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ARTICLE

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 analysed separately because they used renin–angiotensin–aldosterone system inhibition (RAASi), which influences albumin/creatinine ratio (ACR) and estimated (e)GFR. In the other 979 patients (46% men, age 68 years [58–75], ACR 1.8 mg/mmol [0.9–5.7], eGFR 67± 14 ml min^{-1} 1.73 m^{-2}) baseline copeptin (5.3 pmol/l [3.2–9.5]) was significantly associated with log_e[ACR] and eGFR, even after adjustment for sex, age and risk factors for kidney function decline (standardised [std] β 0.13, $p<0.001$, std β −0.20, $p<0.001$ respectively). Follow-up data were available for 756 patients (6.5 years [4.1–9.6]). Baseline copeptin was associated with 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 strength of the association of copeptin with change in eGFR was stronger than that of established risk factors for kidney function decline (e.g. BMI, HbA_{1c}). In patients who used RAASi there was a significant association between baseline copeptin and 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 values and with a decline in eGFR during follow-up. This last association is independent of, and stronger than, most traditional risk factors for kidney function decline.

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Keywords Albumin/creatinine ratio · Copeptin · Diabetic nephropathy · Estimated glomerular filtration rate · Kidney function · Type 2 diabetes mellitus · Vasopressin

Abbreviations

ACE	Angiotensin-converting enzyme
ACR	Albumin/creatinine ratio
ARB	Angiotensin receptor blocker
AVP	Arginine vasopressin
CKD-EPI	Chronic Kidney Disease-Epidemiology Collaboration
eGFR	Estimated GFR
ESRD	End-stage renal disease
IQR	Interquartile range
MDRD	Modification of diet in renal disease
RAASi	Renin–angiotensin–aldosterone system inhibition
std	Standardised (β)
ZODIAC	Zwolle Outpatient Diabetes project Integrating Available Care

Introduction

Arginine vasopressin (AVP), also known as antidiuretic hormone, plays an important role in the regulation of volume status. It is secreted into the blood on 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 V2 receptor [2]. In addition to this role in normal physiology, AVP has been hypothesised 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 in humans 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 between AVP (measured as copeptin) and the natural course of kidney function decline, cross-sectionally as well as longitudinally, in an observational cohort of patients with type 2 diabetes mellitus.

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 mellitus 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 (ACE) inhibitors and/or angiotensin receptor blockers (ARBs); and patients who did not use these medications. The analyses in patients who did not use RAAS inhibition (RAASi) at baseline are presented as primary analyses. This is because the aim of this study was to investigate the association between AVP (measured as copeptin) and the natural course of kidney function decline in type 2 diabetes, and RAASi is 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. BMI was calculated from weight (kg)/height^2 (m).

AVP is difficult to measure in epidemiological studies 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]. It has been shown 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. It 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 (change in) albumin/creatinine ratio (ACR) and estimated (e)GFR. Serum creatinine was measured until March 2007 using a Jaffé method (Modular P Analyzer, Roche Diagnostics, Almere, the Netherlands), 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 creatinine measurements that are not isotope dilution mass spectrometry traceable. Urinary albumin concentration was measured using immunonephelometry (Behring Nephelometer; Mannheim, Germany). Serum creatinine and ACR were measured yearly if possible. Annual change in the log transformed ($\log_e$) 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). In cases where ACE inhibitor/ARB was started during follow-up, the last ACR or eGFR value before the start of this medication was used. This was because the aim of the present study was to investigate the association between copeptin and the natural course of kidney function decline, and the use of ACE inhibitors/ARB medication is known to impact progression in ACR as well as eGFR decline.

Statistical analyses Analyses were performed with SPSS version 18.0 (SPSS, Chicago, IL, USA). Normal probability plots were inspected for deviances of normality. Continuous variables are expressed as mean (± SD) or as median (interquartile range [IQR]) for non-normally distributed variables. Variables with a skewed distribution were log transformed before analysis.

Before analysing the association between copeptin and ACR and eGFR, we tested interactions. The use or non-use of RAASi showed a significant interaction with plasma copeptin level and eGFR at baseline (cross-sectional; std $\beta = -0.21$, $p = 0.003$). There was a significant inverse association between copeptin and eGFR in the group not using RAASi and no significant association in the group that used RAASi. There were no significant interactions of RAASi use/non-use in the relationships between plasma copeptin and ACR, change in eGFR, or change in ACR. RAASi had a significant interaction with BP in all analyses (cross-sectional and prospective for ACR as well as eGFR). Therefore, all analyses were performed separately for patients with and without RAASi. We also tested for interaction between copeptin and sex. This interaction term was not significant in any of our analyses.

The associations between plasma copeptin and ACR and eGFR were analysed cross-sectionally and longitudinally by univariate regression analyses, with baseline copeptin value as the independent variable and (change in) ACR or eGFR as the dependent variable. Using linear multivariable regression analyses these associations were adjusted for covariates that could potentially be confounders in this association. First, crude analyses were performed (model 1). Second, multivariable models were built stepwise, entering possible confounders step by step. In model 2, the association was adjusted for sex, age and baseline ACR or eGFR (baseline ACR or eGFR only for the longitudinal analyses, baseline ACR for change in ACR and baseline eGFR for change in eGFR). Subsequently the association was adjusted for risk factors for progression of diabetic nephropathy (smoking, BP, HbA_{1c}, BMI, total cholesterol) and for duration of diabetes at baseline (model 3).

In the 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 between baseline copeptin concentration and 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).

For all analyses a two-sided p value of less than 0.05 was considered to indicate statistical significance.

Results

In the present study, 1,328 patients were included. Mean age was 66.8±11.6 years, 44.2% were men and the median duration of diabetes mellitus was 4 years (IQR 2–9). Baseline eGFR was inversely associated with baseline ACR. In the two groups, 349 patients used RAASi at baseline and 979 patients did not. Participant characteristics are shown in Table 1.

Cross-sectional analyses ACR was measured at baseline in 955 patients not using RAASi (98% of the group). The median ACR was 1.75 mg/mmol (IQR 0.89–5.71). Baseline copeptin level was positively associated with $\log_e$ACR (Table 2 and Fig. 1a). When adjusted for confounders in the different models, this association remained similar (Table 2). The association remained significant when additionally adjusted for baseline eGFR (std β 0.12; $p<0.001$) or when additionally adjusted for use of antihypertensive drugs (std β 0.13; $p<0.001$).

Serum creatinine was measured at baseline in 979 patients (100%). The median creatinine level was 91 μmol/l (IQR 81–102) and mean eGFR (MDRD) was 66.7±14.3 ml min^{-1}

Table 1 Baseline characteristics, divided in the two populations: patients who did not use RAASi at baseline and patients who did

Characteristic	Non-RAASi	RAASi	*p* value
n	979	349	
Men (%)	45.8	39.8	0.055
Age (years)	68 (58–75)	71 (60–77)	0.003
Weight (kg)	81 (71–91)	80 (73–94)	0.175
Systolic BP (mmHg)	150.9±23.8	155.8±24.7	0.001
Antihypertensive drug use (yes, %)	30.1	100	<0.001
HbA_{1c} (%)	7.1 (6.3–8.2)	6.9 (6.2–7.9)	0.011
HbA_{1c} (mmol/mol)	54.1 (45.4–66.1)	51.9 (44.3–62.8)	0.011
Diabetes therapy (%)			0.792
Diet only	13.9	15.2	
Oral	67.1	66.8	
Insulin	13.4	13.2	
Oral and insulin	5.6	4.9	
C total (mmol/l)	5.6 (4.8–6.3)	5.4 (4.7–6.1)	0.039
C/HDL ratio	4.8 (3.9–6.0)	4.7 (3.9–5.8)	0.340
Lipid-lowering medication (%)	11.5	22.5	<0.001
Serum creatinine (μmol/l)	91 (81–102)	95 (85–109)	<0.001
BMI (kg/m^2)	28.3 (25.5–31.6)	29.4 (26.2–32.9)	0.003
Smoking (%)	20.7	14.6	0.013
eGFR (MDRD [ml min^{-1} 1.73 m^{-2}])	66.7±14.3	60.8±14.7	<0.001
eGFR CKD stages (%)			<0.001
I >90 ml min^{-1} 1.73 m^{-2}	6.0	2.0	
II 60–90 ml min^{-1} 1.73 m^{-2}	61.4	48.7	
IIIa 45–60 ml min^{-1} 1.73 m^{-2}	27.3	36.7	
IIIb 30–45 ml min^{-1} 1.73 m^{-2}	4.7	10.6	
IV 15–30 ml min^{-1} 1.73 m^{-2}	0.6	2.0	
ACR (mg/mmol)	1.75 (0.89–5.71)	2.24 (0.97–7.15)	0.059
ACR CKD stages:			0.321
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	
Diabetes duration (years)	5.0 (2.0–9.0)	3.7 (1.8–9.0)	0.112
Copeptin (pmol/l)	5.3 (3.2–9.5)	5.7 (3.2–10.3)	0.174

Data are presented as mean±SD or median (IQR), unless stated otherwise

C, cholesterol; CKD, chronic kidney disease,

1.73 m^{-2}. Baseline plasma copeptin concentration and eGFR were inversely associated (Table 2 and Fig. 1b): patients with higher copeptin levels had lower eGFR (Fig. 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 when additionally adjusted for baseline ACR (std β −0.20; $p<0.001$) or when additionally adjusted for use of antihypertensive drugs (std β −0.20; $p<0.001$).

Longitudinal analyses In 691 patients (72%) ACR was available at baseline and at least two more measurements were made during a median follow-up of 5.5 years (IQR 3.2–7.8 years), with, on average, six ACR values per patient. Median change in ACR was 1.0 mg/mmol per year (0.9–1.2). Baseline plasma copeptin level was significantly associated with change in ACR (Fig. 2a). This association remained significant after adjustment for age, sex and baseline ACR (Table 2). This association lost significance after additional adjustment for risk factors for renal function decline (Table 2) and after further additional adjustment for baseline eGFR (std β 0.07; $p=0.07$). When additionally adjusted for use of antihypertensive drugs, the association remained the same (std β 0.07; $p=0.08$).

In 756 patients (77%) serum creatinine was available at baseline and at least two more measurements were made during a median follow-up of 6.5 years (IQR 4.1–9.6) with, on average, six creatinine values per patient. The median decline in eGFR was −1.0 ml min^{-1} 1.73 m^{-2} per year (IQR −2.1–0.2). Baseline plasma copeptin level was

Table 2 Associations between baseline copeptin concentration and baseline ACR and eGFR, and changes in ACR and eGFR during follow-up in univariate (model 1) and multivariate models (models 2 and 3)

Model	$\log_e$ACR baseline (n=955)				eGFR baseline (n=979)				Change in $\log_e$ACR (n=691)				Change in eGFR (n=756)			
	Std β	b	95% CI	p	Std β	b	95% CI	p	Std β	b	95% CI	p	Std β	b	95% CI	p
1	0.162	0.297	0.18, 0.41	<0.001	−0.143	−2.727	−3.91, −1.54	<0.001	0.088	0.037	0.01, 0.07	0.020	−0.031	−0.131	−0.44, 0.18	0.402
2	0.157	0.287	0.17, 0.41	<0.001	−0.198	−3.777	−4.79, −2.76	<0.001	0.085	0.035	0.00, 0.07	0.034	−0.085	−0.363	−0.68, −0.04	0.027
3	0.133	0.243	0.13, 0.36	<0.001	−0.201	−3.856	−4.90, −2.81	<0.001	0.072	0.030	−0.00, 0.06	0.075	−0.087	−0.373	−0.71, −0.04	0.029

Copeptin, HbA_{1c}, cholesterol, ACR, BMI and duration of diabetes were log transformed in these analyses of patients with diabetes not using RAASi at baseline

Model 1: crude;

Model 2: as model 1 + age, sex, baseline eGFR (in change eGFR) or ACR (in change ACR)

Model 3: as model 2 + systolic BP, cholesterol, HbA_{1c}, smoking, BMI, duration of diabetes

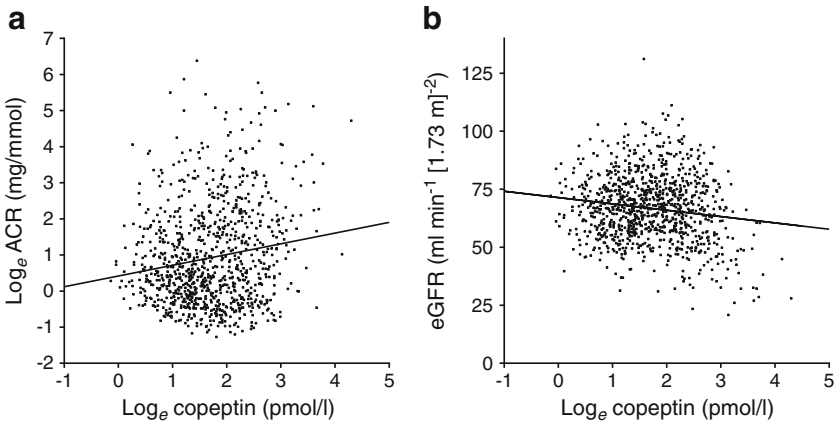

Fig. 1 Individuals with diabetes not using RAASi at baseline: baseline copeptin concentration and (**a**) baseline ACR and (**b**) baseline eGFR

associated with change in eGFR after adjustment for age, sex and baseline eGFR (Table 2 and Fig. 2b), and this association remained significant after adjustment for risk factors for renal function decline (model 3). 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. 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 standardised (std) β values for the covariates in the fully adjusted linear regression model for change in eGFR are shown (Table 3). The std β values of the association of baseline copeptin levels with change in eGFR were higher than the std β values of traditional risk factors for renal function decline, such as smoking, BMI, HbA_{1c} and total cholesterol.

Patients who used RAASi at baseline In patients who used RAASi at baseline, median ACR was 2.24 mg/mmol (0.97–7.15) (n=333), mean eGFR was 60.8±14.7 ml min^{-1} 1.73 m^{-2} (n=349) and median copeptin concentration was 5.7 pmol/l (3.2–10.3) (Table 1). Copeptin was not significantly different between patients who used RAASi and patients who did not. The increase in ACR was 1.0 mg mmol^{-1} year^{-1} (0.8–1.1) and the median decrease in eGFR was −1.1 ml min^{-1} 1.73 m^{-2} year^{-1} (−2.3–0.0). In these patients baseline copeptin level was significantly associated with baseline ACR and eGFR, but not with change in ACR or change in eGFR (electronic supplementary material [ESM] Table 1).

Sensitivity analyses We performed the same 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. Essentially, similar results were obtained, though the associations were slightly less strong (fully adjusted model for change in ACR std β 0.09, p=0.10, and for change in eGFR std β −0.06, p=0.10).

When we used the slope of GFR estimated by the CKD-EPI equation we also found essentially similar, but slightly less strong, results for cross-sectional as well as longitudinal analyses. In the fully adjusted models, the std β of the association between copeptin and baseline eGFR was −0.19 (p<0.001) and between copeptin and change in eGFR it was −0.06 (p=0.13).

When we used linear mixed-effect models, we found significant associations between baseline copeptin

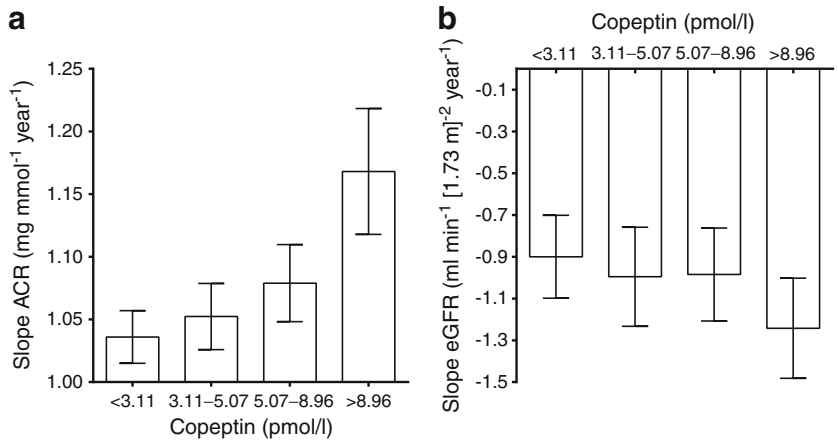

Fig. 2 Individuals with diabetes not using RAASi at baseline: baseline copeptin concentration in quartiles and mean change during follow-up (±SEM) in (**a**) ACR, and (**b**) eGFR

Table 3 Std β values of the multivariable model investigating the association between baseline copeptin and change in eGFR of individuals with diabetes not using RAASi at baseline

	Std β	95% CI	*p* value
Baseline eGFR	−0.315	−0.09, −0.05	<0.001
Copeptin	−0.087	−0.71, −0.04	0.029
Age	−0.081	−0.05, 0.00	0.071
Sex	−0.074	−0.99, 0.07	0.088
Diabetes duration	−0.069	−0.34, 0.01	0.069
Systolic BP	−0.066	−0.02, 0.00	0.088
HbA_{1c}	0.045	−1.01, 4.22	0.244
Cholesterol	0.031	−0.67, 1.64	0.412
BMI	−0.026	−1.94, 0.93	0.489
Smoking	0.019	−0.42, 0.70	0.615

Copeptin, HbA_{1c}, cholesterol, ACR, BMI and duration of diabetes were log transformed in these analyses

concentration and change in ACR and change in eGFR, in the crude as well as in the adjusted models (model 3: std β=0.142; 95% CI 0.05, 0.23; p=0.002 and std β= −0.918; 95% CI −1.70, −0.13; p=0.022, respectively).

Discussion

This study shows 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 decline in eGFR during follow-up. The association with decrease in eGFR was significant after adjustment for sex, age, baseline eGFR and risk factors for renal function decline and appeared stronger than the association between traditional risk factors such as smoking, HbA_{1c} and cholesterol.

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 recognise, especially in patients with diabetes. Copeptin has been found to be associated with albuminuria in the community [23, 24] and with rate of eGFR loss in other patient groups, such as those with autosomal dominant polycystic kidney disease and renal allograft recipients [25, 26]. The present data show that copeptin is also associated with lower kidney function at baseline and a decline in kidney function during follow-up in patients with type 2 diabetes. Therefore, it seems that copeptin is associated with kidney function decline in general and that the association of copeptin and kidney function is not specific for type 2 diabetes.

We found a significant association between baseline copeptin and 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.

From the literature it is known that copeptin is associated with incident microalbuminuria in a general population cohort [23, 24] and with incident diabetes in the general population [11, 12]. 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 summarise 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 higher levels of 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 yet not fully unravelled. With respect to the association between copeptin and change in eGFR, results obtained in rodent models of diabetes suggest that the underlying mechanism may be that AVP leads to hyperfiltration and then to albuminuria and glomerulosclerosis [6]. Other mechanisms have also been mentioned [29].

Interestingly, we found no significant association between copeptin and 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 group 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 between copeptin and change in ACR when the two study groups were combined (with and without RAASi at baseline). 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 BP [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 standardised, copeptin concentration will be more variable. We expect that this would lead to effect dilution and therefore lead to an underestimation rather than an overestimation of the association between copeptin and (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 consequently 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, when we adjusted for HbA_{1c}, the association of copeptin with change eGFR remained significant. 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 serum creatinine 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 kidney outcome in patients with diabetes. Furthermore, our findings suggest that it might be beneficial to lower AVP levels in patients with diabetes. This can be done by increasing water intake, decreasing sodium intake and by blocking the receptors of AVP in the kidney, for instance by specific AVP V2 receptor antagonists. However, before such advice can be given, randomised controlled clinical trials should be performed studying whether these interventions, preferably in addition to RAASi, have a beneficial renal effect.

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

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Duality of interest J. Struck is employed by Thermo Fisher Scientific, a company that manufactures and holds patent rights on the CT-pro-AVP assay. All other authors declare that there is no duality of interest associated with their contribution to this manuscript

Contribution statement HJGB, KHG and NK designed the ZODIAC study. HJGB, NK, ID, AA and JS collected data. WEB, IJR, KHG, SJLB, PEdJ and RTG analysed and interpreted data for this study. WEB drafted the manuscript. HJGB, NK, IJR, KHG, SJLB, ID, AA, JS, PEdJ and RTG reviewed and edited the manuscript critically. All authors gave final approval of this version to be published.

References

1. Robertson GL, Athar S (1976) The interaction of blood osmolality and blood volume in regulating plasma vasopressin in man. J Clin Endocrinol Metab 42:613–620
2. Birnbaumer M (2000) Vasopressin receptors. Trends Endocrinol Metab 11:406–410
3. Bankir L, Kriz W (1995) Adaptation of the kidney to protein intake and to urine concentrating activity: similar consequences in health and CRF. Kidney Int 47:7–24
4. Gellai M, Silverstein JH, Hwang JC, LaRochelle FT Jr, Valtin H (1984) Influence of vasopressin on renal hemodynamics in conscious brattleboro rats. Am J Physiol 246:F819–F827
5. Bardoux P, Martin H, Ahloulay M et al (1999) Vasopressin contributes to hyperfiltration, albuminuria, and renal hypertrophy in diabetes mellitus: study in vasopressin-deficient brattleboro rats. Proc Natl Acad Sci USA 96:10397–10402
6. Bankir L, Bardoux P, Ahloulay M (2001) Vasopressin and diabetes mellitus. Nephron 87:8–18
7. Bouby N, Bachmann S, Bichet D, Bankir L (1990) Effect of water intake on the progression of chronic renal failure in the 5/6 nephrectomized rat. Am J Physiol 258:F973–F979
8. Zerbe RL, Vinicor F, Robertson GL (1979) Plasma vasopressin in uncontrolled diabetes mellitus. Diabetes 28:503–508
9. Tallroth G, Ryding E, Ekman R, Agardh CD (1992) The response of regulatory peptides to moderate hypoglycaemia of short duration in type 1 (insulin-dependent) diabetes mellitus and in normal man. Diabetes Res 20:73–85
10. Nishikawa T, Omura M, Iizuka T, Saito I, Yoshida S (1996) Short-term clinical trial of 1-(1-[4-(3-acetylaminopropoxy)-benzoyl]-4-piperidyl)-3, 4-dihydro-2(1H)-quinolinone in patients with diabetic nephropathy. Possible effectiveness of the specific vasopressin V1 receptor antagonist for reducing albuminuria in patients with non-insulin dependent diabetes mellitus. Arzneimittelforschung 46:875–878
11. Enhörning S, Wang TJ, Nilsson PM et al (2010) Plasma copeptin and the risk of diabetes mellitus. Circulation 121:2102–2108
12. Abbasi A, Corpeleijn E, Meijer E et al (2012) Sex differences in the association between plasma copeptin and incident type 2 diabetes: the prevention of renal and vascular endstage disease (PREVEND) study. Diabetologia 55:1963–1970
13. Bardoux P, Bichet DG, Martin H et al (2003) Vasopressin increases urinary albumin excretion in rats and humans: involvement of V2 receptors and the renin-angiotensin system. Nephrol Dial Transplant 18:497–506

14. Ubink-Veltmaat LJ, Bilo HJ, Groenier KH, Houweling ST, Rischen RO, Meyboom-de Jong B (2003) Prevalence, incidence and mortality of type 2 diabetes mellitus revisited: a prospective population-based study in the Netherlands (ZODIAC-1). Eur J Epidemiol 18:793–800
15. Preibisz JJ, Sealey JE, Laragh JH, Cody RJ, Weksler BB (1983) Plasma and platelet vasopressin in essential hypertension and congestive heart failure. Hypertension 5:I129–I138
16. Robertson GL, Mahr EA, Athar S, Sinha T (1973) Development and clinical application of a new method for the radioimmunoassay of arginine vasopressin in human plasma. J Clin Invest 52:2340–2352
17. Morgenthaler NG, Struck J, Alonso C, Bergmann A (2006) Assay for the measurement of copeptin, a stable peptide derived from the precursor of vasopressin. Clin Chem 52:112–119
18. Morgenthaler NG, Struck J, Jochberger S, Dunser MW (2008) Copeptin: clinical use of a new biomarker. Trends Endocrinol Metab 19:43–49
19. Fenske W, Stork S, Blechschmidt A, Maier SG, Morgenthaler NG, Allolio B (2009) Copeptin in the differential diagnosis of hyponatremia. J Clin Endocrinol Metab 94:123–129
20. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D (1999) A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of diet in renal disease study group. Ann Intern Med 130:461–470
21. Levey AS, Stevens LA, Schmid CH et al (2009) A new equation to estimate glomerular filtration rate. Ann Intern Med 150:604–612
22. Ritz E, Rychlik I, Locatelli F, Halimi S (1999) End-stage renal failure in type 2 diabetes: a medical catastrophe of worldwide dimensions. Am J Kidney Dis 34:795–808
23. Enhörning S, Bankir L, Bouby N et al (2013) Copeptin, a marker of vasopressin, in abdominal obesity, diabetes and microalbuminuria: the prospective malmo diet and cancer study cardiovascular cohort. Int J Obes (Lond) 37:598–603
24. Meijer E, Bakker SJ, Halbesma N, de Jong PE, Struck J, Gansevoort RT (2010) Copeptin, a surrogate marker of vasopressin, is associated with microalbuminuria in a large population cohort. Kidney Int 77:29–36
25. Boertien WE, Meijer E, Zittema D et al (2012) Copeptin, a surrogate marker for vasopressin, is associated with kidney function decline in subjects with autosomal dominant polycystic kidney disease. Nephrol Dial Transplant 27:4131–4137
26. Meijer E, Bakker SJ, de Jong PE et al (2009) Copeptin, a surrogate marker of vasopressin, is associated with accelerated renal function decline in renal transplant recipients. Transplantation 88:561–567
27. Mogensen CE, Vestbo E, Poulsen PL et al (1995) Microalbuminuria and potential confounders. A review and some observations on variability of urinary albumin excretion. Diabetes Care 18:572–581
28. Fenske W, Wanner C, Allolio B et al (2011) Copeptin levels associate with cardiovascular events in patients with ESRD and type 2 diabetes mellitus. J Am Soc Nephrol 22:782–790
29. Meijer E, Boertien WE, Zietse R, Gansevoort RT (2011) Potential deleterious effects of vasopressin in chronic kidney disease and particularly autosomal dominant polycystic kidney disease. Kidney Blood Press Res 34:235–244
30. Bolignano D, Zoccali C (2010) Vasopressin beyond water: implications for renal diseases. Curr Opin Nephrol Hypertens 19:499–504
31. Singer DL, Drolette ME, Hurwitz D, Freinkel N (1962) Serum osmolality and glucose in maturity onset diabetes mellitus. Arch Intern Med 110:758–762
32. Grimaldi A, Pruszczynski W, Jacquin V, Thervet F, Ardaillou R (1988) Vasopressin secretion during hyperglycemia in insulin-dependent diabetics. Diabete Metab 14:37–39