Salt, but not protein intake, is associated with accelerated disease progression in autosomal dominant polycystic kidney disease

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In autosomal dominant polycystic kidney disease (ADPKD), there are only scarce data on the effect of salt and protein intake on disease progression. Here we studied association of these dietary factors with the rate of disease progression in ADPKD and what the mediating factors are by analyzing an observational cohort of 589 patients with ADPKD. Salt and protein intake were estimated from 24-hour urine samples and the plasma copeptin concentration measured as a surrogate for vasopressin. The association of dietary intake with annual change in the estimated glomerular filtration rate (eGFR) and height adjusted total kidney volume (htTKV) growth was analyzed with mixed models. In case of significant associations, mediation analyses were performed to elucidate potential mechanisms. These patients (59% female) had a mean baseline age of 47, eGFR 64 mL/min/1.73m^2 and the median htTKV was 880 mL. The mean estimated salt intake was 9.1 g/day and protein intake 84 g/day. During a median follow-up of 4.0 years, eGFR was assessed a median of six times and 24-hour urine was collected a median of five times. Salt intake was significantly associated with annual change in eGFR of −0.11 (95% confidence interval 0.20 – −0.02] mL/min/1.73m^2) per gram of salt, whereas protein intake was not (−0.00001 [−0.01 – 0.01] mL/min/1.73m^2) per gram of protein). The effect of salt intake on eGFR slope was significantly mediated by plasma copeptin (crude analysis: 77% mediation, and, adjusted analysis: 45% mediation), but not by systolic blood pressure. Thus, higher salt, but not higher protein intake may be detrimental in ADPKD. The substantial mediation by plasma copeptin suggests that this effect is primarily a consequence of a salt-induced rise in vasopressin.

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KEYWORDS: ADPKD; salt; vasopressin; urea

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In chronic kidney disease (CKD), salt restriction is advocated to slow disease progression.[1] Salt restriction lowers blood pressure and potentiates renoprotective effects of renin-angiotensin-aldosterone system (RAAS) blockade.[2] The role of dietary protein restriction in slowing progression of CKD is more controversial, although several meta-analyses indicate a beneficial, albeit small effect.[3,4] In autosomal dominant polycystic kidney disease (ADPKD) specifically, there are only scarce data on the renal effects of salt and protein intake.

In the Consortium for Radiologic Imaging Studies in Polycystic Kidney Disease (CRISP) cohort, an observational study in 241 patients with ADPKD with early stage disease, higher urinary sodium excretion (indicating higher salt intake) was associated with more rapid kidney volume growth. In a *post hoc* analysis of the HALT Progression of Polycystic Kidney Disease (HALT-PKD) study, a randomized controlled trial in 1044 patients with later-stage ADPKD, sodium excretion was associated with steeper estimated glomerular filtration rate (eGFR) decline in patients with later-stage ADPKD but not in patients with early-stage ADPKD.[5,6] It has been suggested that an association with eGFR decline may be caused by salt restriction potentiating the renal protective effects of RAAS blockade, similar to non-ADPKD CKD.[6] An alternative explanation could be that salt intake leads to accelerated disease progression in ADPKD by stimulation of vasopressin secretion. Vasopressin is known to be causally related to disease progression in ADPKD.[7–9] One of the main factors for vasopressin secretion is plasma sodium concentration,[10] which increases after salt ingestion.

As urinary urea excretion was not measured in the HALT-PKD trial, it is unclear whether protein intake was also associated with eGFR decline.[6] The effect of a low protein intake on rate of eGFR decline has been studied in a *post hoc* analysis of the Modification of Diet in Renal Disease (MDRD) study, in which low protein diet was

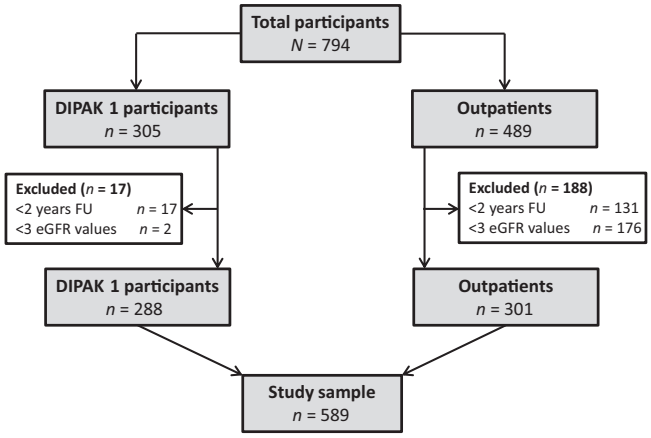

Figure 1 | Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) flow diagram. DIPAK, Developing Interventions to Halt Progression of Autosomal Dominant Polycystic Kidney Disease; eGFR, estimated glomerular filtration rate; FU, follow-up.

compared to a usual diet (study A) and a very low protein diet was compared to a low protein diet (study B). In the subgroup of 200 patients with ADPKD, there were no significant differences in either substudy; however, the results were deemed inconclusive by the investigators, among others due to lack of power.[11,12]

Given these scarce and inconclusive data, we aimed to investigate the relation between salt and protein intake and renal function decline in ADPKD. To address this aim, we analyzed data of patients with ADPKD in a large observational cohort. We also aimed to study whether a potential association was mediated by vasopressin or by other potential mechanisms.

RESULTS

The cohort flow is detailed in Figure 1. The baseline characteristics are shown in Table 1. Mean age was 47 ± 11 years, 59% of participants were female, eGFR was 64 ± 24 ml/min per 1.73 m^2 and median height-adjusted total kidney volume (htTKV) was 880 ml (interquartile range [IQR]: 549, 1352). There were no significant differences in age, sex, eGFR, and htTKV in the 205 patients that were excluded due to insufficient follow-up data. Sodium excretion was 156 ± 65 mmol/24 hours at baseline, corresponding with an estimated salt intake of 9.1 ± 3.8 g. Urea excretion was 390 ± 132 mmol/24 hours, corresponding with an estimated protein intake of 84 ± 25 g. Sodium excretions and urea excretion during all visits in the Developing Interventions to Halt Progression of Autosomal Dominant Polycystic Kidney Disease (DIPAK) 1 trial and the DIPAK observational cohort are shown Figure 2.

During a median follow-up time of 4.0 years (IQR: 2.6, 5.0), eGFR was assessed 6 times (IQR: 5, 14) and 24-hour urine was collected 5 times (IQR: 4, 7). Average annual change in eGFR was −3.50 ml/min per 1.73 m^2 per year (95% confidence interval [CI]: −3.70 to −3.29).

Sodium excretion and urea excretion

Sodium excretion was strongly correlated with urea excretion (standardized β = 0.61, unstandardized β = 1.8 mmol urea per mmol sodium; 95% CI: 1.6 to 2.0; $P < 0.001$). In mixed model analysis, sodium excretion was univariably associated with change in eGFR (−0.16 ml/min per 1.73 m^2 per year per 18 mmol of sodium; 95% CI: −0.24 to −0.08; $P < 0.001$), as was urea excretion (−0.03 ml/min per 1.73 m^2 per year per 40 mmol of urea; 95% CI: −0.05 to −0.001; $P = 0.04$). In multivariable analysis, adjusted for age, sex, body surface area (BSA), baseline htTKV, and DNA mutation, the association of sodium excretion with change in eGFR remained statistically significant (Table 2). In contrast, the association between urea excretion and eGFR slope lost significance after adjustment for potential confounders (Table 2). Figure 3 graphs the relationship among sodium excretion and urea excretion and eGFR slope. Based on the excretions of sodium and urea, we estimated salt and protein intake. In the multivariable model, the association of salt intake with change in eGFR was −0.11 ml/min per 1.73 m^2 per year per gram salt (95% CI: −0.20 to −0.02; $P = 0.02$), the association of protein intake with change in eGFR was not significant (−0.00001 ml/min per 1.73 m^2 per year per gram protein; 95% CI: −0.01 to 0.01; $P = 0.9$) (Supplementary Table S1A). When we excluded the

Table 1 | Baseline characteristics

Characteristics	n = 589
Age, yr	47 ± 11
Female sex	245 (59)
Weight, kg	
Females	76 ± 15
Males	90 ± 14
Height, m	
Females	1.70 ± 0.07
Males	1.84 ± 0.07
SBP, mm Hg	133 ± 14
DBP, mm Hg	82 ± 10
RAAS blockade, yes	415 (71)
eGFR, ml/min per 1.73 m^2[a]	64 ± 24
htTKV, ml/m	880 (549, 1352)
Copeptin, pmol/l	7.6 (4.5, 13.2)
Mayo risk class	
1A/1B, low-risk disease	145 (26)
1C/1D/1E, high-risk disease	385 (69)
2, atypical	27 (5)
PKD genotype	
PKD1 truncating	241 (42)
PKD1 nontruncating	151 (26)
PKD2	128 (22)
Unknown/not detected	50 (9)
Sodium excretion, mmol/24 h	156 ± 65
Estimated salt intake, g/24 h	9.1 ± 3.8
Urea excretion, mmol/24 h	390 ± 132
Estimated protein intake, g/24 h	84 ± 25
Urine volume, l/24 h	2.3 ± 0.8

DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; htTKV, height-adjusted total kidney volume; PKD, polycystic kidney disease; RAAS, renin-angiotensin-aldosterone system; SBP, systolic blood pressure.
Variables are presented as mean ± SD, as median (interquartile range) in case of nonnormal distribution, or as n (%) for categorical variables.
[a]Estimated by Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.

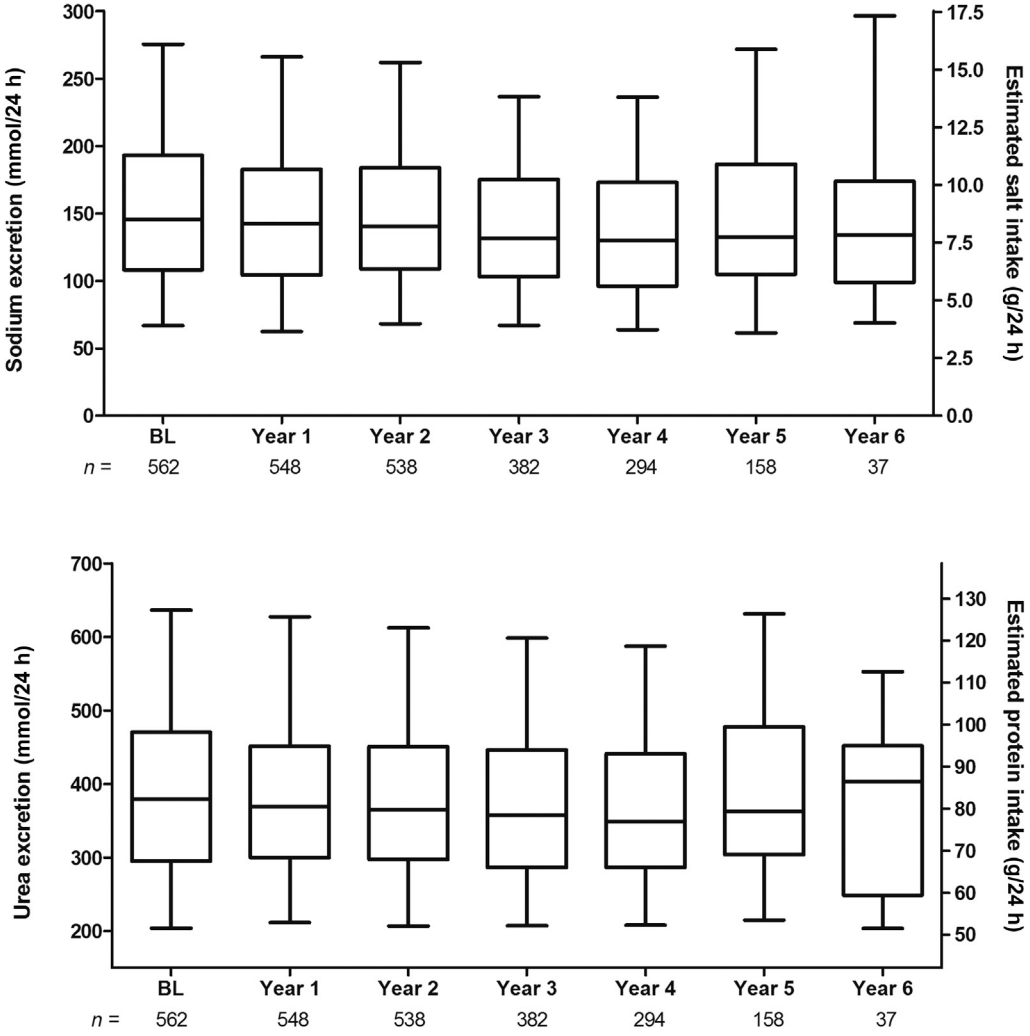

Figure 2 | Mean sodium excretion and urea excretion at the yearly visits, with estimated salt and protein intake. Whiskers indicate 5% to 95% range. BL, baseline.

Table 2 | Associations of sodium and urea excretion with eGFR slope (n = 553)

Sodium and urea excretion vs. eGFR slope (ml/min per 1.73 m^2 per yr)						
	Model 1		Model 2		Model 3	
	Est. (95% CI)	*P* Value	Est. (95% CI)	*P* Value	Est. (95% CI)	*P* Value
Sodium excretion, per 18 mmol[a]	−0.12 (−0.20 to −0.03)	0.006			−0.11 (−0.21 to −0.02)	0.02
Urea excretion, per 40 mmol[a]			−0.02 (−0.05 to 0.01)	0.2	−0.002 (−0.03 to 0.03)	0.9
Age, per yr[a]	0.01 (−0.01 to 0.03)	0.4	0.01 (−0.01 to 0.03)	0.3	0.01 (−0.01 to 0.03)	0.4
Female sex[a]	−0.08 (−0.56 to 0.40)	0.7	−0.01 (−0.50 to 0.47)	0.9	−0.08 (−0.57 to 0.40)	0.7
BSA, per m^2[a]	0.07 (−1.05 to 1.20)	0.9	−0.16 (−1.28 to 0.97)	0.8	0.08 (−1.06 to 1.22)	0.9
Log_{10} htTKV, ml/m	−2.98 (−3.70 to −2.27)	<0.001	−3.05 (−3.76 to −2.33)	<0.001	−2.99 (−3.70 to −2.27)	<0.001
DNA mutation (ref: PKD2)						
PKD 1 truncating	−1.25 (−1.78 to −0.72)	<0.001	−1.24 (−1.77 to −0.71)	<0.001	−1.25 (−1.78 to −0.73)	<0.001
PKD 1 nontruncating	−1.18 (−1.73 to −0.62)	<0.001	−1.14 (−1.70 to −0.58)	<0.001	−1.18 (−1.73 to −0.62)	<0.001
Unknown	−0.64 (−1.33 to 0.05)	0.07	−0.64 (−1.33 to 0.06)	0.07	−0.64 (−1.33 to 0.06)	0.07

BSA, body surface area; CI, confidence interval; eGFR, estimated glomerular filtration rate; Est, estimation; htTKV, height-adjusted total kidney volume; PKD, polycystic kidney disease; ref, reference.

[a]Estimations and *P* values shown for the interactions of variables with time. The interaction with time signifies the effect of said variable on eGFR over time: that is, the effect on eGFR slope. Model 1 shows the association of sodium excretion with eGFR slope. Model 2 shows the association of urea excretion with eGFR slope. Model 3 shows the associations of sodium and urea excretion with eGFR slope in the same model. All models were adjusted for time, age, sex, BSA, and their interactions with time. The estimations for the variables not interacted with time (not shown) signify the effect of said variable on baseline eGFR (the intercept).

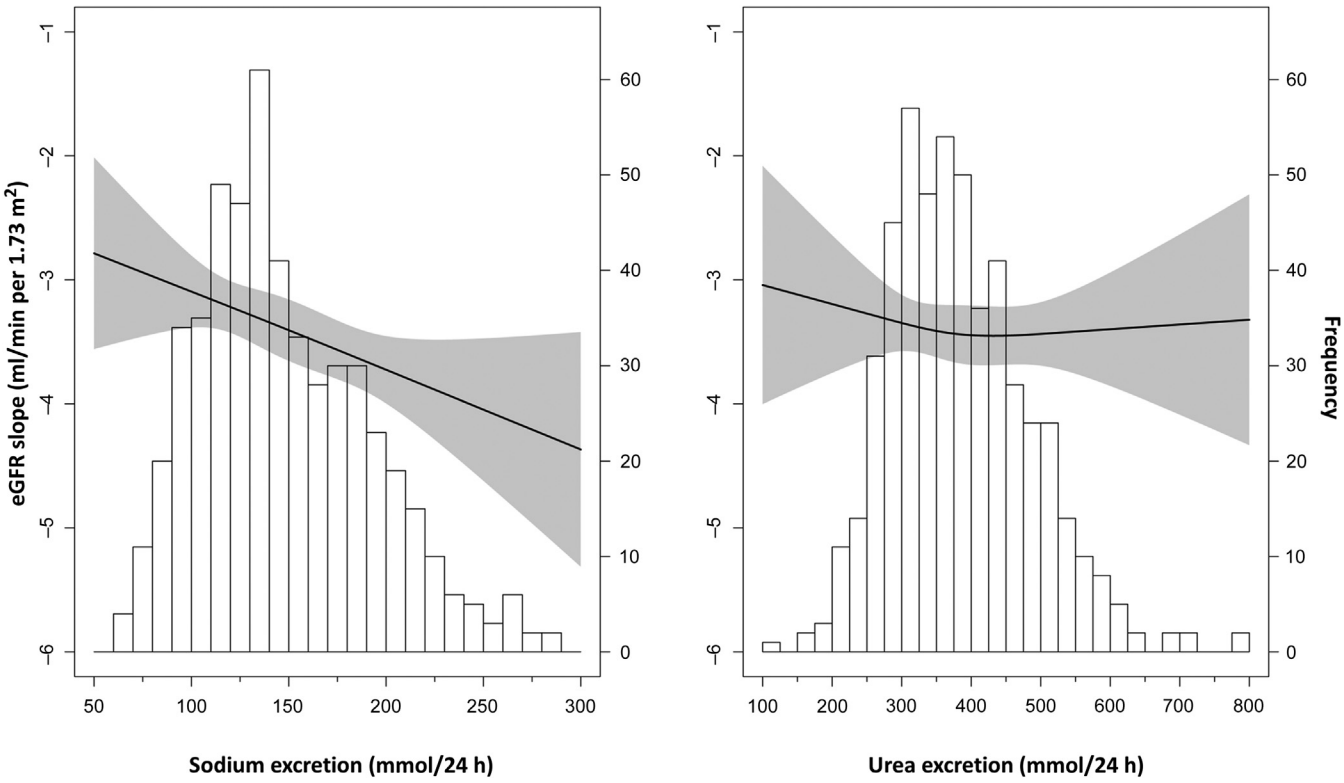

Figure 3 | Distribution of sodium excretion and urea excretion and their association with estimated glomerular filtration rate (eGFR) slope.

patients that used lanreotide during the DIPAK-1 trial, the results were essentially the same (Supplementary Table S1B).

In univariate analysis, both sodium excretion and urea excretion were associated with htTKV growth (0.63% per year per 18 mmol sodium; 95% CI: 0.40 to 0.87; $P < 0.001$, and 0.18% per year per 40 mmol urea; 95% CI: 0.09 to 0.28; $P < 0.001$, respectively). The association of sodium excretion with htTKV growth remained significant after adjustment for age, sex, BSA, baseline htTKV, and DNA mutation, whereas the association of urea excretion lost significance (Table 3).

Sensitivity analyses

As sensitivity analyses, we repeated the linear mixed model analyses with salt and protein intake per kilogram ideal body weight, and salt and protein per kilogram actual body weight (Supplementary Table S1C and D). As additional sensitivity analyses, we repeated the analyses using baseline 24-hour excretions instead of mean excretions, we subsequently used median excretions instead of mean excretions. We excluded 24-hour urine collections if the 24-hour creatinine excretion was >30% different from that participant's average creatinine excretion. Finally, we performed a sensitivity analysis in which we adjusted for albuminuria. All of these analyses yielded essentially the same results.

Subgroup analyses

We tested for differences in the association between salt intake and annual change in eGFR across several subgroups (Figure 4). Higher salt intake was associated with more rapid eGFR decline or neutral eGFR in all subgroups. The interaction term between use of RAAS blockade and salt intake was significant ($P = 0.02$), with a stronger negative association in patients who did not use RAAS blockade. There was a trend toward a significant interaction with age ($P = 0.06$) and baseline eGFR ($P = 0.07$). Compared with patients that did not use RAAS blockade, RAAS blockade users had similar salt intake, but they were older, more often female, had lower eGFR, and had other baseline differences (Supplementary Table S2). There was significantly higher average salt intake in the younger patients than in the older patients (9.0 ± 2.7 g vs. 8.3 ± 2.5 g; $P = 0.002$). Salt intake was similar in patients with higher eGFR (8.8 ± 2.8 g) and patients with lower eGFR (8.4 ± 2.5 g; $P = 0.07$).

Mediation by blood pressure, RAAS, or copeptin

We performed structural equation models to test for possible mediators of the association of excretion and eGFR slope. First, we tested whether the effect was mediated by an effect on blood pressure. In this model, the total effect of estimated salt intake on eGFR slope was estimated as −0.13 ml/min per 1.73 m^2 per year per gram table salt (95% CI: −0.23 to −0.02; $P = 0.03$). The direct effect of blood pressure on eGFR slope was significant (−0.02 ml/min per 1.73 m^2 per year per mm Hg; 95% CI: −0.03 to −0.01; $P = 0.02$). However, the direct effect of salt intake on systolic blood pressure was insignificant ($P = 0.3$). Thus, the indirect effect through

Table 3 | Association of estimated salt intake and protein intake with annual htTKV growth (n = 283)

Sodium and urea excretion vs. htTKV growth (% per yr)	Model 1		Model 2		Model 3	
	Est. (95% CI)	*P* Value	Est. (95% CI)	*P* Value	Est. (95% CI)	*P* Value
Sodium excretion, per 18 mmol[a]	0.31 (0.09 to 0.53)	0.007			0.44 (0.18 to 0.71)	0.001
Urea excretion, per 40 mmol[a]			0.01 (−0.07 to 0.09)	0.8	−0.09 (−0.19 to 0.01)	0.09
Age, per yr[a]	−0.05 (−0.12 to 0.02)	0.2	−0.07 (−0.14 to 0.00)	0.05	−0.05 (−0.12 to 0.02)	0.2
Female sex[a]	−2.87 (−3.94 to −1.79)	<0.001	−3.09 (−4.13 to −2.04)	<0.001	−3.01 (−4.05 to −1.95)	<0.001
BSA, per m^{2}[a]	0.37 (−2.36 to 3.18)	0.9	1.55 (−1.11 to 4.29)	0.3	0.75 (−1.93 to 3.51)	0.6
Log_{10} baseline htTKV, ml/m	0.46 (−1.32 to 2.27)	0.6	0.58 (−1.14 to 2.32)	0.5	0.40 (−1.32 to 2.14)	0.7
DNA mutation (ref: PKD2)						
PKD 1 truncating	−1.38 (−2.64 to −0.10)	0.03	−1.48 (−2.70 to −0.25)	0.02	−1.41 (−2.63 to −0.17)	0.03
PKD 1 nontruncating	−0.92 (−2.29 to 0.48)	0.2	−1.08 (−2.40 to 0.26)	0.1	−0.85 (−2.19 to 0.50)	0.2
Unknown	−0.77 (−2.56 to 1.05)	0.4	−0.90 (−2.63 to 0.86)	0.3	−0.77 (−2.50 to 1.00)	0.4
Randomization group (lanreotide)	−2.07 (−2.93 to −1.20)	0.004	−2.15 (−3.00 to −1.30)	<0.001	−2.21 (−3.05 to 1.35)	<0.001

BSA, body surface area; CI, confidence interval; eGFR, estimated glomerular filtration rate; Est, estimation; htTKV, height-adjusted total kidney volume; PKD, polycystic kidney disease; ref, reference.
[a]Estimations and *P* values shown for the interactions of variables with time. The interaction with time signifies the effect of said variable on eGFR over time: that is, the effect on eGFR slope. Model 1 shows the association of sodium excretion with eGFR slope. Model 2 shows the association of urea excretion with eGFR slope. Model 3 shows the associations of sodium and urea excretion with eGFR slope in the same model. All models were adjusted for time, age, sex, BSA, and their interactions with time. The estimations for the variables not interacted with time (not shown) signify the effect of said variable on baseline eGFR (the intercept).

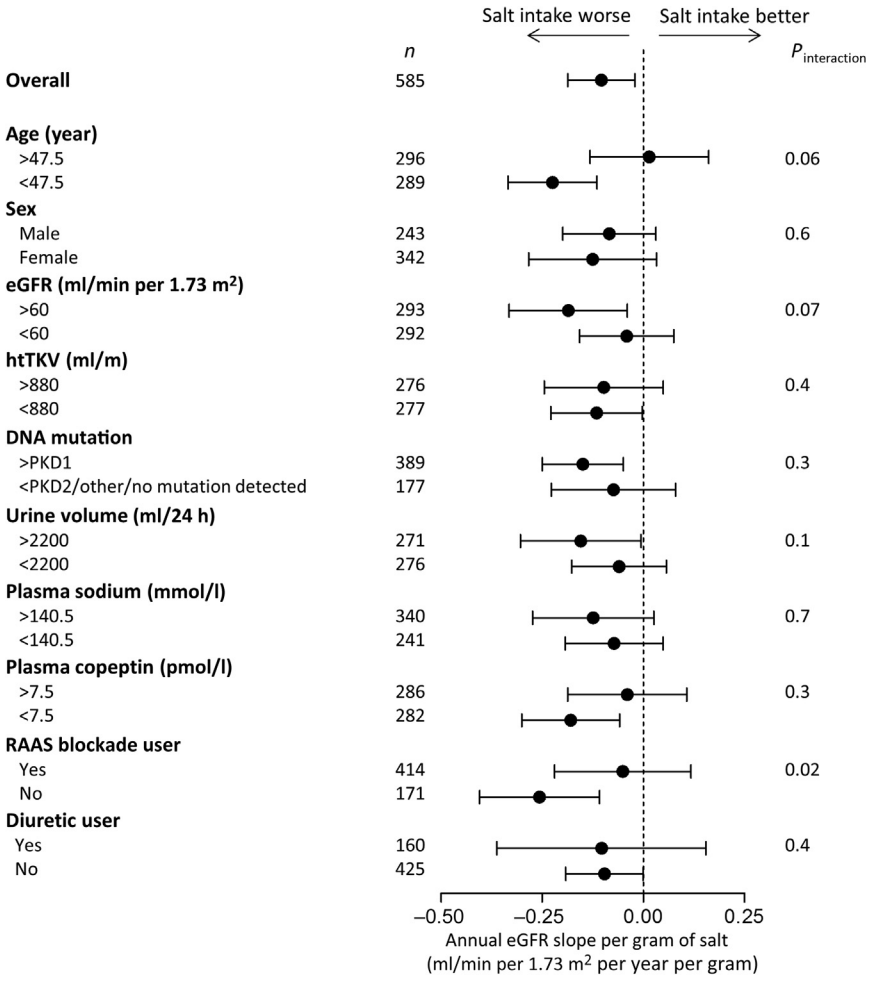

Figure 4 | Association of salt intake with slope of estimated glomerular filtration rate (eGFR) (ml/min per 1.73 m^{2} per year) in subgroups. htTKV, height-adjusted total kidney volume; PKD, polycystic kidney disease; RAAS, renin-angiotensin-aldosterone system.

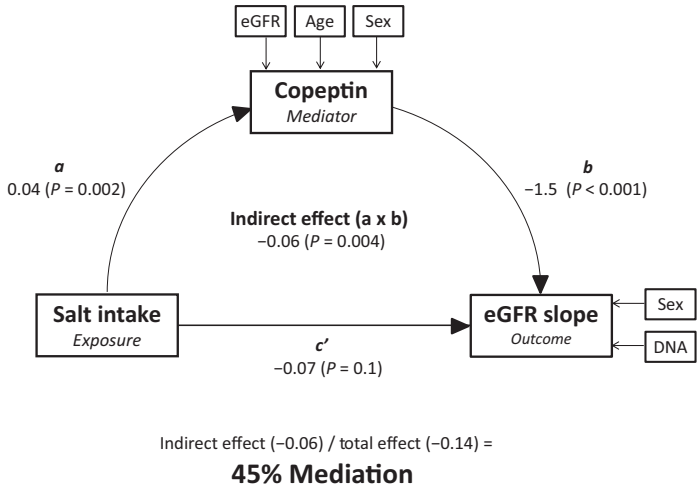

Figure 5 | Mediation analyses of the effect of sodium excretion on estimated glomerular filtration rate (eGFR) slope. Copeptin values are natural log–transformed. The total effect of salt intake on eGFR slope is estimated as −0.14 ml/min per 1.73 m^2 per year per gram of table salt. The indirect effect of salt intake (per gram) on eGFR slope (ml/min per 1.73 m^2 per year) via copeptin (pmol/l) is −0.06 ml/min per 1.73 m^2 per year per gram of table salt ($P < 0.001$). Mediation of total effect of copeptin is calculated as indirect effect (−0.06)/total effect (−0.14) = 45% ($P < 0.001$). Analysis adjusted for baseline eGFR, age, sex, and DNA mutation as shown.

systolic blood pressure was not significant (estimate: −0.005; 95% CI: −0.01 to 0.003; $P = 0.3$). Ergo, there was no significant mediation by systolic blood pressure.

We tested whether the effect of salt intake on eGFR was mediated by plasma renin and plasma aldosterone in patients that did not use RAAS blockade ($n = 58$). In these patients, median plasma renin was 10.6 pg/ml (IQR: 6.5, 16.7) and median plasma aldosterone was 265 pg/ml (IQR: 181, 363). Both indirect effects were not significant ($P = 0.3$ and 0.4, respectively), indicating there was no statistically significant mediation by plasma renin or plasma aldosterone.

Next, we investigated whether the association of sodium excretion and eGFR slope was mediated by copeptin (average of 2 values). There was high correlation between the 2 plasma copeptin measurements (Spearman coefficient: 0.85; $P < 0.001$) (Supplementary Figure S1). In a crude model, the total effect of salt intake on eGFR slope was estimated as −0.16 (95% CI: −0.23 to −0.09) ml/min per 1.73 m^2 per year per gram table salt ($P < 0.001$). The indirect effect, mediated by copeptin, was estimated to be −0.12 (95% CI: −0.18 to −0.08) ml/min per 1.73 m^2 per year per gram table salt ($P < 0.001$). Thus, the effect of salt intake on eGFR slope is mediated by copeptin by 77% (95% CI: 32% to 100%).

After this crude analysis, the mediation model with copeptin was adjusted for potential confounders. In multivariable analysis, baseline age, sex, and eGFR were significantly associated with plasma copeptin on top of estimated salt intake. Sex and DNA mutation were significantly associated with eGFR slope on top of either salt intake or plasma copeptin. After adjustment for these variables, the total effect of salt intake on eGFR slope was −0.14 (95% CI: −0.23 to −0.04) ml/min per 1.73 m^2 per year per gram table salt ($P = 0.004$), and the indirect effect was −0.06 (95% CI: −0.10 to −0.02; $P = 0.004$) (Figure 5). Thus, in the adjusted analysis, the effect of salt intake on eGFR slope is mediated by copeptin by 45% (95% CI: 1% to 89%). There was no indication of an impact of unmeasured confounding on these results (Supplementary Table S3).

We repeated the mediation analysis with htTKV slope as a dependent variable. The total effect of salt intake on htTKV slope was 0.59% htTKV growth per year per gram table salt (95% CI: 0.33 to 0.86; $P < 0.001$). There was no significant mediation by systolic blood pressure ($P = 0.5$). The indirect effect, mediated by plasma copeptin, was statistically significant (0.15% htTKV growth per year per gram table salt; 95% CI: 0.04 to 0.25; $P = 0.006$). Thus, the effect of salt intake on htTKV slope is mediated by plasma copeptin by 25% (95% CI: 4% to 45%).

We performed an exploratory mediation analysis to evaluate whether the effect of copeptin on eGFR slope was in turn mediated by htTKV growth. Rate of htTKV change was significantly associated with rate of eGFR change −0.38% per ml/min per 1.73 m^2 ($P < 0.001$). In the crude analysis, the total effect of natural log–transformed plasma copeptin on eGFR slope was −1.51 (95% CI: −1.88 to −1.14; $P < 0.001$), the indirect effect through htTKV growth was −0.20 (95% CI: −0.34 to −0.05; $P = 0.008$), ergo 13% (95% CI: 3% to 23%) mediation. However, after adjustment for confounders, this mediation lost statistical significance with an indirect effect of −0.12 (95% CI: −0.26 to 0.02; $P = 0.09$) and 8% (95% CI: −2% to 8%) mediation.

DISCUSSION

In this study, we found that salt, and not protein intake, is associated with kidney function decline in ADPKD. The effect is significantly mediated by plasma copeptin level, suggesting that salt intake may have detrimental effects by increasing vasopressin.

The association of salt intake with eGFR decline is in line with scarce earlier findings. An association between sodium excretion and ADPKD disease progression was first shown in the CRISP cohort, where a significant association with kidney volume growth was found.[5] In multivariable analysis, a significant association with eGFR decline was not found. However, this was a cohort of patients with early-stage ADPKD where GFR had not yet started to decline. Finding associations with rate of eGFR decline in such a cohort is difficult. Sodium excretion in the CRISP cohort was higher than in our cohort (193 ± 86 vs. 156 ± 65 mmol/24 hours). Similarly, in a *post hoc* analysis of the HALT-PKD study A, which included patients with early-stage ADPKD, the association between salt intake and eGFR decline was not significant ($P = 0.09$). Conversely, in HALT-PKD study B (which included patients with later-stage ADPKD, average sodium excretion was 178 ± 80), the association of salt intake with eGFR decline did point toward beneficial effects of salt restriction.[6] We were able to confirm this finding in a cohort of patients with ADPKD with a wide range of renal function. Within the present cohort, there was significantly higher average salt intake (and variance) in the younger patients than in older patients. This may have contributed to finding a trend toward a significantly stronger association between salt intake and eGFR decline within this subgroup.

In theory, protein intake could be detrimental through vasodilatory effects that cause intraglomerular hyperfiltration or through increment in vasopressin.[13–15] To our knowledge, only 2 studies assessed the association between protein intake and eGFR decline in ADPKD. Similar to sodium excretion, in the CRISP cohort no significant association between urea excretion and kidney function decline could be shown.[5] A *post hoc* analysis of the MDRD study also did not show a statistically significant effect of a low protein diet on eGFR slope in ADPKD.[12] However, the investigators suggest that this may be due to the acute hemodynamic effect of a lower protein intake, which caused acute lowering of GFR. This effect may have negated a subsequent beneficial effect of slower GFR decline because the follow-up was not long enough.[11] Furthermore, the investigators suggest that they may have lacked power to show the effect.[11] One advantage of the observational nature of our study is the lack of an acute effect of a diet intervention. Therefore, an acute hemodynamic effect cannot be the explanation why we did not find an association with kidney function decline. Although we cannot strictly exclude lack of power as an explanation for not finding an association between protein intake and eGFR decline, there also did not seem to be a trend toward a positive association after adjustment for potential confounders, which makes it less likely.

A possible mediating mechanism of the effect of salt intake on disease progression could be through blood pressure. Salt-sensitive hypertension is common in CKD. Furthermore, the HALT-PKD trial has shown beneficial effects of rigorous blood-pressure control regarding TKV growth in ADPKD and also regarding eGFR decline in the subgroup of patients with most severe disease.[16,17] Indeed, in in our study, there was a negative association between blood pressure and eGFR decline. However, we were not able to show a significant association between salt intake and blood pressure. In line with these results, the mediation analysis did not show significant mediation by systolic blood pressure.

Another mechanism that has been suggested to underlie a beneficial effect of restricted salt intake on ADPKD disease progression is potentiation of RAAS blockade.[6] A *post hoc* analysis of the Irbesartan Diabetic Nephropathy Trial (IDNT) and Reduction of Endpoints in NIDDM With the Angiotensin II Antagonist Losartan (RENAAL) studies showed that lower dietary sodium intake enhances the beneficial effects of RAAS blockade in 1137 patients with diabetic nephopathy.[18] The notion that salt restriction potentiates the renal protective effects of these medicaments is widely accepted nowadays. However, if potentiation of RAAS blockade would be the mechanism of effect in ADPKD, we would expect a stronger beneficial effect of salt restriction in RAAS blockade users than in patients who did not use RAAS blockade. As shown in Figure 4, this was not the case; the association with salt intake was most pronounced in patients who did not use RAAS blockade. Potentiation of RAAS blockade therefore does not seem to be the primary mechanism behind a possible beneficial effect of salt restriction in ADPKD. We were not able to show significant mediation effect of either renin or aldosterone in patients that did not use RAAS blockade; however, we lacked sufficient power to draw definite conclusions.

We did find significant mediation of the salt intake effect by copeptin, the surrogate marker of vasopressin. It is known that an increase in salt intake will cause an increase in plasma osmolality, triggering vasopressin secretion.[10] Vice versa, in their short-term pilot study of 34 patients with ADPKD, Amro *et al.*[19] showed that a combined salt and protein restriction in combination with adjusted water intake led to reduction in vasopressin secretion. In CKD in general, vasopressin can cause relative glomerular hyperfiltration that is potentially detrimental.[20] In ADPKD specifically, vasopressin causes cystogenesis and is associated with GFR decline.[19] Treatment with antagonists of the vasopressin V2 receptor ameliorate kidney volume growth and eGFR decline.[8,9] In the present study, not finding significant mediation of the plasma copeptin effect on eGFR decline by htTKV growth suggests that the detrimental effect of copeptin may not primarily have been a consequence of cyst growth. Mediation of the salt effect on eGFR decline by vasopressin could also explain not finding an independent effect of protein intake. Experiments have shown that urea is an ineffective osmole in plasma: that is, that infusion of sodium causes a much greater increase in vasopressin secretion than an equal infusion of osmoles of urea would.[10] If urea does not have an effect on vasopressin, and the detrimental effects of dietary factors in ADPKD are mainly through vasopressin, one would not expect a detrimental effect of protein intake.

There are limitations to this study that need to be addressed. Due to the lack of a standardized diet, there

probably were variations in salt and protein intake within subjects during the study, which may obscure associations with rate of disease progression. For that reason, we used the average values of all 24-hour urines that were collected throughout the study. The average excretions are probably a reasonable measure of average intake. Furthermore, due to the observational nature of this study, there can be no definite conclusions with regard to causality.

The main strengths of this study include frequent follow-up visits, allowing for accurate eGFR slope estimations, and estimation of protein and salt intake by gold standard measures: that is, by collection of multiple 24-hour urine samples over the entire study period. Previous studies have shown that multiple 24-hour collections are necessary to obtain accurate associations, and we obtained a median of 5 (IQR: 4, 7) samples per patient.[21] Finally, this is the first study that shows an association between salt intake and eGFR slope in a cohort where patients with early-stage and those with later-stage ADPKD are both well represented.

Our finding that 1 g of salt intake is associated with −0.11 ml/min per 1.73 m^2 annual change in eGFR suggests that adherence to the current sodium restriction guidelines could significantly postpone end-stage kidney disease. If a 30-year-old male patient with an eGFR of 110 ml/min per 1.73 m^2 would adhere to the currently advised maximum of 5 g of table salt per day, instead of 9.1 g (average in this cohort), he would hypothetically ameliorate his annual change in eGFR from −3.50 ml/min per 1.73 m^2 per year (average in this cohort) to −3.05 ml/min per 1.73 m^2 per year. This hypothetical patient would postpone end-stage kidney disease by 4 years, from age 57 to 61. This hypothesis needs confirmation in an intervention study. As this cohort included few patients with very low estimated salt intake, we cannot investigate the potential effect of lower sodium intake and therefore cannot make a recommendation for lower sodium restrictions than the current guidelines. Based on our data, there is no indication that protein restriction is beneficial. Additional benefits of salt restriction could be reduction of polyuria both in late-stage ADPKD and in patients using the vasopressin V2 receptor antagonist tolvaptan. In both cases, osmolar excretion is the main determinant of urine volume due to urine concentrating defects.[22] Whether protein intake and salt intake have the same effects on reduction of polyuria remains the subject of future studies.

In conclusion, this study shows that 24-hour urinary sodium excretion is associated with the rate of eGFR decline in ADPKD and suggests that salt restriction should be an important focus of clinicians in treatment of ADPKD.

METHODS

For this study, we used the data of the DIPAK observational cohort study that was designed to investigate the natural course of PKD. This cohort study was initiated to continue follow-up of patients with ADPKD that participated in the DIPAK 1 randomized controlled trial in which the renoprotective effect of the somatostatin analogue lanreotide was assessed (n = 305). Inclusion into the observational cohort was extended to patients with ADPKD from the outpatient clinic (n = 489) and is still ongoing. Data were collected in the University Medical Centers of Groningen, Leiden, Nijmegen, and Rotterdam. Design, methods, and the main outcomes of the DIPAK 1 trial have been published elsewhere.[23,24] In brief, patients were included between 2012 and 2015 if they were 18 to 60 years of age, had ADPKD (modified Ravine criteria[25]), and an eGFR between 30 and 60 ml/min per 1.73 m^2. Following a baseline visit, patients were seen at 4, 8, 12, 48, 96, 120, and 132 weeks, and blood was collected every 12 weeks. After the end of the trial, 175 patients agreed to continue follow-up. Inclusion criteria for the observational cohort study were age ≥18 years and an eGFR ≥15 ml/min per 1.73 m^2. Until December 31, 2017, all eligible patients who were seen at the outpatient clinics of any of the 4 centers were asked to participate in the observational study. Contraindications for participation in the trial and the observational cohort were concomitant diseases or medication use that may influence the natural course of ADPKD (e.g., diabetes mellitus or chronic nonsteroidal anti-inflammatory drug use). For the present analyses, we included patients with ADPKD with a minimal number of eGFR assessments of 3 during at least 2 years of follow-up, leaving 589 patients for analysis (Strengthening the Reporting of Observational Studies in Epidemiology [STROBE] flow diagram, Figure 1). The DIPAK observational study was approved by the Institutional Review Board of the University Medical Center Groningen and conducted in adherence to the International Conference on Harmonization–Good Clinical Practice guidelines. Written informed consent was obtained from all patients.

Measurements

Creatinine was measured using an isotope dilution mass spectrometry–traceable enzymatic method in samples stored at −80 °C. eGFR was estimated using the creatinine based Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula.[26] Fasting plasma copeptin concentrations were measured using a sandwich immunoassay (B·R·A·H·M·S, Thermo Fisher Scientific, Berlin, Germany), at baseline in all patients and also at week 12 in patients in the DIPAK 1 study. Renin (Renin III Generation RIA; Cisbio Bioassays, Codelet, France) and aldosterone (Demeditec Diagnostics GmbH, Kiel, Germany) were measured at baseline by radioimmunoassay. Osmolality was measured by the freezing point depression method, sodium and potassium concentration by ion specific electrodes, and urea by an enzyme kinetic assay. Magnetic resonance imaging was performed using a standardized magnetic resonance imaging protocol without the use of intravenous contrast. TKV was assessed by manual tracing of T_2-weighted coronal magnetic resonance images using Analyze direct 9.0 software (AnalyzeDirect, Inc., Overland Park, KS).

24-hour urine

Twenty-four–hour urine samples were collected at baseline; at weeks 12, 48, 96, 120, and 132; and in case of early end of treatment during the DIPAK 1 trial and yearly thereafter. For all analyses, the average values of all available 24-hour urine samples were used. Sodium was measured by ion-specific electrodes and urea by enzyme kinetic essay. Salt intake was estimated by multiplying sodium excretion by sum of the molar mass of sodium and chloride: salt intake = sodium excretion (mol) × (22.99 + 35.45). Total protein intake was estimated from urea excretion by the method of Maroni *et al.*[27]: protein intake = [urea excretion (mmol) × 0.028 + 0.031 × body weight (kg)] × 6.25.

Statistical analyses

For statistical analyses, we used SPSS version 23 (IBM Corp., Armonk, NY) or Stata SE 14 (StataCorp, College Station, TX) in case of linear mixed model analyses. For all analyses, a 2-sided $P < 0.05$ was considered statistically significant.

Mixed model repeated measure analyses were used to evaluate associations of dietary intake with slope of eGFR slope, and all available eGFR assessments were included for slope analysis. For sodium and urea excretions, the average values of all available 24-hour urine collections were used. Intercept and slope were allowed to vary randomly, with an unstructured covariance matrix. Fixed effects in the models were time, sodium excretion (or estimated salt intake), BSA, age, sex, htTKV, and DNA mutation, as well as the interactions of these variables with time. A significant interaction time × sodium excretion signifies an association with annual eGFR decline. Similar analyses were performed for urea excretion (or estimated protein intake). Patients were included in the analysis if all data was available listwise (complete case analysis). Follow-up magnetic resonance imaging was performed during the DIPAK 1 trial. Change in htTKV was assessed using $\log_{10}$-transformed htTKV data, the antilog of the estimated effect was derived from the mixed model analysis to provide annual percentage change of htTKV. Model validation was performed by visual inspection of the residual plots. We plotted histograms of the level 1 residuals and histograms of residuals of random slopes and intercepts. Standardized residuals were plotted versus predicted values and time.

We performed a number of sensitivity analyses. Salt and protein intake were corrected for actual body weight and ideal body weight. Ideal body weight was derived using a body mass index of 22 kg/m^2 as reference. Furthermore, the analyses were repeated excluding the 142 patients who received lanreotide treatment during the DIPAK 1 trial. We also performed sensitivity analyses to investigate the effect of urine collection errors. We repeated the analyses with median urinary excretion values instead of the mean, and we excluded follow-up urine collections if creatinine excretion was >30% different from the mean. Finally, we performed a sensitivity analysis in which we used baseline 24-hour urine collections instead of average, and we performed a sensitivity analysis in which we adjusted for albuminuria.

Structural equation models were used to perform mediation analysis with eGFR slope as an outcome variable; estimated salt intake as an exposure; and systolic blood pressure, plasma copeptin, plasma renin, and plasma aldosterone as potential mediators. For copeptin, the average of 2 measurements was used for mediation analysis. Copeptin was natural log–transformed. Mediation analyses with plasma renin and aldosterone were performed only in patients who did not use RAAS blockade. eGFR slope and intercept were added as latent variables. In contrast to longitudinal mixed effects models, latent growth structural equation models require time-structured data (i.e., data collected at the same time from baseline for every subject). Therefore, data from the DIPAK observational study could not be combined with data from the DIPAK 1 trial, and only the data collected during the DIPAK 1 trial were included for this analysis. The same analysis was repeated with htTKV growth as outcome variable.

In case of significant mediation effect on eGFR slope, we investigated the role of potential measured and unmeasured confounding. As potential confounders, we evaluated age, sex, BSA, baseline eGFR, baseline TKV ($\log_{10}$-transformed), DNA mutation (PKD1 or PKD2), use of RAAS blockade, and use of diuretics. We evaluated exposure-mediator confounding, mediator-outcome confounding, and exposure-mediator confounding. Using structural equation models, we estimated univariable associations of potential confounders with mediator and outcome. Subsequently, we added all potential confounders that were univariably associated to a multivariable model. Variables that were associated with exposure or outcome in multivariable analysis ($P < 0.05$) after backward elimination were included in the structural equation mediation model. The impact of unmeasured confounding was evaluated by a series of sensitivity analyses, according to the method of Imai *et al.*[28]

Subgroup analyses were performed for the association between salt intake and eGFR slope by including an interaction term between subgroup and salt intake (salt intake × subgroup) to the multivariable mixed model, adjusted for age, sex, and BSA. If the interaction term was significant, the subgroup was considered a significant moderator for the association.

DISCLOSURE

The authors received an unrestricted grant from Ipsen (manufacturer of a somatostatin analogue) as cofunding for an investigator-driven randomized, controlled trial (the DIPAK 1 study).

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SUPPLEMENTARY MATERIAL

Supplementary File (Word)

Figure S1. Scatterplot of copeptin measurement at baseline and at week 12.

Table S1. Sensitivity analyses: associations with slope of estimated GFR.

Table S2. Baseline characteristics of patients that use RAAS blockade and patients that do not use RAAS blockade.

Table S3. Sensitivity analysis mediation.

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