Plasma Copeptin and Renal Outcomes in Patients With Type 2 Diabetes and Albuminuria

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OBJECTIVE—Plasma copeptin, a surrogate for vasopressin, was associated with albuminuria in population-based studies. These associations are consistent with the effect of vasopressin on albuminuria observed in humans and rodents. The objective of this study was to determine whether plasma copeptin is an independent marker of risk of renal events in people with type 2 diabetes and albuminuria.

RESEARCH DESIGN AND METHODS—We studied 3,101 participants of the DIABHYCAR trial (6-year follow-up) with type 2 diabetes and albuminuria. A renal event was defined as doubling of serum creatinine or development of end-stage renal disease.

RESULTS—During follow-up, 86 renal events occurred in 76 subjects (2.45%). Incidences by tertiles of baseline plasma copeptin were 1.06% (T1), 1.45% (T2), and 4.84% (T3). They were 2.43% (T1), 5.11% (T2), and 11.81% (T3) for the subset of subjects with macroalbuminuria at baseline (n = 729). Hazard ratio for plasma copeptin tertiles as a risk for renal events was 4.79 (95% CI, 2.48–9.24; $P < 0.0001$; for T3 vs. T1). In a stepwise regression analysis, urinary albumin excretion and plasma copeptin remained positively associated and HDL cholesterol and estimated glomerular filtration rate were inversely associated with the incidence of renal events. These independent predictors explained ~18% of the variance of the outcome. The yearly variations of estimated glomerular filtration rate by copeptin tertiles were -1.43 ± 0.51 (T1), -2.29 ± 0.49 (T2), and -3.52 ± 0.44 mL/min/1.73 m^2 per year (T3) (P = 0.005) in subjects with macroalbuminuria.

CONCLUSIONS—Plasma copeptin may help to identify subjects with diabetic chronic kidney disease who are at high risk for renal function decline.

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Type 2 diabetes is a major cause of kidney and cardiovascular diseases, and screening for albuminuria and proteinuria is recommended for stratification of risk of these comorbidities (1). Care of patients with diabetes and albuminuria improved markedly in the past decades. However, as shown in recent randomized trials, even with optimal treatment up to 20% of patients with diabetes and proteinuria develop end-stage renal disease (ESRD) within a 3-year follow-up (2,3). There is still an important unmet therapeutic need and many innovative therapeutic options have been or are being evaluated. Some of these new strategies were disappointing because of severe side effects (4). Because not all patients with type 2 diabetes and proteinuria will progress to ESRD, the identification of those at higher risk would improve the benefit-to-risk ratio of intensification of treatment.

Copeptin, the stable COOH-terminal portion of the precursor of vasopressin, is an easily measurable surrogate marker of vasopressin. Studies in healthy subjects have shown plasma copeptin and vasopressin concentrations to correlate strongly over a wide range of osmolalities (5,6). We hypothesized that vasopressin might contribute to the progression of kidney damage and, thus, that plasma copeptin could be a good candidate for the identification of subjects at high risk for progression of nephropathy. Vasopressin is elevated in diabetes (7), and copeptin was associated with albuminuria in a recent, cross-sectional, population-based study (8). Moreover, infusion of DDAVP (a selective vasopressin V2 receptor agonist) was shown to increase urinary albumin excretion (UAE) in healthy subjects and in normal rats (9), and treatment with a selective vasopressin V2-receptor antagonist prevented the increase in albuminuria in rats with streptozotocin-induced diabetes (10).

The aim of the present investigation was to evaluate whether vasopressin could represent a risk factor for the decline of kidney function in patients with diabetic nephropathy. We examined the association of plasma copeptin with the risk of doubling plasma creatinine concentration and the risk of ESRD in subjects with type 2 diabetes and microalbuminuria or macroalbuminuria from the prospective DIABHYCAR study (11,12).

RESEARCH DESIGN AND METHODS

Participants

We studied 3,101 unrelated French type 2 diabetic patients from the DIABHYCAR

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cohort. DIABHYCAR was a 6-year clinical trial conducted in men and women with type 2 diabetes selected on the basis of persistent microalbuminuria (UAE, 20–200 mg/L) or macroalbuminuria (UAE >200 mg/L) without renal failure (plasma creatinine <150 μmol/L) at baseline. The trial tested whether a low dose of ramipril, an ACE inhibitor able to reduce UAE, also would reduce cardiovascular and/or renal events such as myocardial infarction, stroke, acute heart failure, ESRD, and cardiovascular death. For the purpose of the original DIABHYCAR trial, a renal event was defined as the doubling of serum creatinine levels or the requirement of hemodialysis or renal transplantation (ESRD) during follow-up. An independent committee reviewed all case records to validate selection criteria, to grade the renal involvement of each patient, and to adjudicate the clinical events during follow-up (11). Results were negative regarding the drug effect and were published previously (11,12). Participants gave written informed consent and study protocols were approved by the Ethics Committee of Angers University Hospital.

Procedures

Copeptin concentration was measured in fasting plasma-EDTA samples, collected at baseline, and kept frozen at −80°C. An automated immunofluorescent sandwich immunoassay was used (BRAHMS Copeptin US KRYPTOR CT-proAVP; Thermofisher Scientific, Hennigsdorf, Germany) (13). Urinary albumin was measured by nephelometry (14). Estimated glomerular filtration rate (eGFR) was calculated according to the Modification of Diet in Renal Disease formula (15). Variation of eGFR during the study was computed as the difference between values at the end of follow-up and at baseline divided by the duration of follow-up.

Statistical analysis

Results are expressed as mean ± SD unless otherwise stated. Tertiles of plasma copeptin concentration were computed separately for women and men to take into account sex-related differences in copeptin levels. Differences between groups were assessed by Pearson χ^2 test, Wilcoxon/Kruskal-Wallis test, ANOVA, and ANCOVA. When ANOVA or ANCOVA were significant, comparisons between pairs were made using the Tukey-Kramer honestly significant difference test. Kaplan-Meier curves were used to plot survival (renal event-free) rates over time by sex-specific tertiles of plasma copeptin. Cox proportional hazards survival regression analyses were used to examine the effect of explanatory variables on time-related survival (renal event-free) rates in prospective analyses. Hazard ratios with their 95% CIs were computed in these analyses. Competing risk regression analysis (Fine and Gray model) was performed to estimate subhazard ratios of risk factors assuming death as a competing risk (16). Adjustments for clinical and biological parameters were performed by including these parameters as covariates in the regression models. Interaction between plasma copeptin tertiles and sex or study treatment (randomization group in the original DIABHYCAR study: ramipril vs. placebo) on the risk of renal events or in the comparison of eGFR decline were assessed by including in the regression model (Cox or ANCOVA) a "crossed" compound covariate (copeptin tertiles/sex or copeptin tertiles/study treatment). C-statistics were used to compare areas of receiver operating characteristic (ROC) curves. Data were log-transformed for the analyses when the normality of the distribution was rejected by the Kolmogorov-Smirnov-Lilliefors goodness-of-fit test. Statistics were performed with JMP (SAS Institute, Cary, NC) and Stata (StataCorp, College Station, TX) software. $P < 0.05$ was considered significant.

RESULTS

Clinical characteristics at baseline

Characteristics of participants at baseline by plasma copeptin tertiles and by sex are shown in Table 1. For both men and women, subjects in the highest tertile were older, had higher HbA_{1c} levels, had lower eGFR, and more often had arterial hypertension and proteinuria. Renal events during follow-up comprised 67 cases of doubling of serum creatinine and 19 cases of ESRD in 76 subjects (2.45%). Individuals who presented a renal event, as compared with those who did not, at baseline had a longer duration of diabetes, and higher copeptin, HbA_{1c}, and triglyceride levels, and had lower HDL cholesterol levels. They had lower eGFR, higher UAE, more often had arterial hypertension and proteinuria, and more often were treated by diuretics. A history of myocardial infarction was more frequent in individuals who presented a renal event (Supplementary Table 1).

Plasma copeptin and renal events during follow-up

The incidences of renal events during follow-up by sex-specific tertiles of plasma copeptin were 1.06% (T1), 1.45% (T2), and 4.84% (T3) for the whole population (n = 3,101). They were 2.43% (T1), 5.11% (T2), and 11.81% (T3) for the subset of subjects with macroalbuminuria at baseline (n = 729). Cox proportional hazards survival regression analyses showed a positive association of the highest tertile of plasma copeptin with the incidence of renal events in the whole population, as well as in the subset of subjects with macroalbuminuria at baseline (Fig. 1 and Table 2, model 1). This association remained significant when adjusted for baseline UAE and eGFR (Table 2, model 2). The highest tertile of plasma copeptin was associated with the incidence of both outcomes comprising the renal events (doubling of plasma creatinine and ESRD) in subset analyses stratified by outcomes (Supplementary Table 2). Despite the smaller number of renal events in the subset of subjects with microalbuminuria at baseline (25 events in 2,372 subjects), the highest tertile of plasma copeptin also was associated with the outcome (data not shown). Plasma copeptin levels were, on average, 15% higher in subjects treated with diuretics as compared with those who were not (mean ± SEM, 7.55 ± 0.3 vs. 6.58 ± 0.02 pmol/L; $P < 0.0001$, adjusted for age and sex). However, associations of copeptin tertiles with renal events were similar in subjects treated or not treated by diuretics, and no interaction copeptin/use of diuretics was observed (data not shown).

A stepwise multiple regression analysis was performed to evaluate the independence of the association of plasma copeptin levels with renal events from other potentially confounding covariates (Table 3). The renal event status during follow-up (yes or no) was entered in the model as the dependent variable. Sex, age, duration of diabetes, arterial hypertension status (yes or no), use of diuretics (yes or no), HbA_{1c}, triglycerides, HDL cholesterol, eGFR, UAE, and copeptin (tertiles) at baseline were entered as independent covariates. UAE and copeptin remained positively associated and HDL cholesterol and eGFR were inversely associated with the incidence of renal events. These independent predictors explained ~18% of the variance of the outcome. Another potential confounder, smoking behavior, was not associated

with plasma copeptin levels and had no impact on the association of copeptin with renal events (data not shown). In an additional analysis, the inclusion in the regression model of blood glucose levels and blood pressure at baseline and changes in blood glucose levels and blood pressure during follow-up had no impact on the results. We constructed ROC curves with UAE, plasma copeptin levels, or both as markers of renal events (data not shown). ROC areas were similar for UAE and plasma copeptin (0.78 vs. 0.73; C-statistics $P = 0.16$) adjusted for age, sex, and study treatment. We observed an added effect for the combined markers (UAE plus copeptin ROC area, 0.82) as compared with the UAE effect ($P = 0.03$).

Renal events and death as competing risks

Death occurred in 454 subjects (14.65%) during follow-up, including 19 subjects who had presented a renal event. The association between tertiles of plasma copeptin and the incidence of renal events evaluated with the Cox model might be biased if many patients died of other causes related to or associated with copeptin levels before achieving the renal end point (17). Consequently, we performed competing risk regression analyses to estimate subhazard ratios of plasma copeptin tertiles as a risk for renal events assuming death as a competing risk. Subhazard ratios and hazard ratios from the Cox model were similar, indicating that death was not a competing risk in the association of plasma copeptin levels with renal events (Table 2, model 3). Moreover, we observed no independent association of plasma copeptin levels with mortality during follow-up in our cohort (Supplementary Table 3).

Plasma copeptin and eGFR

The yearly variations of eGFR during follow-up by tertiles of plasma copeptin were −0.65 ± 0.24 (T1), −0.77 ± 0.24 (T2), and −1.91 ± 0.24 mL/min/1.73 m^2 per year (T3) (mean ± SEM, ANCOVA $P = 0.0001$, adjusted for sex, age, and study treatment in the original DIABHYCAR trial) for the whole population. They were −1.43 ± 0.51 (T1), −2.29 ± 0.49 (T2), and −3.52 ± 0.44 mL/min/1.73 m^2 per year (T3) ($P = 0.005$) for the subset of subjects with macroalbuminuria at baseline (Supplementary Fig. 1). These differences remained significant after additional adjustment for the duration of diabetes, HbA_{1c}, triglycerides, HDL

Table 1—*Clinical characteristics at baseline by tertiles of plasma copeptin and by sex*

	Men				Women			
	T1	T2	T3	*P*	T1	T2	T3	*P*
N	757	757	756		277	277	277	
Age, years	65 ± 8	65 ± 8	67 ± 8*†	<0.0001	65 ± 8	65 ± 9	69 ± 9*†	<0.0001
Plasma copeptin, pmol/L‡	4.9 (0.9–5.8)	7.7* (5.9–10.0)	13.7*† (10.1–143)	<0.0001	2.9 (1.0–4.3)	6.2* (4.4–8.5)	12.6*† (8.5–47.8)	<0.0001
Age at diagnosis of diabetes, years	55 ± 9	55 ± 9	56 ± 10*†	0.01	55 ± 9	55 ± 10	58 ± 10*†	0.0001
Duration of diabetes, years	10 ± 8	10 ± 8	11 ± 9*	0.01	10 ± 7	11 ± 8	11 ± 7	0.44
BMI, kg/m^2	29.0 ± 4.0	29.2 ± 4.1	28.9 ± 4.1	0.42	30.2 ± 5.5	30.5 ± 5.5	30.5 ± 5.9	0.76
Fasting plasma glucose, mmol/L	9.2 ± 2.8	9.2 ± 2.8	9.6 ± 3.0*	0.02	9.7 ± 3.0	10.1 ± 3.5	9.8 ± 3.4	0.34
HbA_{1c}, % (mmol/mol)	7.5 ± 1.6 (58 ± 5)	7.5 ± 1.6 (58 ± 5)	8.0 ± 1.8*† (64 ± 8)	<0.0001	8.1 ± 1.8 (65 ± 8)	8.4 ± 1.9 (68 ± 9)	8.5 ± 2.1* (69 ± 11)	0.04
Systolic BP, mmHg	144 ± 13	144 ± 14	144 ± 13	0.72	147 ± 15	147 ± 16	148 ± 16	0.48
Diastolic BP, mmHg	82 ± 8	82 ± 8	82 ± 8	0.82	83 ± 9	83 ± 9	83 ± 9	0.84
Arterial hypertension, %	49.0	51.4	58.1	0.001	59.9	63.9	72.2	0.008
eGFR, mL/min/1.73 m^2	84 ± 19	81 ± 19*	75 ± 20*†	<0.0001	73 ± 19	74 ± 24	64 ± 20*†	<0.0001
Microalbuminuria and macroalbuminuria, %	80.3 and 19.7	77.3 and 22.7	73.5 and 26.5	0.007	79.4 and 20.6	77.3 and 22.7	68.6 and 31.4	0.008
UAE, mg/24 h§	70 (28–437)	78* (29–577)	85* (30–649)	0.002‖	64 (28–385)	63 (28–522)	86*† (29–914)	0.009‖
Previous myocardial infarction, %	7.7	6.9	5.4	0.21	3.3	1.4	2.5	0.38

Data expressed as mean ± SD unless otherwise indicated. Statistics for quantitative parameters are ANOVA with log-transformed data unless otherwise indicated. Tukey-Kramer honestly significant difference test after ANOVA or Wilcoxon for each pair: significantly different ($P < 0.05$) from *T1 or †T2. Arterial hypertension: systolic blood pressure >140 mmHg and/or diastolic blood pressure >90 mmHg or presence of antihypertensive medication and history of hypertension. BP, blood pressure. ‡Expressed as median and (range). §Expressed as median and (10th–90th percentiles). ‖Wilcoxon test.

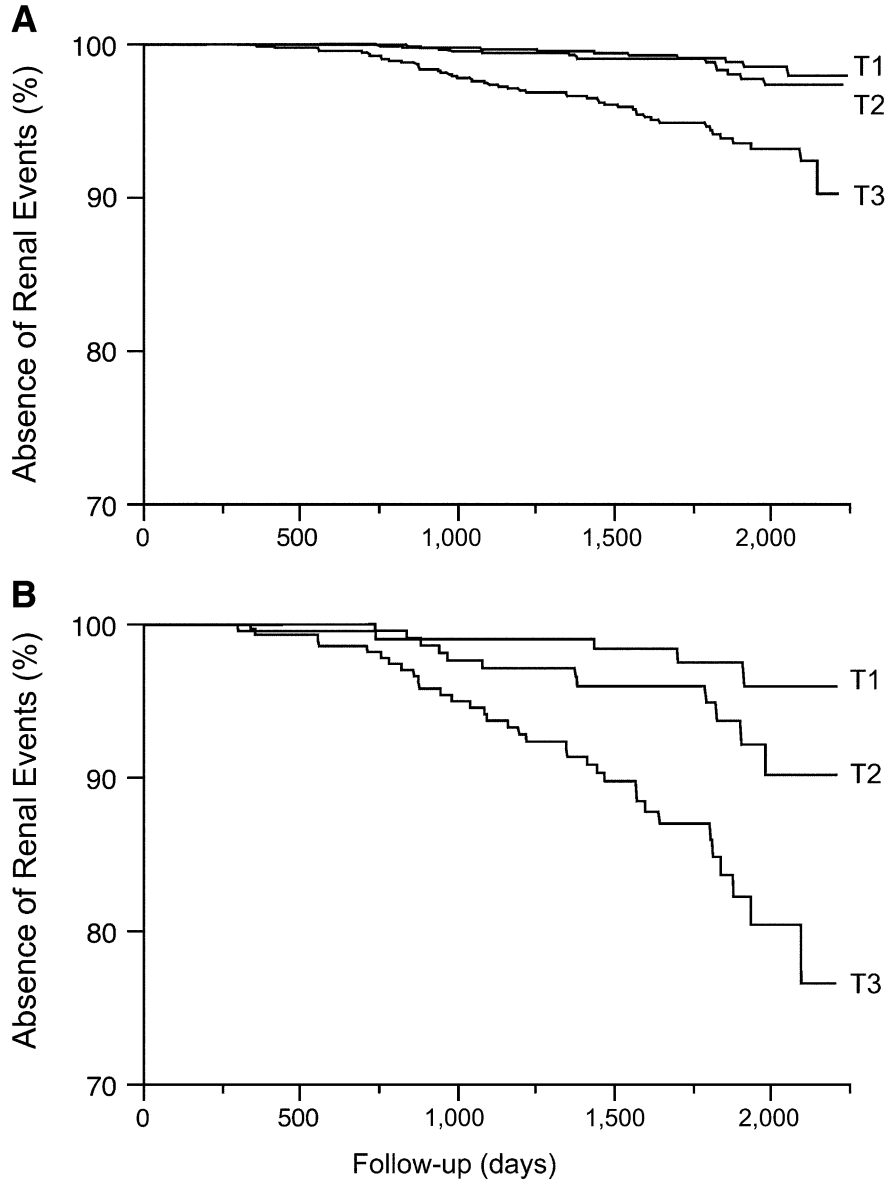

Figure 1—*Kaplan-Meier survival (renal event-free) curves during follow-up by tertiles of plasma copeptin. Renal events were defined as the doubling of the serum creatinine levels or ESRD during follow-up.* A*: Subjects with microalbuminuria or macroalbuminuria at baseline* (n = *3,101).* B*: Subjects with macroalbuminuria at baseline* (n = *729).*

cholesterol, UAE, arterial hypertension status (yes or no), and use of diuretics (yes or no) at baseline ($P = 0.003$ for the whole population and $P = 0.03$ for the subjects with microalbuminuria). In all comparisons, either of the risk of renal events or of the variation of eGFR during follow-up, we observed no interaction between copeptin tertiles and sex or between copeptin tertiles and study treatment (ramipril vs. placebo) in the original DIABHYCAR study (data not shown).

CONCLUSIONS—In this study, we demonstrated that high plasma copeptin concentration was strongly associated with the risk of severe renal outcomes (doubling of plasma creatinine concentration and/or ESRD) in patients with type 2 diabetes and albuminuria. This association was independent of relevant covariates such as age, duration of diabetes, blood pressure, and baseline levels of HbA_{1c}, UAE, and eGFR. Plasma copeptin and UAE similarly predicted the incidence of renal outcomes, and a 4% added effect was observed for the combined markers.

To our knowledge, this is the first clinical study that investigates the association between vasopressin or copeptin concentration and accelerated renal function decline in type 2 diabetic patients during a long-term follow-up. Our study confirms and extends previous results on copeptin concentration and kidney diseases. Plasma copeptin has been shown to be positively associated with the prevalence of microalbuminuria in cross-sectional observational and long-term follow-up studies in the general population (8,18). In autosomal-dominant polycystic kidney disease, copeptin is associated with various markers of disease severity including albuminuria and GFR (19–21). In renal transplant recipients, higher copeptin level at baseline was associated with a more rapid decline in effective GFR during 3- to 4-year follow-up (22). Moreover, studies in Australian and Canadian community-based cohorts of the general population showed, respectively, an inverse linear relationship between fluid intake and prevalence of chronic kidney disease (CKD) (23) and a faster decline of eGFR over time in people with lower 24-h urine volume (24). The results of those population studies are in agreement with an adverse effect of vasopressin on kidney function.

As in any epidemiological study, the association we observed may be confounded by other factors. Among them, age and sex have been shown to influence urinary concentrating activity and vasopressin levels (25). We observed a positive association of age with plasma copeptin levels in men and women. We also found copeptin levels to be higher in men than in women as previously observed (8,18,26). However, we observed no interaction between copeptin levels and sex on the risk of renal events. Moreover, when adjusted for confounding covariates, neither age nor sex was associated with renal events. In a recent study of 1,241 hemodialysis patients with type 2 diabetes in Germany, high levels of copeptin were found to be associated with increased risk for all-cause mortality (17). In our study, death was not a competing risk in the association of copeptin levels with renal events and we observed no association of plasma copeptin levels with mortality. However, it is noteworthy that all subjects in the German cohort had ESRD and were more severely ill than our patients. It is possible to speculate that the association between copeptin and renal events could be explained, at least in part, by vasopressin-mediated changes in blood pressure. Vasopressin may contribute to hypertension through activation

Table 2—*Risk of renal events during the follow-up by tertiles of plasma copeptin at baseline*

	All subjects (n = 3,101; 76 renal events)		Subjects with macroalbuminuria (n = 729; 51 renal events)	
	Hazard ratio (95% CI)	*P*	Hazard ratio (95% CI)	*P*
Model 1: adjusted for sex, age, and study treatment				
T3 vs. T1	4.79 (2.48–9.24)	<0.0001	5.42 (2.09–14.01)	<0.0001
T3 vs. T2	3.43 (1.92–6.15)	<0.0001	2.41 (1.23–4.70)	0.009
T2 vs. T1	1.39 (0.64–3.03)	0.41	2.25 (0.79–6.39)	0.13
Model 2: adjusted for sex, age, study treatment, UAE, and eGFR				
T3 vs. T1	2.97 (1.56–6.14)	0.0006	3.33 (1.37–9.96)	0.006
T3 vs. T2	2.78 (1.58–5.19)	0.0003	1.96 (1.02–4.04)	0.04
T2 vs. T1	1.07 (0.49–2.40)	0.87	1.69 (0.62–5.37)	0.31
	Subhazard ratio (95% CI)	*P*	Subhazard ratio (95% CI)	*P*
Model 3: adjusted for sex, age, and study treatment, and with death as a competing risk				
T3 vs. T1	4.69 (2.43–9.05)	<0.0001	5.14 (1.98–6.06)	<0.0001
T3 vs. T2	3.41 (1.91–6.07)	<0.0001	2.40 (1.24–4.62)	0.009
T2 vs. T1	1.38 (0.63–3.00)	0.42	2.15 (0.76–6.05)	0.15

Renal event during follow-up defined as the doubling of the serum creatinine levels or ESRD. Models 1 and 2: hazard ratios computed by Cox proportional hazards survival regression analyses. Model 3: subhazard ratios computed by competing risk regression analysis. Age, UAE, and eGFR are baseline values and were log-transformed for the analyses. Study treatment: randomization group in the original DIABHYCAR study (ramipril vs. placebo).

of V1a receptors on the vascular smooth muscle or by V2-receptor–dependent tubular effects on sodium reabsorption (27). However, the association between copeptin and renal outcomes was independent of blood pressure as previously noted for the association of copeptin and UAE (8).

Experimental evidence strongly supports a causal role of vasopressin in aggravation of diabetic CKD through V2-receptor activation. Besides well-known antidiuretic effects at the collecting duct level, a V2-receptor agonist was shown to induce glomerular hyperfiltration and to increase UAE in normal rats (9,28). Moreover vasopressin has been shown to participate in progression to renal failure in rats with five-sixths reduction in renal mass (29,30) and in rodent models of type 1 diabetes (10,31). Acute administration of DDAVP has been shown to increase UAE in healthy humans but not in patients with diabetes insipidus caused by mutations in the V2-receptor (9). The mechanisms of these deleterious effects of vasopressin are not clear. They may involve changes in the composition of the tubular fluid at the macula densa that influence tubuloglomerular feedback control of GFR, as well as an increase in intraglomerular pressure subsequent to afferent arteriole vasodilatation (32). The latter hemodynamic effect is supposed to be one of the main drivers in the decline of renal function in diabetic CKD since the pioneering works by Brenner and colleagues (33,34). However, our study cannot preclude or confirm any of these mechanisms because of its observational nature. Further experimental studies are needed in that regard.

Table 3—*Covariates associated with the incidence of renal events in a stepwise regression analysis*

	Cumulated R^2	β-Coefficient	*P*
UAE	0.129	0.78	<0.0001
Copeptin: T3 vs. T2 and T1	0.161	0.57	<0.0001
HDL cholesterol	0.173	−1.31	0.003
eGFR	0.179	−0.93	0.03
HbA_{1c}	0.182	0.76	0.16
Use of diuretics	0.185	0.22	0.18
Sex, male	0.187	0.04	0.21
Triglycerides	0.188	0.25	0.52
Duration of diabetes	0.188	0.18	0.55
Arterial hypertension	0.188	0.12	0.82
Copeptin: T2 vs. T1	0.188	0.08	0.88
Age	0.188	0.79	0.97

The cumulated R^2 expresses the percentage of the variance of the dependent variable (renal events) as explained by the stepwise inclusion of independent variables in the model (1 = 100%). Statistical analyses test the probability for the independent variable effect (β-coefficient) to be different from zero. Quantitative parameters were log-transformed for the analysis.

Our study has limitations. First, the design did not allow any conclusion regarding the possible causal relationship between copeptin and disease progression. Second, we have not measured the true GFR, but we used an estimation index based on plasma creatinine levels. Finally, half of the patients of DIABHYCAR received ramipril during the follow-up and this drug may influence blood pressure. However, we observed no interaction between copeptin and study treatment (ramipril vs. placebo) in any of our results, and adjustment for ramipril treatment had no impact on the results.

The strong and independent associations of copeptin with baseline albumin excretion rate and with progression to CKD offer new tools to identify patients with highest risk of progression. Several novel therapeutic strategies have been

proposed for slowing the progression of CKD (4). Some of them, like double-blockade of the renin-angiotensin system (VA-nephron-D study, NCT00555217) or antibodies against tumor growth factor-β1 (NCT01113801), are still undergoing evaluation but their use will induce a higher risk of side effects, as suggested by the ONTARGET trial (35). We propose that plasma copeptin can help target patients with the highest risk of CKD progression to optimize the benefit/risk ratio. Our observations also raise exciting hypotheses of new therapeutic options in diabetic CKD. Increasing water intake may lower vasopressin secretion; such "medicinal use of water" has been discussed recently for kidney diseases other than diabetic nephropathy (36). Also, vaptans (vasopressin receptor antagonists) could selectively target the V2 receptor–mediated deleterious effects of vasopressin. Advantages and disadvantages of both options are debatable (37). If the increase of water intake or V2 receptor antagonists were shown to be beneficial, then this could open the way to individualized therapy. These interventions then could be used specifically in subjects with high copeptin levels who, presumably, would benefit the most from the reduction of vasopressin effects. Further studies are needed to test these hypotheses.

In conclusion, our study is the first investigation studying the association between endogenous vasopressin and kidney dysfunction in type 2 diabetic patients. It highlights the role of copeptin as a risk marker for long-term renal outcomes in these patients. Furthermore, it provides a rationale for intervention studies aiming to reduce vasopressin secretion or action.

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G.V. and N.B. researched data and wrote the manuscript. S.H. contributed to discussion and reviewed and edited the manuscript. N.M. and K.M. researched data. F.F. contributed to discussion and reviewed and edited the manuscript. L.P., N.B.-M., and C.T. researched data. F.A.-G., L.B., and M.M. contributed to discussion and reviewed and edited the manuscript. R.R. researched data and wrote the manuscript. R.R. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

References

1. National Kidney Foundation. KDOQI Clinical Practice Guideline for Diabetes and CKD: 2012 Update. Am J Kidney Dis 2012;60:850–886
2. Brenner BM, Cooper ME, de Zeeuw D, et al.; RENAAL Study Investigators. Effects of losartan on renal and cardiovascular outcomes in patients with type 2 diabetes and nephropathy. N Engl J Med 2001;345:861–869
3. Lewis EJ, Hunsicker LG, Clarke WR, et al.; Collaborative Study Group. Renoprotective effect of the angiotensin-receptor antagonist irbesartan in patients with nephropathy due to type 2 diabetes. N Engl J Med 2001; 345:851–860
4. MacIsaac RJ, Ekinci EI, Kelly DJ, Jerums G. New treatments for diabetic chronic kidney disease. J Nephrol Therapeutic 2012;2(Suppl. 2). DOI: 10.4172/2161-0959.S2-006
5. Szinnai G, Morgenthaler NG, Berneis K, et al. Changes in plasma copeptin, the c-terminal portion of arginine vasopressin during water deprivation and excess in healthy subjects. J Clin Endocrinol Metab 2007;92:3973–3978
6. Balanescu S, Kopp P, Gaskill MB, Morgenthaler NG, Schindler C, Rutishauser J. Correlation of plasma copeptin and vasopressin concentrations in hypo-, iso-, and hyperosmolar States. J Clin Endocrinol Metab 2011;96:1046–1052
7. Zerbe RL, Vinicor F, Robertson GL. Plasma vasopressin in uncontrolled diabetes mellitus. Diabetes 1979;28:503–508
8. Meijer E, Bakker SJ, Halbesma N, de Jong PE, Struck J, Gansevoort RT. Copeptin, a surrogate marker of vasopressin, is associated with microalbuminuria in a large population cohort. Kidney Int 2010;77: 29–36
9. Bardoux P, Bichet DG, Martin H, et al. Vasopressin increases urinary albumin excretion in rats and humans: involvement of V2 receptors and the renin-angiotensin system. Nephrol Dial Transplant 2003;18: 497–506
10. Bardoux P, Bruneval P, Heudes D, Bouby N, Bankir L. Diabetes-induced albuminuria: role of antidiuretic hormone as revealed by chronic V2 receptor antagonism in rats. Nephrol Dial Transplant 2003;18: 1755–1763
11. Lièvre M, Marre M, Chatellier G, et al.; DIABHYCAR Study Group. The non-insulin-dependent diabetes, hypertension, microalbuminuria or proteinuria, cardiovascular events, and ramipril (DIABHYCAR) study: design, organization, and patient recruitment. Control Clin Trials 2000;21: 383–396
12. Marre M, Lievre M, Chatellier G, Mann JF, Passa P, Ménard J; DIABHYCAR Study Investigators. Effects of low dose ramipril on cardiovascular and renal outcomes in patients with type 2 diabetes and raised excretion of urinary albumin: randomised, double blind, placebo controlled trial (the DIABHYCAR study). BMJ 2004; 328:495
13. Morgenthaler NG, Struck J, Alonso C, Bergmann A. Assay for the measurement of copeptin, a stable peptide derived from the precursor of vasopressin. Clin Chem 2006;52:112–119
14. Marre M, Claudel JP, Ciret P, Luis N, Suarez L, Passa P. Laser immunonephelometry for routine quantification of urinary albumin excretion. Clin Chem 1987;33: 209–213
15. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D; Modification of Diet in Renal Disease Study Group. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Ann Intern Med 1999;130:461–470
16. Fine JP, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. J Am Stat Assoc 1999;94:496–509
17. Fenske W, Wanner C, Allolio B, et al.; German Diabetes, Dialysis Study Investigators. Copeptin levels associate with cardiovascular events in patients with ESRD and type 2 diabetes mellitus. J Am Soc Nephrol 2011;22:782–790
18. Enhorning S, Bankir L, Bouby N, et al. Copeptin, a marker of vasopressin, in abdominal obesity, diabetes and microalbuminuria: the prospective Malmo Diet and Cancer Study cardiovascular cohort. Int J Obes (Lond) 2013;37:598–603
19. Meijer E, Bakker SJ, van der Jagt EJ, et al. Copeptin, a surrogate marker of vasopressin, is associated with disease severity in autosomal dominant polycystic kidney

disease. Clin J Am Soc Nephrol 2011;6:361–368
20. Boertien WE, Meijer E, Li J, et al.; Consortium for Radiologic Imaging Studies of Polycystic Kidney Disease CRISP. Relationship of copeptin, a surrogate marker for arginine vasopressin, with change in total kidney volume and GFR decline in autosomal dominant polycystic kidney disease: results from the CRISP cohort. Am J Kidney Dis 2013;61:420–429
21. Boertien WE, Meijer E, Zittema D, et al. Copeptin, a surrogate marker for vasopressin, is associated with kidney function decline in subjects with autosomal dominant polycystic kidney disease. Nephrol Dial Transplant 2012;27:4131–4137
22. Meijer E, Bakker SJ, de Jong PE, et al. Copeptin, a surrogate marker of vasopressin, is associated with accelerated renal function decline in renal transplant recipients. Transplantation 2009;88:561–567
23. Strippoli GFM, Craig JC, Rochtchina E, Flood VM, Wang JJ, Mitchell P. Fluid and nutrient intake and risk of chronic kidney disease. Nephrology (Carlton) 2011;16:326–334
24. Clark WF, Sontrop JM, Macnab JJ, et al. Urine volume and change in estimated GFR in a community-based cohort study. Clin J Am Soc Nephrol 2011;6:2634–2641
25. Perucca J, Bouby N, Valeix P, Bankir L. Sex difference in urine concentration across differing ages, sodium intake, and level of kidney disease. Am J Physiol Regul Integr Comp Physiol 2007;292:R700–R705
26. Bhandari SS, Loke I, Davies JE, Squire IB, Struck J, Ng LL. Gender and renal function influence plasma levels of copeptin in healthy individuals. Clin Sci (Lond) 2009;116:257–263
27. Bankir L, Bichet DG, Bouby N. Vasopressin V2 receptors, ENaC, and sodium reabsorption: a risk factor for hypertension? Am J Physiol Renal Physiol 2010;299:F917–F928
28. Bouby N, Ahloulay M, Nsegbe E, Déchaux M, Schmitt F, Bankir L. Vasopressin increases glomerular filtration rate in conscious rats through its antidiuretic action. J Am Soc Nephrol 1996;7:842–851
29. Bouby N, Bachmann S, Bichet D, Bankir L. Effect of water intake on the progression of chronic renal failure in the 5/6 nephrectomized rat. Am J Physiol 1990;258:F973–F979
30. Bouby N, Hassler C, Bankir L. Contribution of vasopressin to progression of chronic renal failure: study in Brattleboro rats. Life Sci 1999;65:991–1004
31. Bardoux P, Martin H, Ahloulay M, et al. Vasopressin contributes to hyperfiltration, albuminuria, and renal hypertrophy in diabetes mellitus: study in vasopressin-deficient Brattleboro rats. Proc Natl Acad Sci USA 1999;96:10397–10402
32. Bankir L, Bardoux P, Ahloulay M. Vasopressin and diabetes mellitus. Nephron 2001;87:8–18
33. Hostetter TH, Troy JL, Brenner BM. Glomerular hemodynamics in experimental diabetes mellitus. Kidney Int 1981;19:410–415
34. Brenner BM. Nephron adaptation to renal injury or ablation. Am J Physiol 1985;249:F324–F337
35. Mann JF, Anderson C, Gao P, et al.; ONTARGET investigators. Dual inhibition of the renin-angiotensin system in high-risk diabetes and risk for stroke and other outcomes: results of the ONTARGET trial. J Hypertens 2013;31:414–421
36. Wang CJ, Grantham JJ, Wetmore JB. The medicinal use of water in renal disease. Kidney Int 2013;8:45–53
37. Bankir L, Bouby N, Ritz E. Vasopressin: a novel target for the prevention and retardation of kidney disease? Nat Rev Nephrol 2013;9:223–239