# Kidney Function and Plasma Copeptin Levels in Healthy Kidney Donors and Autosomal Dominant Polycystic Kidney Disease Patients

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## Abstract

**Background and objectives** Plasma copeptin, a marker of arginine vasopressin, is elevated in patients with autosomal dominant polycystic kidney disease and predicts disease progression. It is unknown whether elevated copeptin levels result from decreased kidney clearance or as compensation for impaired concentrating capacity. Data from patients with autosomal dominant polycystic kidney disease and healthy kidney donors before and after donation were used, because after donation, overall GFR decreases with a functionally normal kidney.

**Design, setting, participants, & measurements** Data were obtained between October of 2008 and January of 2012 from healthy kidney donors who visited the institution for routine measurements predonation and postdonation and patients with autosomal dominant polycystic kidney disease who visited the institution for kidney function measurement. Plasma copeptin levels were measured using a sandwich immunoassay, GFR was measured as $^{125}$I-iothalamate clearance, and urine concentrating capacity was measured as urine-to-plasma ratio of urea. In patients with autosomal dominant polycystic kidney disease, total kidney volume was measured with magnetic resonance imaging.

**Results** Patients with autosomal dominant polycystic kidney disease ($n$=122, age=40 years, men=56%) had significantly higher copeptin levels (median=6.8 pmol/L; interquartile range=3.4–15.7 pmol/L) compared with donors ($n$=134, age=52 years, men=49%) both predonation and postdonation (median=3.8 pmol/L; interquartile range=2.8–6.3 pmol/L; $P$<0.001; median=4.4 pmol/L; interquartile range=3.6–6.1 pmol/L; $P$<0.001). In donors, copeptin levels did not change after donation, despite a significant fall in GFR (from $105\pm17$ to $66\pm10$; $P$<0.001). Copeptin and GFR were significantly associated in patients with autosomal dominant polycystic kidney disease ($\beta$=−0.45, $P$<0.001) but not in donors. In patients with autosomal dominant polycystic kidney disease, GFR and total kidney volume were both associated significantly with urine-to-plasma ratio of urea ($\beta$=0.84, $P$<0.001; $\beta$=−0.51, $P$<0.001, respectively).

**Conclusions** On the basis of the finding in donors that kidney clearance is not a main determinant of plasma copeptin levels, it was hypothesized that, in patients with autosomal dominant polycystic kidney disease, kidney damage and associated impaired urine concentration capacity determine copeptin levels.

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## Introduction

In autosomal dominant polycystic kidney disease (ADPKD), arginine vasopressin (AVP) is assumed to be involved in cyst formation by promoting intracellular cAMP production, which leads to cyst growth and kidney function decline (1–3). Indeed, it has been found that blocking the AVP V2 receptor improves prognosis in both experimental ADPKD models (4,5) and patients with ADPKD (6).

AVP is difficult to measure (7,8). Copeptin consists of the C-terminal portion of Pro-AVP, the precursor of AVP, and is produced in equimolar amounts as AVP during precursor processing (9). Copeptin has been shown to be a relatively easy to measure, stable substitute for circulating AVP concentration (10,11).

Recently, we found cross-sectional associations between copeptin concentration and various markers of disease severity in ADPKD (12). In these patients, copeptin is elevated in proportion to the impairment of renal function. In addition, we showed that, in patients with ADPKD, a high copeptin concentration is associated with accelerated growth of total kidney volume (TKV) (13), more rapid loss of measured GFR during short-term follow-up, and loss of eGFR during long-term follow-up (14). These data suggest that copeptin is a risk marker or even risk factor for detrimental kidney outcome in ADPKD and that measurement of copeptin concentration may be of help to assess prognosis in this patient group.

The question arises of why copeptin is increased in some patients with ADPKD. We previously hypothesized that increased levels are the result of an impaired urine concentrating capacity caused by cyst-induced abnormality in medullary osmolar gradient. However, a role of diminished kidney clearance in elevated plasma copeptin levels cannot be excluded. Copeptin has a molecular mass of only 5 kD, which makes it subject to kidney clearance. Indeed, several studies showed inverse associations between kidney function and copeptin (15–18).

To gain more insight in the role of kidney function and urine concentrating capacity in determining copeptin levels in ADPKD, we assessed copeptin levels in living kidney donors before and after donation and patients with ADPKD. In these patients, kidney function was measured as clearance of $^{125}$I-iothalamate. In addition, we measured plasma osmolality, TKV, and urine-to-plasma concentration ratio of urea (U/P urea). U/P urea has been suggested to reflect the severity of the urine concentrating defect in ADPKD, with lower values reflecting more severe disease (19).

## Materials and Methods
### Study Population
For this study, data were used from 134 participants who donated a kidney for transplantation at our institution between October of 2008 and December of 2011 and had blood samples available. In these kidney donors, kidney function was routinely measured predonation and postdonation, which is described in more detail by Tent *et al.* (20). Furthermore, we used data from 122 patients with ADPKD who visited our institution for kidney function measurement between November of 2007 and January of 2012. Diagnosis of ADPKD was made using the criteria by Ravine *et al.* (21). Exclusion criteria for both groups were indications for kidney disease other than ADPKD, diabetes, or cardiovascular events. In addition, patients with ADPKD were excluded if they received kidney replacement therapy or were unable to undergo magnetic resonance imaging (MRI). This study was performed in adherence to the Declaration of Helsinki. All participants gave written informed consent.

### Measurements and Definitions
The day before kidney function measurement, kidney donors and patients with ADPKD collected a 24-hour urine sample, in which concentrations of sodium, potassium, and urea were measured using standard methods. BP was assessed for 15 minutes with an automatic device (Dinamap) during the kidney function measurement. Blood samples were drawn in the morning without standardized fluid intake. Blood was used for immediate determination of hemoglobin, sodium, potassium, urea, and glucose using standard laboratory methods. Creatinine was measured with the Roche enzymatic creatinine assay. Furthermore, plasma samples were stored frozen at −80°C and thawed later to measure plasma copeptin levels using a sandwich immunoassay (Thermo Fisher Scientific BRAHMS, Hennigsdorf/Berlin, Germany) (10). All samples were analyzed for copeptin concentration at one single time point to eliminate interassay variation. The lower limit of detection was

0.4 pmol/L, and the functional assay sensitivity (interassay coefficient of variation<20%) was <1 pmol/L.

GFR was measured using a constant infusion method with $^{125}$I-iothalamate. The intrapatient day-to-day coefficient of variation of this method is 2.2% (22–25). GFR was adjusted for body surface area using the equation by Du Bois and Du Bois (26).

Patients with ADPKD underwent a standardized abdominal MRI protocol without the use of intravenous contrast. Scanning was performed on a 1.5-Tesla MRI Magnetom Avento (Siemens, Erlangen, Germany) with the use of body matrix and spine matrix coils. TKV was measured on T2-weighted coronal images using the commercially available software Analyze Direct 8.0 (Analyze Direct, Inc., Overland Park, KS), and adjusted for height. Intrareviewer and inter-reviewer coefficients of variation were 1.8% and 2.3%, respectively.

Urine osmolality was calculated using the equation 2× (urine sodium concentration+urine potassium concentration)+urine urea concentration (27). Plasma osmolality was calculated using the equation 1.9×(plasma sodium concentration+plasma potassium concentration)+plasma glucose concentration+plasma urea concentration×0.5+5 (28). U/P urea was also assessed.

### Statistical Analyses
To display characteristics of participants, parametric values were expressed as mean±SD, whereas nonparametric variables were presented as median (interquartile range). Significance between donors predonation and postdonation was tested using a paired *t* test for parametric values or a Wilcoxon signed rank test for nonparametric values. Differences between donors and patients with ADPKD were analyzed using a *t* test, a chi-squared test, or a Mann–Whitney *U* test as appropriate.

Several regression analyses were performed to test associations between plasma copeptin, GFR, plasma osmolality, urine osmolality, U/P urea, and TKV. Copeptin, urine osmolality, U/P urea, urine volume, TKV, change in copeptin, change in plasma sodium, change in mean arterial pressure (MAP), and change in urine volume in donors from predonation to postdonation have a nonnormal distribution and therefore, were ln log transformed. Standardized *β*-values and *P* values are given for all regression analyses. Regression analyses were adjusted for age and sex when indicated, as well as copeptin analyses for variables that may influence AVP levels (*i.e.*, plasma sodium concentration, urine volume [as indicator of fluid intake], MAP and use of diuretics). A sensitivity analysis was performed excluding all participants using diuretics. All analyses were performed using the statistical package IBM SPSS Statistics, version 20.0 (International Business Machines Corp., Chicago, IL). A two-sided *P* value <0.05 was considered to indicate statistical significance.

## Results
Characteristics of participants are presented in Table 1. Overall, 134 donors and 122 patients with ADPKD were studied. The median time period between the predonation measurement and donation was 16 weeks (minimum=3 weeks, maximum=101 weeks) and between donation and

**Table 1. Characteristics of all participants**

| Characteristics | Donors Predonation (n=134) | Donors Postdonation (n=134) | ADPKD (n=122) | P Value Predonation/ Postdonation | P Value Predonation/ ADPKD | P Value Postdonation/ ADPKD |
|---|---|---|---|---|---|---|
| Age (yr) | 52±10 | 52±10 | 40±12 | <0.001 | <0.001 | <0.001 |
| Men (%) | 49 | 49 | 56 | — | 0.30 | 0.30 |
| Use of antihypertensives (%) | 15 | 15 | 85 | >0.99 | <0.001 | <0.001 |
| Use of diuretics (%) | 5 | 4 | 20 | 0.32 | <0.001 | <0.001 |
| Body surface area (m$^2$) | 1.96±0.20 | 1.95±0.20 | 2.02±0.23 | <0.01 | 0.02 | <0.01 |
| Mean arterial pressure (mmHg) | 92±10 | 90±9 | 95±9 | 0.002 | <0.01 | <0.001 |
| Hemoglobin (mg/dl) | 14.3±1.1 | 13.5±1.1 | 13.4±1.5 | <0.001 | <0.001 | 0.51 |
| Plasma osmolality (mosM/kg) | 291±4 | 290±4 | 289±4 | 0.11 | <0.001 | 0.002 |
| Plasma sodium (mEq/L) | 142.3±1.9 | 141.6±1.8 | 140.4±1.8 | <0.001 | <0.001 | <0.001 |
| Plasma urea (mg/dl) | 16.0±3.4 | 19.3±4.2 | 24.1±12.6 | <0.001 | <0.001 | <0.001 |
| 24-h urine volume (L) | 2600 (2200–3050) | 2208 (1713–2700) | 2200 (1785–2673) | <0.001 | <0.001 | 0.51 |
| 24-h urine osmolality (mosM/kg) | 372 (320–459) | 412 (343–530) | 396 (314–474) | <0.001 | 0.54 | 0.06 |
| 24-h urine sodium (mmol/24 h) | 197 (156–249) | 169 (132–216) | 161 (128–215) | <0.001 | <0.001 | 0.34 |
| 24-h urine urea (mg/24 h) | 1140 (983–1350) | 1297 (1028–1669) | 1089 (905–1311) | <0.001 | <0.16 | <0.001 |
| GFR (ml/min per 1.73 m$^2$) | 105±17 | 66±10 | 74±32 | <0.001 | <0.001 | 0.01 |
| U/P urea | 29.2 (23.3–35.1) | 27.1 (20.9–34.5) | 23.3 (15.2–31.1) | 0.10 | <0.001 | 0.001 |
| Total kidney volume (ml) | — | — | 1535 (931–2356) | — | — | — |
| Plasma copeptin (pmol/L) | 3.8 (2.8–6.3) | 4.4 (3.1–6.1) | 6.8 (3.4–15.7) | 0.17 | <0.001 | <0.001 |

Data are given as mean ± SD for parametric data or median (interquartile range) for nonparametric data. Significance between predonation and postdonation was tested using a paired $t$ test or a Wilcoxon signed rank test when appropriate. Significance of difference between predonation and postdonation was tested using a paired $t$ test or a Wilcoxon signed rank test when appropriate. Significance of differences between predonation/postdonation and ADPKD was tested using a $t$ test, a chi-squared test, or a Mann–Whitney $U$ test when appropriate. U/P urea, urine-to-plasma concentration ratio of urea; ADPKD, autosomal dominant polycystic kidney disease.

postdonation measurement was 7 weeks (minimum=4 weeks, maximum=20 weeks). Plasma copeptin levels were significantly higher in patients with ADPKD compared with donors both predonation and postdonation. Patients with ADPKD had a significantly lower GFR compared with donors predonation but significantly higher GFR compared with donors postdonation. Men had higher copeptin levels than women in donors both predonation (men: 4.5 pmol/L; interquartile range=3.3–7.3 pmol/L; women: 3.3 pmol/L; interquartile range=2.2–5.7 pmol/L; $P=0.003$) and postdonation (men: 5.3 pmol/L; interquartile range=4.0–7.5 pmol/L; women: 3.5 pmol/L; interquartile range=2.5–5.2 pmol/L; $P<0.001$) as well as in patients with ADPKD (men: 10.7 pmol/L; interquartile range= 4.8–21.8 pmol/L; women: 4.8 pmol/L; interquartile range=2.5–9.4 pmol/L; $P<0.001$.). Because sex is an independent predictor for copeptin, we adjusted all regression analyses for sex.

We found differences in the associations of copeptin with GFR between donors and patients with ADPKD. No significant association between copeptin and GFR was found in donors predonation ($\beta=0.15$, $P=0.08$) or postdonation ($\beta=0.008$, $P=0.93$), whereas a significant inverse association was found in patients with ADPKD ($\beta=-0.45$, $P<0.001$, which corresponds with a 19% increase in copeptin per 10-ml/min per 1.73 m$^2$ decrease in GFR). In line, the interaction term between study group (ADPKD or donors predonation and postdonation) and GFR in the association between copeptin and GFR was significant ($P<0.001$ and $P=0.03$, respectively). Adjustment for age, sex, and variables that may influence AVP levels did not materially change our results (donors predonation and postdonation: $P=0.85$ and $P=0.87$, respectively; patients with ADPKD: $\beta=-0.53$, $P<0.001$) (Figure 1, Tables 2, 3, and 4). When only studying patients with ADPKD with a GFR in the range similar to the range in donors (GFR>48 ml/min per 1.73 m$^2$, $n=93$), the association between copeptin and GFR remained significant ($\beta=-0.26$, $P=0.01$; i.e., a 13% increase in copeptin per 10-ml/min per 1.73 m$^2$ decrease in GFR), even after additional adjustment for age, sex, and variables that may influence AVP levels ($\beta=-0.28$, $P=0.01$) (Tables 2–4).

Because we wanted to test whether a decline in GFR influences copeptin values, we visualized change in GFR in

relation to change in copeptin levels in percentages in donors from predonation to postdonation in Figure 2. Univariate regression analysis showed no association between change in GFR and change in copeptin ($P=0.97$). Also, when adjusting for change in plasma sodium, change in MAP, and change in 24-hour urine volume, no association was found between change in GFR and change in copeptin ($P=0.92$).

To assess the role of the urine concentrating capacity, we calculated the U/P urea and plotted it against copeptin levels and markers of disease severity in ADPKD (Figure 3). In patients with ADPKD, a negative association was found between U/P urea and copeptin ($\beta=-0.32$, $P<0.001$; i.e., a 52% decrease of copeptin per doubling in U/P urea): the lower the U/P urea and thereby the concentrating capacity, the higher the copeptin levels. This finding is in contrast to the positive association found in donors predonation ($\beta=0.19$, $P=0.03$; i.e., a 46% increase of copeptin per doubling in U/P urea) and postdonation ($\beta=0.39$, $P<0.001$; i.e., a doubling of copeptin per doubling in U/P urea) (Figure 3). In line, the interaction term between study group (ADPKD or donors predonation and postdonation) and U/P urea in the association between copeptin and U/P urea was significant ($P<0.001$ and $P<0.001$, respectively). After additional adjustment for age and sex, the associations between U/P urea and copeptin in patients with ADPKD ($\beta=-0.31$, $P=0.001$) and donors postdonation ($\beta=0.29$, $P=0.001$) remained similar, whereas in donors predonation, this association was no longer significant ($P=0.40$). Additional adjustment of the association between U/P urea and copeptin for factors that may influence AVP levels made no essential difference in patients with ADPKD ($\beta=-0.46$, $P<0.001$) and donors predonation ($P=0.41$), whereas in donors postdonation, the association lost significance ($P=0.35$) (Tables 2–4).

To link concentrating capacity to disease severity in patients with ADPKD, we investigated the associations of GFR and TKV with U/P urea. In patients with ADPKD, we found a strong positive association between GFR and U/P urea ($\beta=0.84$, $P<0.001$; i.e., a 14% decrease of U/P urea per 10-ml/min per 1.73 m$^2$ decrease in GFR), that, in donors predonation and postdonation, was also present but considerably weaker ($\beta=0.32$, $P<0.001$; a 7% decrease of U/P
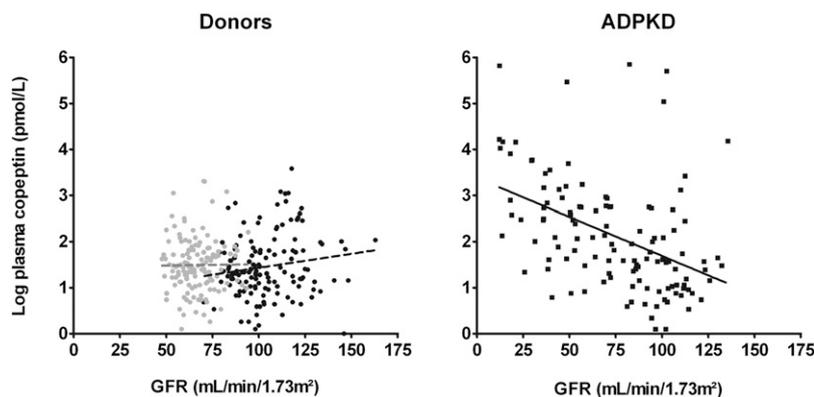


Figure 1. | No association between plasma copeptin and GFR in donors whereas patients show a significant inverse association ($\beta=-0.45$, $P<0.001$).

**Table 2. Multivariable linear regression analyses showing the association of GFR and urine-to-plasma concentration ratio of urea with plasma copeptin (dependent variable) in 134 healthy kidney donors predonation and postdonation**

| Donors | Model 1 | | | | Model 2 | | | | Model 3 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Predonation | | Postdonation | | Predonation | | Postdonation | | Predonation | | Postdonation | |
| | St. $\beta$ | P Value | St. $\beta$ | P Value | St. $\beta$ | P Value | St. $\beta$ | P Value | St. $\beta$ | P Value | St. $\beta$ | P Value |
| **GFR** | | | | | | | | | | | | |
| GFR | 0.15 | 0.08 | 0.008 | 0.93 | 0.02 | 0.84 | −0.08 | 0.41 | 0.02 | 0.85 | −0.16 | 0.87 |
| Age (yr) | | | | | −0.25 | <0.01 | −0.13 | 0.18 | −0.27 | <0.01 | −0.11 | 0.26 |
| Men | | | | | −0.18 | 0.04 | −0.35 | <0.001 | −0.14 | 0.10 | −0.32 | <0.001 |
| Plasma sodium | | | | | | | | | 0.03 | 0.73 | 0.03 | 0.44 |
| 24-h urine volume | | | | | | | | | −0.20 | 0.02 | −0.28 | 0.001 |
| MAP | | | | | | | | | 0.02 | 0.79 | −0.09 | 0.29 |
| Diuretics | | | | | | | | | 0.003 | 0.98 | 0.03 | 0.73 |
| **U/P urea** | | | | | | | | | | | | |
| U/P urea | 0.19 | 0.03 | 0.39 | <0.001 | 0.08 | 0.40 | 0.29 | 0.001 | −0.09 | 0.41 | 0.12 | 0.35 |
| Age (yr) | | | | | −0.24 | <0.01 | −0.01 | 0.86 | −0.31 | 0.001 | −0.07 | 0.48 |
| Men | | | | | −0.16 | 0.07 | −0.27 | 0.002 | −0.16 | 0.08 | −0.30 | 0.001 |
| Plasma sodium | | | | | | | | | 0.02 | 0.78 | 0.06 | 0.45 |
| 24-h urine volume | | | | | | | | | −0.26 | 0.02 | −0.20 | 0.09 |
| MAP | | | | | | | | | 0.03 | 0.76 | −0.08 | 0.33 |
| Diuretics | | | | | | | | | 0.006 | 0.94 | 0.02 | 0.79 |

MAP, mean arterial pressure; U/P urea, urine-to-plasma concentration ratio of urea.

**Table 3. Multivariable linear regression analyses showing the association of GFR with plasma copeptin (dependent variable) in all 122 patients with ADPKD and 93 patients with ADPKD with a GFR>48 ml/min per 1.73 m².**

| ADPKD | Model 1 | | | | Model 2 | | | | Model 3 | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | All Patients | | GFR>48 ml/min per 1.73 m² | | All Patients | | GFR>48 ml/min per 1.73 m² | | All Patients | | GFR>48 ml/min per 1.73 m² | |
| | St. β | P Value | St. β | P Value | St. β | P Value | St. β | P Value | St. β | P Value | St. β | P Value |
| GFR | −0.45 | <0.001 | −0.26 | 0.01 | −0.50 | <0.001 | −0.31 | 0.01 | −0.53 | <0.001 | −0.28 | 0.01 |
| Age (yr) | | | | | −0.16 | 0.08 | −0.18 | 0.12 | −0.11 | 0.20 | −0.09 | 0.43 |
| Men | | | | | −0.28 | <0.001 | −0.33 | 0.001 | −0.32 | <0.001 | −0.41 | <0.001 |
| Plasma sodium | | | | | | | | | −0.13 | 0.08 | 0.17 | 0.08 |
| 24-h urine volume | | | | | | | | | −0.31 | <0.001 | −0.36 | 0.001 |
| MAP | | | | | | | | | 0.08 | 0.28 | 0.05 | 0.63 |
| Diuretics | | | | | | | | | 0.004 | 0.96 | 0.03 | 0.77 |

urea per 10-ml/min per 1.73 m² decrease in GFR; $\beta$=0.27, $P$=0.02; a 9% decrease of U/P urea per 10-ml/min per 1.73 m² decrease in GFR, respectively) (Figure 4). In line, the interaction term between study group (ADPKD or donors predonation and postdonation) and GFR in the association between GFR and U/P urea was significant ($P$<0.001 and $P$<0.001, respectively). Adjustment for age and sex resulted in a similar significant association of GFR and U/P urea in patients with ADPKD ($\beta$=0.81, $P$<0.001) and donors predonation ($\beta$=0.23, $P$=0.02), whereas the association in donors postdonation was lost ($\beta$=0.13, $P$=0.16). In addition, in patients with ADPKD, an inverse association was found between TKV and U/P urea ($\beta$=−0.51, $P$<0.001; i.e., a 38% decrease in U/P urea when TKV doubles) (Figure 4), which remained significant after adjustment for age and sex ($\beta$=−0.43, $P$<0.001) and additional adjustment for GFR ($\beta$=−0.13, $P$=0.05) (Table 5).

TKV and GFR were also inversely associated ($\beta$=−0.49, $P$<0.001; i.e., an 11% increase of TKV corresponds with a 10-ml/min per 1.73 m² decrease in GFR), and this association remained significant after adjustment for age and sex ($\beta$=−0.45, $P$<0.001). Lastly, a significant positive association was found between TKV and plasma osmolality ($\beta$=0.44, $P$<0.001; i.e., a doubling of TKV corresponds with a 3.3-mosM/kg increase in plasma osmolality) (Figure 4), which remained significant after adjustment for age and sex ($\beta$=0.34, $P$<0.001) and additional adjustment for GFR ($\beta$=0.22, $P$<0.01) (Table 5).

Interestingly, in the aforementioned multivariable model, it seemed that U/P urea and GFR were significantly associated with TKV. However, when both were entered in the model simultaneously, U/P urea was significantly associated with TKV ($\beta$=−0.29, $P$=0.05), whereas GFR was not ($\beta$=−0.22, $P$=0.14) (Table 5). This finding suggests that U/P urea may be an earlier and more sensitive sign of renal dysfunction in ADPKD than GFR. To further explore this finding, we substituted measured GFR with eGFR (Chronic Kidney Disease Epidemiology Collaboration), a measure for kidney function often used in clinical practice to evaluate disease severity. Again, we found that U/P urea was significantly associated with TKV ($\beta$=−0.34, $P$=0.02), whereas eGFR was not ($\beta$=−0.15, $P$=0.33).

Because use of diuretics can influence urine concentrating capacity and plasma osmolality and consequently, copeptin levels, we performed a sensitivity analysis excluding all participants using diuretics (25 patients with ADPKD, six donors predonation, and five donors postdonation). No differences were present between participants with or without the use of diuretics in copeptin values in donors predonation ($P$=0.68), donors postdonation ($P$=0.53), and patients with ADPKD ($P$=0.14). Using logistic regression adjusted for age and sex, we found, as may be expected, that patients with ADPKD with lower GFR and, thus, more advanced disease, were more likely to use diuretics ($P$<0.01). Importantly, excluding participants using diuretics did not materially change our results.

## Discussion

In this study, we aimed to gain insight into the role of kidney function and urine concentrating capacity in determining plasma copeptin levels in patients with ADPKD.

**Table 4. Multivariable linear regression analyses showing the association of urine-to-plasma concentration ratio of urea with plasma copeptin (dependent variable) in 122 patients with ADPKD.**

| ADPKD | Model 1: All Patients | | Model 2: All Patients | | Model 3: All Patients | |
|---|---|---|---|---|---|---|
| | St. $\beta$ | P Value | St. $\beta$ | P Value | St. $\beta$ | P Value |
| U/P urea | −0.32 | <0.001 | −0.31 | 0.001 | −0.46 | <0.001 |
| Age (yr) | | | −0.06 | 0.53 | −0.04 | 0.63 |
| Men | | | −0.28 | 0.001 | −0.32 | <0.001 |
| Plasma sodium | | | | | 0.12 | 0.14 |
| 24-h urine volume | | | | | −0.42 | <0.001 |
| MAP | | | | | 0.10 | 0.23 |
| Diuretics | | | | | 0.01 | 0.89 |
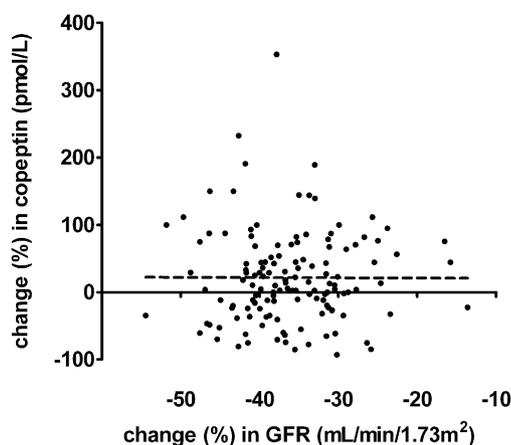


**Figure 2. | No significant association between change in GFR and change in copeptin in donors comparing predonation with post-donation values ($\beta$=-0.02, p=0.84).**

We found no association between GFR and copeptin levels in healthy kidney donors predonation or postdonation. Most importantly, in these participants, copeptin levels did not change after kidney donation, despite a significant decrease in GFR. In contrast, in patients with ADPKD, a significant association was found between GFR and copeptin levels. Moreover, in these patients, copeptin was associated with U/P urea, and in turn, U/P urea was associated with TKV and GFR. These data indicate that GFR, as such, is not a determinant of copeptin levels.

In the literature, the association between kidney function and plasma copeptin has been described in several studies, with higher copeptin concentrations when kidney function is lower (15–18). In our opinion, two mechanisms can underlie the negative association in these studies. First, it could be that copeptin is cleared by kidney excretion, leading to an increase in copeptin levels when kidney function deteriorates. Second, it could also be that, in patients with lower kidney function, more copeptin is released, because the AVP system is activated. When patients in these cross-sectional studies have a lower kidney function, it is caused by kidney damage, which is known to be associated with an impaired urine concentrating capacity (29). Patients with an impaired urine concentrating capacity show a compensatory increase in AVP to maintain water homeostasis

(30). Our study results can distinguish between these two mechanisms. Kidney donors have a considerable decrease in GFR after the donation procedure but without a concomitant increase in kidney damage. Because in these participants no increase in copeptin levels was observed after the donation procedure, it is unlikely that kidney clearance plays a predominant role in determining plasma copeptin levels (at least at the range of GFR observed here). In line with this assumption are our findings of higher copeptin values in patients with ADPKD compared with kidney donors postdonation, despite better kidney function in these patients with ADPKD, as well as the significant inverse correlation between GFR and copeptin in patients with ADPKD.

Various studies in patients with ADPKD have shown that, before kidney function starts to decline, an impaired concentrating capacity can already be observed (31–33). Bankir and Bichet (19) suggested that this phenomenon is probably because of a urea-selective concentrating defect caused by a cystic distortion of the medullary countercurrent mechanism, and they proposed U/P urea as measure of impaired concentrating capacity (19). Using data from water deprivation tests in patients with ADPKD with normal and impaired kidney function, we found that, indeed, baseline U/P urea was strongly associated with maximal urine osmolality during water deprivation ($\beta$=0.82, P=0.001) (Supplemental Figure 1 and Supplemental Table 1 show patient characteristics) (30). These data support U/P urea as a marker for urine concentrating capacity in patients with ADPKD. In this study, we found a negative association between U/P urea and copeptin levels in patients with APDKD. Furthermore, TKV was strongly associated with U/P urea and plasma osmolality. We interpret these data as, when disease progresses in patients with ADPKD, the cystic kidney develops a concentrating defect with a compensatory rise in AVP and copeptin levels. Another possible explanation for the association between copeptin, U/P urea, and TKV is that patients with higher copeptin (*i.e.*, AVP) show a more rapid disease progression and therefore, are more likely to have impaired kidney function and high TKV. In this explanation, it remains unresolved why many patients with ADPKD with relatively preserved GFR have such elevated copeptin levels.

Our data may have an additional consequence. It is generally acknowledged that, in patients with ADPKD,
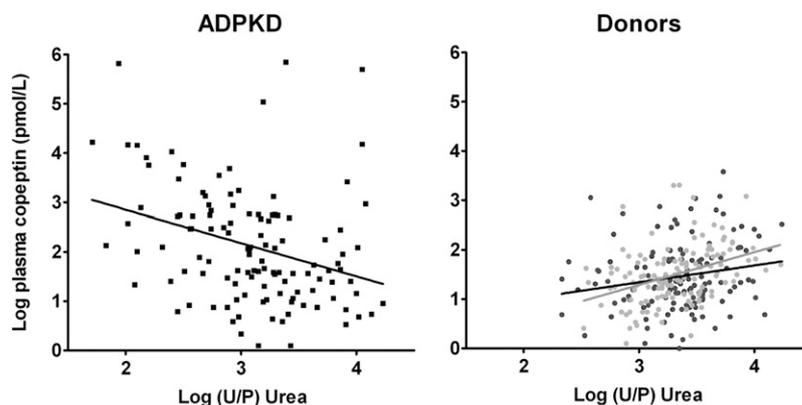
**Figure 3.** | **Significant inverse association between U/P urea and copeptin in patients with ADPKD ($\beta=-0.32$, $P<0.001$) whereas donors show a significant positive association predonation and postdonation ($\beta=0.19$, $P=0.03$; $\beta=0.39$, $P<0.001$).** Because the units of the numerator and the denominator are similar in the U/P urea, this ratio has no unit by itself.
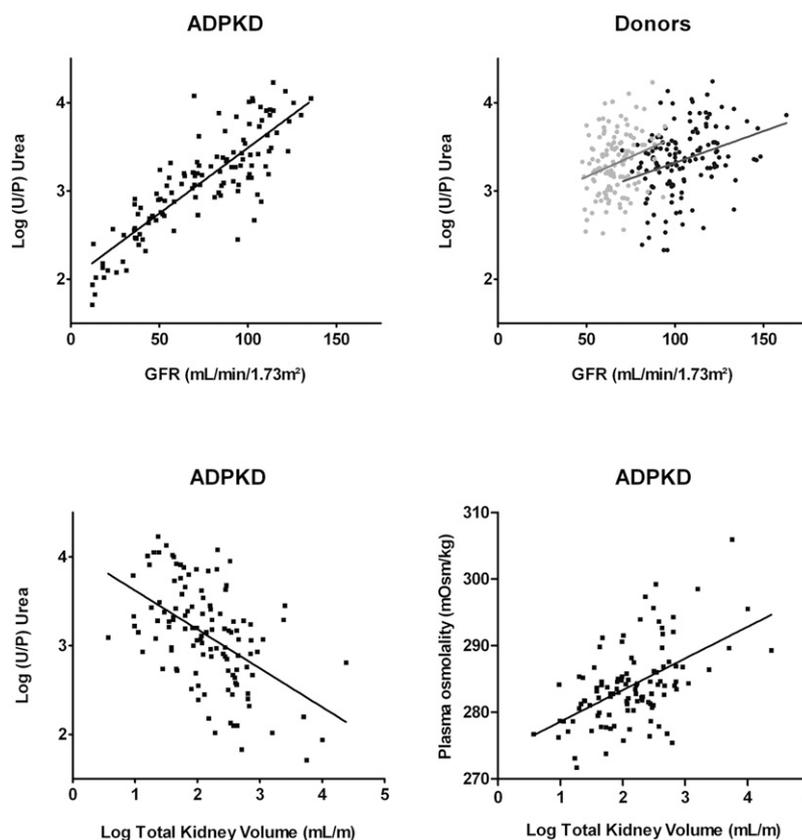


**Figure 4.** | **Significant positive associations between GFR and U/P urea in patients with ADPKD and donors and significant positive associations between total kidney volume, U/P urea and plasma osmolality in patients with ADPKD.** Significant positive associations between GFR and U/P urea in patients with ADPKD ($\beta=0.84$, $P<0.001$) and donors predonation and postdonation ($\beta=0.32$, $P<0.001$; $\beta=0.27$, $P=0.02$). Also shown is the association between total kidney volume and U/P urea and total kidney volume and plasma osmolality in patients with ADPKD ($\beta=-0.51$, $P<0.001$; $\beta=0.44$, $P<0.001$).

GFR remains near normal for a prolonged period of time, whereas disease actually progresses, with progressive development of cysts and an increase in TKV (34). In a multivariable regression analysis adjusted for age and sex, we found that both U/P urea and GFR were associated with TKV. However, when both were entered in the model simultaneously, only U/P urea, and not GFR, was associated with TKV. This finding implies that the urine concentrating capacity estimated by U/P urea or copeptin level may be an earlier and more sensitive marker of disease severity than GFR. This finding should be investigated in future longitudinal studies.

**Table 5. Multiple linear regression analyses showing the association of GFR, plasma osmolality and the urine-to-plasma ratio of urea with total kidney volume (dependent variable) in 122 Patients with ADPKD**

| ADPKD | Model 1 | | Model 2 | | Model 3 | |
|---|---|---|---|---|---|---|
| | St. $\beta$ | P Value | St. $\beta$ | P Value | St. $\beta$ | P Value |
| **GFR** | −0.49 | <0.001 | −0.45 | <0.001 | | |
| Age (yr) | | | 0.02 | 0.82 | | |
| Men | | | −0.29 | <0.001 | | |
| **Plasma osmolality** | 0.44 | <0.001 | 0.34 | <0.001 | 0.22 | <0.01 |
| Age (yr) | | | 0.22 | 0.01 | 0.05 | 0.54 |
| Men | | | −0.28 | 0.001 | −0.27 | 0.001 |
| GFR | | | | | −0.41 | <0.001 |
| **U/P urea** | −0.51 | <0.001 | −0.46 | <0.001 | −0.29 | 0.05 |
| Age (yr) | | | 0.03 | 0.76 | −0.001 | 0.99 |
| Men | | | −0.26 | 0.002 | −0.26 | 0.001 |
| GFR | | | | | −0.22 | 0.14 |

We acknowledge that this study has limitations. First, the study design comparing predonation and postdonation values has a fixed rather than a random order, and consequently, there may be potential time-related confounders. It is theoretically possible that adaptive changes postdonation modify the renal handling of copeptin beyond influencing GFR. However, we think that this design is the best available with the knowledge that we have on this topic. Second, the moment of data collection pre- and potsdonation was not standardized. Predonation, this variability is not likely to have influenced our results, because all participants involved were healthy and had stable kidney function. Postdonation, a period of at least 4 weeks was implemented between donation and patient visit to verify that patients reached steady state postdonation. Third, fluid and dietary intake was not standardized at the moment that blood was drawn for assessing copeptin levels. Urine output in kidney donors predonation was high compared with donors postdonation and patients with ADPKD. Because donors did not receive advice as to water load, we cannot explain this difference. However, it is not of major importance for our main conclusion. In case there was unanticipated water loading predonation, it may be expected to have resulted in a lower copeptin concentration predonation, which means that our finding that there is no rise in copeptin concentration postdonation is not threatened and may only be an underestimation. Fourth, living donors as a model for GFR reduction focuses on the upper GFR range. Therefore,we cannot draw conclusions about the role of kidney function on copeptin levels in the lower GFR spectrum. However, because a significant association was found between copeptin and GFR in patients with ADPKD, whereas GFR was significantly higher compared with donors postdonation who showed no association between copeptin and GFR, we can conclude that, in patients with ADPKD, other disease-related factors are of more importance in determining copeptin levels than GFR. Finally, this study did not include a control group with non-ADPKD. We, therefore, cannot conclude whether an increase in copeptin in more advanced disease is specific for ADPKD, or can also be found in other kidney diseases. However, answering this question is beyond the scope of this study and needs additional investigations. Strengths of this study are the use of a healthy population that donated a kidney, which offers a unique potential to disentangle the role of kidney function *per se* and urine concentrating impairment in determining plasma copeptin levels. The included study group was, furthermore, well phenotyped with a gold standard measurement of GFR (constant infusion of $^{125}$I- Iothalamate) and measurement of TKV by MRI.

In conclusion, our data indicate that kidney function *per se* is not the main determinant of plasma copeptin levels. We hypothesize that, in patients with ADPKD, disease severity is reflected by an impaired urine concentrating capacity, which causes disturbances in plasma osmolality stimulating AVP and copeptin release.

**Disclosures**
None.

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**Supplementary Table 1**: Characteristics of 28 ADPKD patients who underwent a water deprivation test.

|  | ADPKD N=28 |
|---|---|
| Age (y) | 42±14 |
| Male (%) | 61 |
| eGFR (mL/min/1,73 m$^2$) | 74±34 |
| Plasma osmolality (mOsm/kg) | 287±6 |
| Plasma urea (mg/dL) | 24.4±10.6 |
| 24h urine volume (L) | 2181 (1813-2958) |
| 24h urine osmolality (mOsm/kg) | 455±167 |
| 24h urine urea (mg/24h) | 1176±328 |
| U/P Urea ratio | 23.6 (14.6-36.9) |
| Maximal urine osmolality (mOsm/kg) | 641±172 |

*Supplementary table 1: Data are given as mean ± SD for parametric data or median (IQR) for non-parametric data. Abbreviations are: eGFR, estimated glomerular filtration rate; U/P urea, urine-to-plasma concentration ratio of urea.*

**Supplementary Figure 1:** Association between maximal urine concentrating capacity after water deprivation and U/P Urea in 28 ADPKD patients (β=0.82, p<0.001).