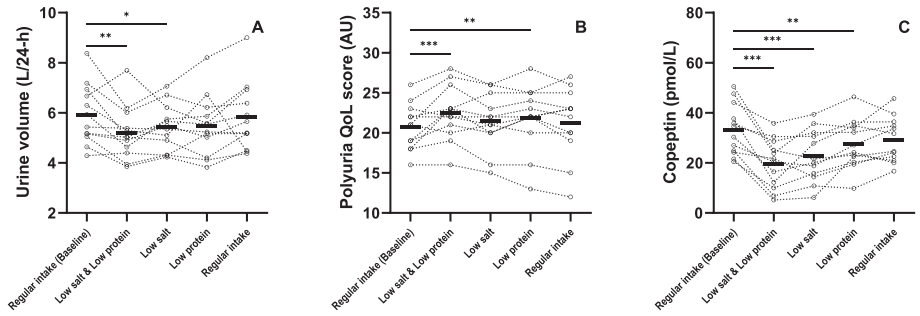


Figure 3: Endpoints per treatment period. (**A**) Twenty-four-hour urine volume (primary outcome). (**B**) Polyuria quality of life score (secondary outcome). A higher score indicates a better quality of life. (**C**) Copeptin plasma levels (co-secondary outcome). AU, artificial unit. Linear mixed effects models were used to compare treatment periods with baseline: *$P < .05$; **$P < .01$; ***$P < .001$.

Table 3: Quality of life scores during the various treatment phases.

	Baseline	Low salt and low protein	Low salt	Low protein	Regular intake
ADPKD-IS physical	1.21 (1.11–1.39)	1.14 (1.00–2.07)	1.07 (1.00–1.86)	1.21 (1.00–2.21)	1.14 (1.00–2.36)
ADPKD-IS emotional	1.25 (1.25–1.56)	1.25 (1.00–1.31)	1.25 (1.00–1.56)	1.25 (1.00–1.25)	1.25 (1.00–1.50)
ADPKD-IS fatigue	1.50 (1.00–2.42)	1.33 (1.00–2.67)	1.50 (1.00–2.42)	1.67 (1.00–2.67)	1.50 (1.00–2.50)
Polyuria QoL score	20.75 ± 2.86	22.50 ± 3.40***	21.42 ± 3.48	21.83 ± 4.04**	21.25 ± 4.39

A lower ADPKD-IS score indicates a better quality of life. A higher polyuria QoL score indicates a better quality of life. Data are presented as median (interquartile range) and mean ± SD.
Linear mixed effects models were used to compare treatment periods with baseline: **$P < .01$; ***$P < .001$.
QoL, quality of life.

Table 4: Adverse events.

Event	Baseline	Low salt and low protein	Low salt	Low protein	Regular intake
Adverse events	13	7	10	7	11
Participants with adverse events	7 (58.3)	6 (50.0)	7 (58.3)	6 (50.0)	6 (50.0)
Serious adverse events	0	0	0	0	0
Headache	3 (23.1)	1 (14.3)	2 (20.0)	1 (14.3)	3 (27.3)
Nausea	3 (23.1)	0	0	0	1 (9.1)
Abdominal pain	1 (15.4)	1 (14.3)	1 (10.0)	1 (14.3)	0
Orthostatic hypotension	0	1 (14.3)	1 (10.0)	1 (14.3)	0
Thirst	1 (7.7)	0	0	1 (14.3)	1 (9.1)
Hematuria	1 (7.7)	1 (14.3)	0	0	0
Diarrhea	1 (7.7)	0	1 (10.0)	0	0
Pain right foot	1 (7.7)	0	1 (10.0)	0	0
Sinusitis	0	0	1 (10.0)	1 (14.3)	0
Fatigue	0	0	0	1 (14.3)	1 (9.1)
Restless legs	0	1 (14.3)	0	0	0
Episodes of sweating	0	1 (14.3)	0	0	0
Sprained wrist	0	0	1 (10.0)	0	0
Abdominal fullness	0	0	1 (10.0)	0	0
Dyspepsia	0	0	1 (10.0)	0	0
Broken molar	0	1 (14.3)	0	0	0
Fasciitis plantaris	0	0	0	1 (14.3)	0
Vomiting	0	0	0	0	1 (9.1)
Hematoma intravenous entry site	0	0	0	0	1 (9.1)
Itch whole body	0	0	0	0	1 (9.1)
Fever after COVID-19 vaccination	0	0	0	0	1 (9.1)
Cramp in calves	0	0	0	0	1 (9.1)
Sleep problems	1 (7.7)	0	0	0	0

Data are presented as *n* (%).

volume of –3.7 L/24-h [9]. In our study, a decrease in osmolar excretion of –344 mOsm/24-h decreases urine volume with –0.7 L/24-h; thus, the effect is 2 to 3 times less.

This smaller effect of osmolar reduction on change in urine volume was contrary to our hypothesis. Several explanations may be possible. It could be that V2RA-treated patients were used to drinking high volumes of water (average tolvaptan use was 4.7 years) and continued to do so even during low osmolar intake. We found a significant increase in free water clearance of ∼450 mL during the low salt and low protein, which was not found during

the separate low salt and low protein periods. It is possible that urine volume would have decreased further during the low salt and low protein period if the participants were instructed to only drink based on thirst. Because of safety issues, this was not done in the current study. Another possible explanation for this difference could be that V2RA treatment did not cause full inhibition of vasopressin activity, causing the kidney to still be able to concentrate urine to some extent. In turn, this results in a smaller effect of changes in osmotic load on urine volume than would be expected in a situation of complete V2R blockage. This hypothesis is supported by a phase 2 study wherein tolvaptan was given in higher dosages than presently used in clinical practice, resulting in progressively lower urine osmolality, indicating additional competitive blockade of the V2R beyond the dosage of tolvaptan that at present is assumed to cause maximal V2R blockade [29]. Incomplete blockage of the V2R could also explain the trend towards a difference in the effect of change in osmotic load on urine volume between patients with high and low urine osmolality in our study. Patients with a low urine osmolality at baseline, which indicates more complete vasopressin blockage by the V2RA and consequently less concentrating capacity, tended to have a more substantial change in urine volume after reduction of osmolar intake of –1.1 L (–15.8%), compared with –0.4 L (–7.6%) in those with a relatively high urine osmolality.

The study population of our study contained more females than males. Our study is too small to formally investigate a potential gender difference in polyuria upon osmolar load. However, we do not expect this to occur because in literature, gender does not affect urine volume in V2RA-treated patients [10] and we found no statistically significant effect of gender on urine volume in V2RA-treated patients.

It is known that copeptin is increased during V2RA treatment, probably as a result of feedback mechanisms, and the baseline copeptin concentration of the current study was similar to previous studies [21, 30]. What happens with copeptin during change in salt and protein intake while on V2RA treatment was unknown. Vasopressin is excreted by the posterior pituitary gland in response to an increase in plasma osmolality sensed by the osmoreceptors in the hypothalamus [31]. In a situation without V2RA treatment, copeptin levels increase as an effect of increasing dietary osmolar intake [32–34]. In line with this, we found that during V2RA treatment, the reduction in protein intake decreased plasma osmolality and copeptin levels. Remarkably, salt intake reduction also diminished copeptin, even though plasma osmolality remained similar to baseline. This might indicate a direct effect of salt on the osmoreceptor and, therefore, vasopressin levels, and supports findings by Spinelli *et al.*, who found a rise in vasopressin levels after a dietary salt load, prior to the rise in plasma osmolality [35]. The initial half-life of copeptin is 26 min [36]. Due to this short half-life, we assume the changes in copeptin concentrations after 2-week interventions reflect changes as a result of the intervention.

Whether this decrease in copeptin due to lower osmolar intake may have a long-term impact is unknown. Previous studies in untreated ADPKD patients showed that vasopressin is positively correlated with the rates of kidney growth and eGFR decline, whereas interventions with V2RAs have indicated a causal role for vasopressin in the pathophysiology of ADPKD [30, 37]. If V2RA treatment blocks the vasopressin V2R incompletely, a high vasopressin concentration could thus negatively influence disease progression in ADPKD patients treated with a V2RA. The other way around, a low dietary intake of especially salt could potentially increase V2RA efficacy by lowering vasopressin levels.

This study implicates a role for dietary salt and protein restriction in V2RA-treated patients with ADPKD. Reduction of salt to ~6 g per day is already recommended in patients with ADPKD by the KDOQI guideline [38]. Patients treated with V2RAs can be incentivized to lower their osmolar and, specifically, salt intake. This will lower urine volume and consequently also polyuria complaints, with the additional possibility that this might also increase the renoprotective effect of V2RAs by lowering vasopressin, although this must be investigated further. The antipolyuric effect of salt reduction is especially important for younger patients with good functioning kidneys, as kidney function is positively correlated to the amount of polyuria, and these patients have to use a V2RA for a longer period of time [10, 39]. Furthermore, patients in this study adhered successfully to the low-salt and low protein diet for a total of 13 weeks, indicating that patients can adhere to such a low-osmolar diet for longer periods of time.

This randomized controlled trial has limitations, the main limitation being the relatively small size of the included study population. To overcome this limitation, we aimed to include representative patients, implemented a crossover design wherein patients are their own controls, resulting in greater power, and performed a power analysis.

The main strength of this study is that it is a randomized, controlled, double-blind, crossover trial with well-controlled and monitored dietary interventions. Laboratory measurements, including 24-h excretion of osmoles, were performed at the end of the study to prevent bias by keeping the researchers blinded to the intervention, and GFR was measured using a gold standard method.

In conclusion, in this randomized control trial, we found that changing dietary osmolar intake had only a minor effect on urine volume in V2RA-treated ADPKD patients. Additionally, reduction of osmotic (and especially salt) intake decreased copeptin levels, a surrogate for vasopressin. As vasopressin is known to cause cyst growth and V2RA treatment leads only to partial blockage of vasopressin V2R activity, lower dietary intake of osmoles, and specifically salt, might increase the degree of renoprotection induced by the V2RA in ADPKD patients.

SUPPLEMENTARY DATA

Supplementary data are available at *ndt* online.

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AUTHORS' CONTRIBUTIONS

Research idea and study design: I.W.K., E.M., R.T.G. and K.J.R.I.; data acquisition: P.G. and I.W.K.; data analysis/interpretation: P.G., E.M. and R.T.G.; statistical analysis: P.G. and B.J.K.; supervision or mentorship: N.F.C., E.M. and R.T.G. Manuscript drafting and revision: all authors.

DATA AVAILABILITY STATEMENT

The data underlying this article will be shared upon reasonable request to the corresponding author.

CONFLICT OF INTEREST STATEMENT

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REFERENCES

1. Cornec-Le Gall E, Alam A, Perrone RD. Autosomal dominant polycystic kidney disease. *Lancet North Am Ed* 2019;**393**:919–35. https://doi.org/10.1016/S0140-6736(18)32782-X
2. Spithoven EM, Kramer A, Meijer E *et al.* Renal replacement therapy for autosomal dominant polycystic kidney disease (ADPKD) in Europe: prevalence and survival - an analysis of data from the ERA-EDTA Registry. *Nephrol Dial Transplant* 2014;**29**:iv15–25. https://doi.org/10.1093/ndt/gfu017
3. Chebib FT, Torres VE. Recent advances in the management of autosomal dominant polycystic kidney disease. *Clin J Am Soc Nephrol* 2018;**13**:1765–76. https://doi.org/10.2215/CJN.03960318
4. Torres VE, Chapman AB, Devuyst O *et al.* Tolvaptan in patients with autosomal dominant polycystic kidney disease. *N Engl J Med* 2012;**367**:2407–18. https://doi.org/10.1056/NEJMoa1205511
5. Torres VE, Chapman AB, Devuyst O *et al.* Tolvaptan in later-stage autosomal dominant polycystic kidney disease. *N Engl J Med* 2017;**377**:1930–42. https://doi.org/10.1056/NEJMoa1710030
6. Blair HA, Keating GM. Tolvaptan: a review in autosomal dominant polycystic kidney disease. *Drugs* 2015;**75**:1797–806. https://doi.org/10.1007/s40265-015-0475-x
7. Harris PC, Torres VE. Genetic mechanisms and signaling pathways in autosomal dominant polycystic kidney disease. *J Clin Invest* 2014;**124**:2315–24. https://doi.org/10.1172/JCI72272
8. Bockenhauer D, Bichet DG. Pathophysiology, diagnosis and management of nephrogenic diabetes insipidus. *Nat Rev Nephrol* 2015;**11**:576–88. https://doi.org/10.1038/nrneph.2015.89
9. Blalock T, Gerron G, Quiter E *et al.* Role of diet in the management of vasopressin responsive and resistant diabetes insipidus. *Am J Clin Nutr* 1977;**30**:1070–6. https://doi.org/10.1093/ajcn/30.7.1070
10. Kramers BJ, van Gastel MDA, Boertien WE *et al.* Determinants of urine volume in ADPKD patients using the vasopressin V2 receptor antagonist tolvaptan. *Am J Kidney Dis* 2019;**73**:354–62. https://doi.org/10.1053/j.ajkd.2018.09.016
11. Van Gastel MDA, Meijer E, Scheven LE *et al.* Modifiable factors associated with copeptin concentration: a general population cohort. *Am J Kidney Dis* 2015;**65**:719–27. https://doi.org/10.1053/j.ajkd.2014.10.009
12. Pei Y, Obaji J, Dupuis A *et al.* Unified criteria for ultrasonographic diagnosis of ADPKD. *J Am Soc Nephrol* 2009;**20**:205–12. https://doi.org/10.1681/ASN.2008050507
13. Kramers BJ, Koorevaar IW, Drenth JPH *et al.* Salt, but not protein intake, is associated with accelerated disease progression in autosomal dominant polycystic kidney disease. *Kidney Int* 2020;**98**:989–98. https://doi.org/10.1016/j.kint.2020.04.053
14. Maroni BJ, Steinman TI, Mitch WE. A method for estimating nitrogen intake of patients with chronic renal failure. *Kidney Int* 1985;**27**:58–65. https://doi.org/10.1038/ki.1985.10
15. Bröchner-Mortensen J. A simple method for the determination of glomerular filtration rate. *Scand J Clin Lab Invest* 1972;**30**:271–4. https://doi.org/10.3109/00365517209084290
16. Gaspari F, Perico N, Ruggenenti P *et al.* Plasma clearance of nonradioactive Iohexol as a measure of glomerular filtration rate. *J Am Soc Nephrol* 1995;**6**:257–63. https://doi.org/10.1681/ASN.V62257
17. Gaspari F, Perico N, Matalone M *et al.* Precision of plasma clearance of iohexol for estimation of GFR in patients with renal disease. *J Am Soc Nephrol* 1998;**9**:310–3. https://doi.org/10.1681/ASN.V92310
18. Bird NJ, Michell AR, Peters AM. Accurate measurement of extracellular fluid volume from the slope/intercept technique after bolus injection of a filtration marker. *Physiol Meas* 2009;**30**:1371–9. https://doi.org/10.1088/0967-3334/30/12/006
19. Oberdhan D, Cole JC, Krasa HB *et al.* Development of the Autosomal Dominant Polycystic Kidney Disease Impact Scale: a new health-related quality-of-life instrument. *Am J Kidney Dis* 2018;**71**:225–35. https://doi.org/10.1053/j.ajkd.2017.08.020
20. Nozaki A, Ando T, Akazawa S *et al.* Quality of life in the patients with central diabetes insipidus assessed by Nagasaki Diabetes Insipidus Questionnaire. *Endocrine* 2016;**51**:140–7. https://doi.org/10.1007/s12020-015-0637-3
21. Boertien WE, Meijer E, De Jong PE *et al.* Short-term effects of tolvaptan in individuals with autosomal dominant polycystic kidney disease at various levels of kidney function. *Am J Kidney Dis* 2015;**65**:833–41. https://doi.org/10.1053/j.ajkd.2014.11.010
22. Rose BD, Post TW. Clinical physiology of acid-base and electrolyte disorders, 5th edn. McGraw Hill Professional, 2001.
23. Scrimshaw NS, Perera WD, Young VR. Protein requirements of man: obligatory urinary and fecal nitrogen losses in elderly women. *J Nutr* 1976;**106**:665–70. https://doi.org/10.1093/jn/106.5.665
24. Torres VE, Abebe KZ, Schrier RW *et al.* Dietary salt restriction is beneficial to the management of autosomal dominant polycystic kidney disease. *Kidney Int* 2017;**91**:493–500. https://doi.org/10.1016/j.kint.2016.10.018
25. Sands JM, Bichet DG. Nephrogenic diabetes insipidus. *Ann Intern Med* 2006;**144**:186–94. https://doi.org/10.7326/0003-4819-144-3-200602070-00007
26. Bichet DG. Nephrogenic diabetes insipidus. *Am Physiol Soc* 1998;**105**:431–42.
27. Yamamura Y, Ogawa H, Yamashita H *et al.* Characterization of a novel aquaretic agent, OPC-31260, as an orally effective, nonpeptide vasopressin V2 receptor antagonist. *Br J Pharmacol* 1992;**105**:787–91. https://doi.org/10.1111/j.1476-5381.1992.tb09058.x
28. Efe O, Klein JD, LaRocque LM *et al.* Metformin improves urine concentration in rodents with nephrogenic diabetes insipidus. *JCI Insight* 2016;**1**:e88409. https://doi.org/10.1172/jci.insight.88409
29. Shoaf SE, Chapman AB, Torres VE *et al.* Pharmacokinetics and pharmacodynamics of tolvaptan in autosomal dominant polycystic kidney disease: phase 2 trials for dose selection in the pivotal phase 3 trial. *J Clin Pharmacol* 2017;**57**:906–17. https://doi.org/10.1002/jcph.880
30. Gansevoort RT, van Gastel MDA, Chapman AB *et al.* Plasma copeptin levels predict disease progression and tolvaptan efficacy in autosomal dominant polycystic kidney disease. *Kidney Int* 2019;**96**:159–69. https://doi.org/10.1016/j.kint.2018.11.044
31. Sands JM, Layton HE. Advances in understanding the urine-concentrating mechanism. *Annu Rev Physiol* 2014;**76**:387–409. https://doi.org/10.1146/annurev-physiol-021113-170350
32. Somova L, Zaharieva S, Ivanova M. Humoral factors involved in the regulation of sodium-fluid balance in normal man. I. Effect of dietary sodium chloride intake on renal prostaglandins,

vasopressin and renin-angiotensin-aldosterone system. *Acta Physiol Pharmacol Bulg* 1984;**10**:21–8.
33. Kjeldsen SE, Os I, Forsberg G *et al.* Dietary sodium intake increases vasopressin secretion in man. *J Clin Hypertens* 1985;**1**:123–31.
34. Bankir L, Roussel R, Bouby N. Protein- and diabetes-induced glomerular hyperfiltration: role of glucagon, vasopressin, and urea. *Am J Physiol Renal Physiol* 2015;**309**:F2–23. https://doi.org/10.1152/ajprenal.00614.2014
35. Spinelli L, Golino P, Piscione F *et al.* Effects of oral salt load on arginine-vasopressin secretion in normal subjects. *Ann Clin Lab Sci* 1987;**17**:350–7.
36. Fenske WK, Schnyder I, Koch G *et al.* Release and decay kinetics of copeptin vs AVP in response to osmotic alterations in healthy volunteers. *J Clin Endocrinol Metab* 2018;**103**:505–13. https://doi.org/10.1210/jc.2017-01891
37. Wang X, Wu Y, Ward CJ *et al.* Vasopressin directly regulates cyst growth in polycystic kidney disease. *J Am Soc Nephrol* 2008;**19**:102–8. https://doi.org/10.1681/ASN.2007060688
38. Ikizler TA, Burrowes JD, Byham-Gray LD *et al.* KDOQI Clinical Practice Guideline for Nutrition in CKD: 2020 Update. *Am J Kidney Dis* 2020;**76**:S1–107. https://doi.org/10.1053/j.ajkd.2020.05.006
39. Devuyst O, Chapman AB, Gansevoort RT *et al.* Urine osmolality, response to tolvaptan, and outcome in autosomal dominant polycystic kidney disease: results from the TEMPO 3:4 trial. *J Am Soc Nephrol* 2017;**28**:1592–602. https://doi.org/10.1681/ASN.2016040448

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