Diet-sensitive prognostic markers for cardiovascular and renal disease

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Chapter 3

Copeptin, a Surrogate Marker for Arginine Vasopressin, is Associated with Declining Glomerular Filtration in Patients with Diabetes Mellitus (ZODIAC-33)

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Abstract

Aim/hypothesis. Arginine vasopressin (AVP), the hormone important for maintaining fluid balance, has been shown to cause kidney damage in rodent models of diabetes. We investigated the potential role of AVP in the natural course of kidney function decline in diabetes in an epidemiological study.

Methods. Plasma copeptin, a surrogate for AVP, was measured in baseline samples from patients with type 2 diabetes treated in primary care and included in the Zwolle Outpatient Diabetes project Integrating Available Care (ZODIAC) cohort.

Results. Samples from 1,328 patients were available; 349 were analyzed separately because they used renin-angiotensin-aldosterone system inhibitors (RAASi), which affect urinary albumin to creatinine ratio (ACR) and estimated (e)GFR. In the other 979 patients (46% male, age 66 ± 12 years, ACR 1.8 [0.9-5.7] mg/mmol, eGFR 67 ± 14 mL/min/1.73 m^2), baseline copeptin (5.3 [3.2-9.5] pmol/L) was significantly associated with ln ACR and eGFR, even after adjustment for sex, age, and risk factors for kidney function decline (std β 0.13, $P<0.001$ and std β -0.20, $P<0.001$, respectively). Follow-up data were available for 756 patients (6.5 [4.1-9.6] years). Baseline copeptin was associated with an increase in ACR (std β 0.09, $P=0.02$), but lost significance after adjustment (std β 0.07, $P=0.08$). Copeptin was associated with a decrease in eGFR after adjustment (std β -0.09, $P=0.03$). The association of copeptin with change in eGFR was stronger than the association of established risk factors for kidney function decline (e.g. BMI, HbA_{1c}) with change in eGFR. In patients who used RAASi, there was a significant association of baseline copeptin with ACR and eGFR, but not with change in ACR and eGFR.

Conclusions/interpretation. In patients with diabetes not using RAASi, a higher baseline copeptin concentration is significantly associated with higher baseline ACR and lower eGFR and with a decline in eGFR during follow-up. The latter is independent of, and stronger than, most traditional risk factors for kidney function decline.

Introduction

Arginine vasopressin (AVP), also known as antidiuretic hormone, plays an important role in the regulation of volume status. AVP is secreted into the blood during dehydration (increase in plasma osmolality) or volume loss (1). The primary role of AVP is water reabsorption in the tubules by binding to the AVP V_2 receptor (2). In addition to its role in normal physiology, AVP has been hypothesized to have deleterious renal effects. In various experimental models, including rodent models of diabetes, it has been shown that AVP infusion induces hypertension, glomerular hyperfiltration, albuminuria, and glomerulosclerosis (3-6). In contrast, lowering AVP concentration by water loading resulted in less kidney damage (7).

It is known that AVP levels are higher in patients with diabetes compared with healthy individuals (8,9), especially in patients with diabetes and microalbuminuria (10). Furthermore, it has been shown that copeptin, a surrogate for AVP, is associated with an increased risk for diabetes (11,12) and that infusion of AVP increases albuminuria (13). However, epidemiological studies investigating the association between AVP levels and the rate of kidney function decline are lacking.

The aim of the present study is to investigate the association of AVP, measured as copeptin, with the natural course of kidney function decline, cross-sectionally as well as longitudinally, in an observational cohort of patients with type 2 diabetes.

Materials and Methods

Study sample and design

In 1998, the Zwolle Outpatient Diabetes project Integrating Available Care (ZODIAC) study was initiated, as described in detail previously (14). In the first year, 1,143 patients with type 2 diabetes participated. Briefly, the objective was to investigate the effects of a shared-care project for diabetes. Sixty-one general practitioners participated and were allocated to receive different degrees of support from diabetes specialist nurses for the practical implementation of the national guidelines in patients with known diabetes. The ZODIAC study was approved by the local medical ethics committee and all patients gave informed consent. In 2001, the ZODIAC cohort was extended to include 546 patients, resulting in a total of 1,689 patients. Plasma samples from 1,328 (79%) patients were available for the measurement of copeptin.

We divided the patients into two groups: patients who, at baseline, used medication that interferes with the renin–angiotensin–aldosterone system (RAAS), i.e., angiotensin

converting enzyme inhibitors (ACEi) and/or angiotensin receptor blockers (ARBs); and patients who did not use these medications. The analyses in patients who did not use RAAS inhibitors (RAASi) at baseline are presented as primary analyses because the aim of this study was to investigate the association of AVP with the natural course of kidney function decline in type 2 diabetes, and RAASi are known to influence albuminuria and kidney function.

Data collection

At baseline, a full medical history was obtained including medication use, diabetes duration, and tobacco consumption. Physical and laboratory assessment included measurement of blood pressure (measured twice with a Welch Allyn sphygmomanometer in the supine position after at least 5 min of rest), weight, height, HbA_{1c}, and non-fasting lipid profile.

AVP is difficult to measure because of platelet binding (15), a very short ex vivo half-life (16), and a laborious assay. Copeptin is part of the precursor of AVP (17) and is more stable ex vivo and easier to measure (18). Copeptin was found to be a reliable surrogate for AVP (19). Copeptin was measured in plasma samples collected at baseline and kept frozen at -80°C until analysis. Copeptin was measured by a chemiluminescence immunoassay (CT-proAVP LIA; Thermo Fisher Scientific, B.R.A.H.M.S. Biomarkers, Hennigsdorf/Berlin, Germany) as described previously (17), modified by replacing the capture antibody with a murine monoclonal antibody directed to amino acids 137-144 of copeptin. This modification improved the sensitivity of the assay. The lower limit of detection was 0.4 pmol/L (19).

Kidney function was assessed by measurement of albumin to creatinine ratio (ACR) and estimated (e)GFR. Serum creatinine (SCr) was measured using a Jaffé method (Modular P Analyzer, Roche Diagnostics, Almere, the Netherlands) until March 2007, and thereafter by an enzymatic assay (Roche, Mannheim, Germany). A correction factor was applied to adjust enzymatic values. GFR was estimated using the modification of diet in renal disease (MDRD) equation (20), as advocated for SCr measurements that are not isotope dilution mass spectrometry traceable. Urinary albumin concentration was measured using immunonephelometry (Behring Nephelometer; Mannheim, Germany). SCr and ACR were measured yearly if possible. Annual change in the ln-transformed ACR and eGFR were calculated from the slope of the regression line through all available ACR and eGFR values during follow-up (provided that a minimum of three values were available). If RAASi were started during follow-up, the last ACR or eGFR value before the start of this medication was used because RAASi are known to affect progression of ACR as well as eGFR decline.

Statistical analyses

Analyses were performed using SPSS version 18.0 (SPSS, Chicago, IL, USA). Normal probability plots were inspected for deviances of normality. Continuous variables are expressed as mean (± SD) for normally distributed variables or as median (interquartile range [IQR]) for non-normally distributed variables. Variables with a skewed distribution were ln-transformed before analysis. For all analyses a two-sided *P*-value <0.05 was considered to indicate statistical significance.

Before analyzing the association of copeptin with ACR and eGFR, we tested for interactions. We found a significant interaction between the use of RAASi and plasma copeptin with eGFR at baseline (cross-sectional; std β=-0.21, *P*=0.003). There was a significant inverse association between copeptin and eGFR in the subgroup not using RAASi and no significant association in the subgroup that used RAASi. We found no significant interactions between the use of RAASi and plasma copeptin with ACR, change in eGFR, or change in ACR. We found significant interactions between the use of RAASi and blood pressure in all analyses (cross-sectional and prospective for both ACR and eGFR). Therefore, all analyses were performed separately for patients with and without RAASi. We also tested for interactions between copeptin and sex. This interaction term was not significant in any of our analyses.

We used linear regression analyses to investigate the cross-sectional and longitudinal associations of baseline copeptin with ACR and eGFR. First, crude analyses were performed (model 1). Multivariable models were built stepwise, entering possible confounders step by step. In model 2, we adjusted for sex and age. In longitudinal analyses, we additionally adjusted for baseline ACR for change in ACR or eGFR for change in eGFR. Subsequently, the association was adjusted for risk factors for progression of diabetic nephropathy (i.e., smoking, systolic blood pressure, HbA_{1c}, BMI, and total cholesterol) and for duration of diabetes at baseline (model 3).

In sensitivity analyses, changes in ACR and eGFR were calculated in a different manner: by subtracting the baseline from the last available ACR or eGFR and dividing by follow-up time (with a minimum follow-up of 1 year). Second, change in eGFR was calculated with GFR estimated using the Chronic Kidney Disease-Epidemiology Collaboration (CKD-EPI) equation (21). Third, we used linear mixed effects models to investigate the association of baseline copeptin concentration with ACR and eGFR over time. In addition to a crude analysis, we also performed analyses in which we adjusted for the covariates that were adjusted for in the main analyses (model 3).

Results

In the present study, 1,328 patients were included. Mean age was 67 ± 12 years, 44% was male, and the median duration of diabetes was 4 (2-9) years. Baseline eGFR was inversely associated with baseline ACR. At baseline, 349 patients used RAASi and 979 patients did not. Baseline patient characteristics for subgroups that did not use and used RAASi at baseline are shown in Table 1.

Cross-sectional analyses

Baseline ACR was measured in 955 patients not using RAASi (98%). Median ACR was 1.8 (0.9-5.7) mg/mmol. Baseline copeptin level was positively associated with ln ACR (Table 2 and Figure 1A). This association remained similar after adjustment for potential confounders (Table 2). The association of copeptin with ACR remained significant after additional adjustment for baseline eGFR (std β 0.12, $P<0.001$) or use of antihypertensive drugs (std β 0.13; $P<0.001$).

Baseline SCr was measured in 979 patients (100%). Median SCr was 91 (81-102) μmol/L and mean eGFR (MDRD) was 67 ± 14 mL/min/1.73 m^2. Baseline plasma copeptin concentration was inversely associated with eGFR (Table 2 and Figure 1B): i.e., patients with higher copeptin levels had lower eGFR values (Figure 1). This association remained similar after adjustment for age, sex, and the aforementioned risk factors for renal function decline (Table 2). The association remained significant after additional adjustment for baseline ACR (std β -0.20, $P<0.001$) or use of antihypertensive drugs (std β -0.20, $P<0.001$).

Table 1. Baseline characteristics presented according to use of RAAS inhibitors at baseline.

	Non-RAASi (n=979)	RAASi (n=349)	*P*-value
Copeptin (pmol/L)	5.3 (3.2-9.5)	5.7 (3.2-10.3)	0.2
Demographics			
Male sex (%)	45.8	39.8	0.06
Age (years)	66 ± 12	68 ± 11	0.003
Smoking (%)	20.7	14.6	0.01
Body composition			
BMI (kg/m^2)	28.3 (25.5-31.6)	29.4 (26.2-32.9)	0.003
Blood pressure			
Systolic blood pressure (mmHg)	151 ± 24	156 ± 25	0.001
Antihypertensive drug use (%)	30.1	100	<0.001
Glucose homeostasis			
HbA_{1c} (%)	7.1 (6.3-8.2)	6.9 (6.2-7.9)	0.01
HbA_{1c} (mmol/mol)	54 (45-66)	52 (44-63)	0.01
Duration of diabetes (years)	5.0 (2.0-9.0)	3.7 (1.8-9.0)	0.1
Lipids			
Total cholesterol (mmol/L)	5.6 (4.8-6.3)	5.4 (4.7-6.1)	0.04
Total cholesterol-HDL ratio	4.8 (3.9-6.0)	4.7 (3.9-5.8)	0.3
Lipid lowering medication (%)	11.5	22.5	<0.001
Renal function			
Serum creatinine (µmol/L)	91 (81-102)	95 (85-109)	<0.001
eGFR (mL/min/1.73m^2)	67 ± 14	61 ± 15	<0.001
eGFR-based CKD stages			<0.001
I. >90 mL/min/1.73m^2 (%)	6.0	2.0	
II. 60-90 mL/min/1.73m^2 (%)	61.4	48.7	
IIIa. 45-60 mL/min/1.73m^2 (%)	27.3	36.7	
IIIb. 30-45 mL/min/1.73m^2 (%)	4.7	10.6	
IV. 15-30 mL/min/1.73m^2 (%)	0.6	2.0	
ACR (mg/mmol)	1.8 (0.9-5.7)	2.2 (1.0-7.2)	0.06
ACR-based CKD stages			0.3
I. <3.5 mg/mmol (%)	63.9	59.3	
II. 3.5-35 mg/mmol (%)	28.5	29.5	
III. >35 mg/mmol (%)	4.9	6.6	

Abbreviations: ACR, urinary albumin to creatinine ratio; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate.

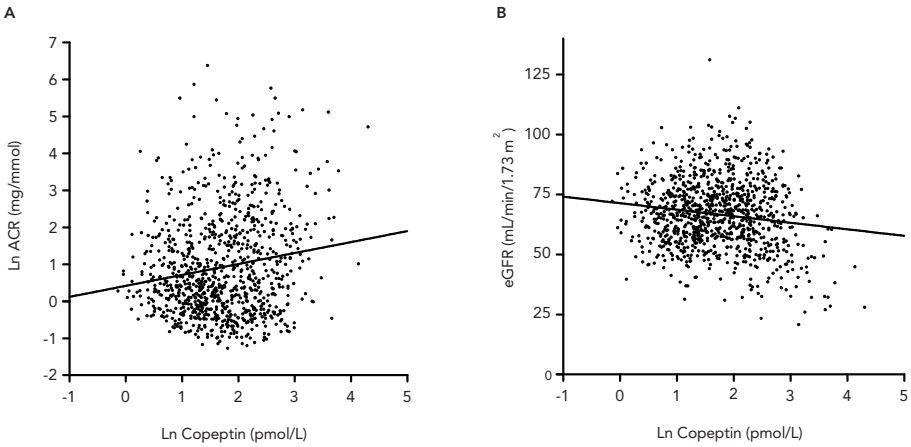

Figure 1. Cross-sectional associations of baseline copeptin concentration with urinary albumin to creatinine ratio (ACR, A) and eGFR (B) in patients with diabetes not using RAAS inhibitors at baseline.

Longitudinal analyses

In 691 patients (72%), ACR was available at baseline and at least two more measurements during a median follow-up of 5.5 (3.2-7.8) years, with on average six ACR measurements per patient. Mean change in ACR (geometric mean with 95% CI) was 1.0 (0.6-1.9) mg/mmol per year. Baseline plasma copeptin level was significantly associated with change in ACR (Figure 2A). This association remained significant after adjustment for age, sex, and baseline ACR (Table 2). This association, however, lost significance after additional adjustment for risk factors for renal function decline (Table 2) and baseline eGFR (std β 0.07, *P*=0.07). When additionally adjusted for use of antihypertensive drugs, the association was materially unchanged (std β 0.07, *P*=0.08).

In 756 patients (77%), SCr was available at baseline and at least two more measurements during a median follow-up of 6.5 (4.1-9.6) years with on average six SCr measurements per patient. Mean change in eGFR was -1.0 ± 3.1 mL/min/1.73 m^2 per year. Baseline plasma copeptin level was associated with change in eGFR after adjustment for age, sex, and baseline eGFR (Table 2 and Figure 2B). This association remained significant after adjustment for risk factors for renal function decline (Table 2). We did not include ACR in the adjustments performed in the models listed in Table 2 because we did not consider ACR a potential confounder, but rather potentially in the causal pathway.

Table 2. Cross-sectional and longitudinal associations of baseline copeptin concentration with ACR and eGFR.

	Baseline						Change in					
	Ln ACR (n=955)			eGFR (n=979)			Ln ACR (n=691)			eGFR (n=756)		
Model	Std β	b (95% CI)	*P*-value	Std β	b (95% CI)	*P*-value	Std β	b (95% CI)	*P*-value	Std β	b (95% CI)	*P*-value
1.	0.162	0.30 (0.18, 0.41)	<0.001	-0.143	-2.73 (-3.91, -1.54)	<0.001	0.088	0.04 (0.01, 0.07)	0.02	-0.031	-0.13 (-0.44, 0.18)	0.4
2.	0.157	0.29 (0.17, 0.41)	<0.001	-0.198	-3.78 (-4.79, -2.76)	<0.001	0.085	0.04 (0.00, 0.07)	0.03	-0.085	-0.36 (-0.68, -0.04)	0.03
3.	0.133	0.24 (0.13, 0.36)	<0.001	-0.201	-3.86 (-4.90, -2.81)	<0.001	0.072	0.03 (-0.00, 0.06)	0.075	-0.087	-0.37 (-0.71, -0.04)	0.03

Abbreviations: ACR, urinary albumin to creatinine ratio; eGFR, estimated glomerular filtration rate.
Model 1. Crude.
Model 2. As model 1 + age, sex, and ACR (change in ln ACR) or baseline eGFR (change in eGFR).
Model 3. As model 2 + systolic blood pressure, cholesterol, HbA_{1c}, smoking, BMI, and duration of diabetes.

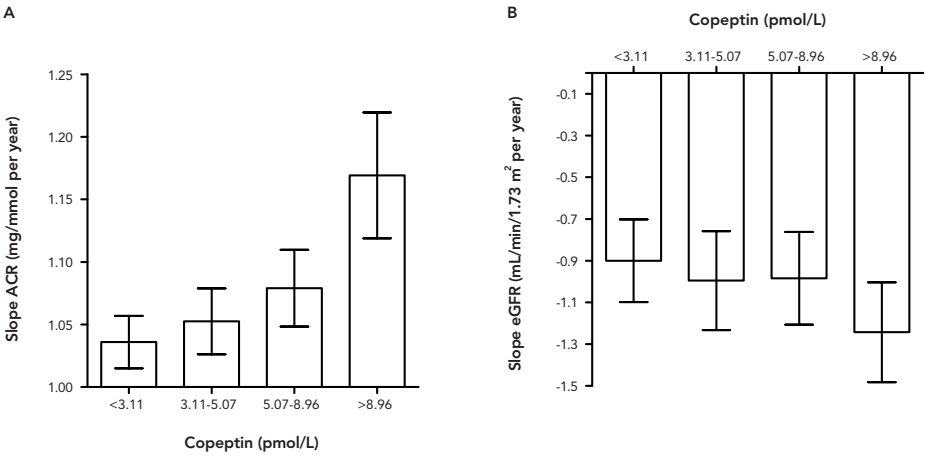

Figure 2. Mean change in ACR (A) and eGFR (B) with standard error of the mean during follow-up according to quartiles of baseline copeptin concentrations for patients with diabetes not using RAAS inhibitors at baseline.

However, further adjustment for baseline ACR did not materially change the association (std β -0.09, *P*=0.03). When additionally adjusted for use of antihypertensive drugs, the association remained significant (std β -0.09, *P*=0.03). The standardized (std) betas for the covariates included in the fully adjusted linear regression model for change in eGFR are shown in Table 3. The std β of baseline copeptin was higher than the std betas of traditional risk factors for renal function decline such as smoking, BMI, HbA_{1c}, and total cholesterol.

Patients who used RAASi at baseline

In the patients who used RAASi at baseline, median ACR was 2.2 (1.0-7.2) mg/mmol (n=333), mean eGFR was 61 ± 15 mL/min/1.73 m^2 (n=349), and median copeptin concentration was 5.7 (3.2-10.3) pmol/L (Table 1). Copeptin concentration was not significantly different between patients who used RAASi and patients who did not. Mean change in ACR (geometric mean with 95% CI) was 1.0 (0.5-1.7) mg/mmol per year and mean change in eGFR was -1.2 ± 2.7 mL/min/1.73 m^2 per year. In these patients, baseline copeptin level was significantly associated with baseline ACR and eGFR, but not with change in ACR or eGFR (Supplementary Table S1).

Table 3. Standardized betas of the multivariable model for the association of baseline ln copeptin with change in eGFR in patients who did not use RAAS inhibitors at baseline.

	Std β	b (95% CI)	*P*-value
Baseline eGFR (mL/min/1.73m^2)	-0.315	-0.07 (-0.09, -0.05)	<0.001
Ln Copeptin (pmol/L)	-0.087	-0.37 (-0.71, -0.04)	0.03
Age (years)	-0.081	-0.02 (-0.05, 0.00)	0.07
Female sex	-0.074	-0.46 (-0.99, 0.07)	0.09
Ln duration of diabetes (years)	-0.069	-0.16 (-0.34, 0.01)	0.07
Systolic blood pressure (mmHg)	-0.066	-0.01 (-0.02, 0.00)	0.09
Ln HbA_{1c} (%)	0.045	1.57 (-1.01, 4.22)	0.2
Ln total cholesterol (mmol/L)	0.031	0.49 (-0.67, 1.64)	0.4
Ln BMI (kg/m^2)	-0.026	-0.51 (-1.94, 0.93)	0.5
Smoking	0.019	0.14 (-0.42, 0.70)	0.6

Abbreviations: ACR, urinary albumin to creatinine ratio; eGFR, estimated glomerular filtration rate.

Sensitivity analyses

We performed linear regression analyses with change in ACR and eGFR calculated as baseline values subtracted from the last values available during follow-up divided by follow-up time. Although the associations were slightly less strong, essentially similar results were obtained (i.e., fully adjusted model for change in ACR std β 0.09, *P*=0.1, and for change in eGFR std β -0.06, *P*=0.1). When we used the slope of GFR estimated by the CKD-EPI equation we also found essentially similar, but slightly less strong results for the cross-sectional and longitudinal analyses. In the fully adjusted models, the std β was -0.19 (*P*<0.001) for the association of copeptin with baseline eGFR and -0.06 (*P*=0.1) for the association of copeptin with change in eGFR.

When we used linear mixed-effect models, we found significant associations of baseline copeptin with change in ln ACR and change in eGFR in the crude as well as in the adjusted models (model 3: std β=0.14 [95% CI 0.05, 0.23], *P*=0.002 and std β=-0.92 [-1.70, -0.13], *P*=0.02, respectively).

Discussion

This study showed that baseline copeptin concentration is significantly associated with higher ACR, which is an early marker for renal damage, and with lower eGFR in patients with type 2 diabetes who were not using RAASi at baseline. Moreover, a higher baseline copeptin concentration was associated with a stronger decline in eGFR during follow-up. The association of copeptin with change in eGFR remained significant after adjustment for sex, age, baseline eGFR, and risk factors for renal function decline and appeared stronger than the association of traditional risk factors such as smoking, HbA_{1c}, and cholesterol with renal function decline.

Diabetes is an important cause of end-stage renal disease (ESRD) worldwide (22). Risk factors for ESRD, particularly those that are modifiable, are therefore important to recognize, especially in patients with diabetes. Copeptin has been found to be associated with albuminuria in the general population (23,24) and with rate of renal function loss in patient groups such as autosomal dominant polycystic kidney disease and renal transplant recipients (25,26). The present study showed that copeptin is also associated with renal function at baseline and change in renal function during follow-up in patients with type 2 diabetes. Therefore, it seems that copeptin is associated with renal function decline in general and that the association of copeptin with renal function is not specific for type 2 diabetes.

We found a significant association of baseline copeptin with change in ACR in our crude model as well as in the model adjusted for sex, age, and baseline ACR. In the fully adjusted model, this association was not significant. This difference in the strength of the association might reflect the smaller sample size for change in ACR compared with change in eGFR and the natural higher variability in ACR (27) than in eGFR, which makes it harder to find significant associations with this chronic kidney disease measure.

It is known that copeptin is associated with incident microalbuminuria (23,24) and with incident diabetes (11,12) in the general population. Furthermore, it was recently shown that higher copeptin levels are associated with higher incidence of cardiovascular and all-cause mortality in patients with diabetes and ESRD (28). Our results add information on the missing link in this chain of events, i.e., copeptin is also associated with renal function decline.

To summarize these conclusions, patients with high AVP levels early in life have a higher risk of developing diabetes mellitus (11), when they have diabetes they have a higher risk of developing albuminuria and renal function decline, and when they reach ESRD, they have a higher mortality risk (28). Given these data, AVP seems to be

an important factor in these patients. The pathophysiological mechanism by which AVP exerts these effects is not yet fully unraveled. With respect to the association of copeptin with change in eGFR, results obtained in rodent models of diabetes suggest that the underlying mechanism may be that AVP leads to hyperfiltration and subsequently to albuminuria and glomerulosclerosis (6). Other mechanisms including RAAS activation have also been mentioned (29).

Interestingly, we found no significant association of copeptin with change in ACR or eGFR in patients with diabetes who were using RAASi at baseline. There may be at least two explanations for this observation. This subgroup of patients was considerably smaller in size. Consequently, the lack of significant association might be a power problem, especially because we did find a significant association of copeptin with change in ACR when the two subgroups (with and without RAASi at baseline) were combined. Our finding may also indicate that the deleterious effect of AVP, measured as copeptin, is mediated at least in part via the renin–angiotensin system, which could also explain the significant interaction that we found between RAASi use and copeptin for the association with eGFR. Indeed, it has been suggested that high AVP levels stimulate RAAS, resulting in vasoconstriction and consequently higher systemic and glomerular blood pressure (30). Unfortunately, from the present dataset it is not possible to firmly conclude which of the explanations is most likely.

Some limitations of this study should be addressed. First, patients were allowed to eat and drink ad libitum at baseline when blood was drawn for copeptin assessment. AVP concentrations are influenced by water and osmolar intake and because intake was not standardized, copeptin concentration will be more variable. We expect that this would lead to effect dilution and therefore to an underestimation rather than an overestimation of the association of copeptin with (change in) ACR or eGFR. Second, fasting glucose levels were not measured. In patients with diabetes, a high glucose level probably leads to an increase in plasma osmolality (31), and subsequently to an increase in AVP (32). Longstanding high glucose levels can cause renal function decline. It could therefore be that copeptin is not directly involved in the association between glucose regulation and renal outcome. However, the association of copeptin with change eGFR remained significant after adjustment for HbA_{1c}. Glucose regulation therefore seems less likely to explain the associations we found. We cannot be sure, though, that the effect of copeptin on glucose metabolism is not the reason for this association. Third, although the associations described were independent of possible confounders in our multivariate analysis, residual confounding cannot be excluded, as in any epidemiological study. We did not correct for multiple testing which may lead to less strong associations as our study is exploratory and the four endpoints (ACR, eGFR,

and changes in ACR and eGFR) are strongly interrelated.

The strengths of this study are that it included a relatively large observational cohort of patients with type 2 diabetes with a long follow-up. Both SCr and ACR were available at baseline and, in most patients, during follow-up. Cross-sectional and longitudinal analyses of both outcome measures rendered generally similar results, i.e., a significant association with copeptin. These data combined suggest that our conclusion that copeptin is associated with renal outcome in patients with diabetes is valid.

Besides offering insight into a potential pathophysiological mechanism explaining the decline in kidney function in diabetes, our data may have clinical implications. This study suggests that copeptin might be a novel marker to predict renal outcome in patients with diabetes. Furthermore, our findings suggest that it might be beneficial to lower AVP levels in patients with diabetes by for example increasing water intake and decreasing sodium intake, or by blocking the effects of vasopressin at the AVP receptors in the kidney e.g., by specific AVP V_2 receptor antagonists. However, before such advice can be given, randomized controlled clinical trials should be performed studying whether these interventions, preferably in addition to RAASi, have a beneficial renal effect.

In conclusion, we found that plasma copeptin concentration is significantly associated with renal function decline in patients with type 2 diabetes not using RAASi. This suggests that copeptin might be a new prognostic marker for renal function decline in these patients and that it might be beneficial to lower AVP levels.

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Supplementary Table S1. Cross-sectional and longitudinal associations of baseline ln copeptin concentration with ln ACR and eGFR in patients who used RAAS inhibitors at baseline.

	Baseline				Change in			
	Ln ACR (n=333)		eGFR (n=349)		Ln ACR (n=274)		eGFR (n=302)	
Model	Std β	*P*-value	Std β	*P*-value	Std β	*P*-value	Std β	*P*-value
1.	0.198	<0.001	-0.332	<0.001	0.051	0.4	-0.076	0.2
2.	0.178	0.003	-0.369	<0.001	0.107	0.08	-0.062	0.3
3.	0.214	<0.001	-0.370	<0.001	0.092	0.2	-0.062	0.4

Abbreviations: ACR, urinary albumin to creatinine ratio; eGFR, estimated glomerular filtration rate.
Model 1: crude.
Model 2: model 1 + age, sex, and baseline eGFR (change in eGFR) or ACR (change in ACR).
Model 3: model 2 + total cholesterol, HbA_{1c}, smoking, BMI, and SBP.