



Plasma Copeptin, Kidney Outcomes, Ischemic Heart Disease, and All-Cause Mortality in People With Long-standing Type 1 Diabetes

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OBJECTIVE

Plasma copeptin, a surrogate for vasopressin, has been associated with a decline in renal function and albuminuria in population-based studies as well as with progression of diabetic nephropathy in people with type 2 diabetes. We assessed the risk of kidney and coronary events and all-cause mortality associated with plasma copeptin in people with type 1 diabetes.

RESEARCH DESIGN AND METHODS

Plasma copeptin was measured in baseline samples of the GENEDIAB ($n = 398$; 56% male; mean \pm SD age 45 ± 12 years and diabetes duration 28 ± 10 years) and GENESIS ($n = 588$; 52% male; age 42 ± 11 years; diabetes duration 27 ± 9 years) cohorts. Follow-up data were available for 218 GENEDIAB and 518 GENESIS participants. Median duration of follow-up was 10.2 and 5.0 years, respectively.

RESULTS

Upper sex-specific tertiles of copeptin were associated with a higher incidence of end-stage renal disease (ESRD) during follow-up (hazard ratio [HR] for third vs. first tertile 26.5 [95% CI 8.0–163.3; $P < 0.0001$]; analysis in pooled cohorts adjusted for age, sex, duration of diabetes, and cohort membership). The highest tertile of copeptin was also associated with incidence of myocardial infarction or coronary revascularization (HR 2.2 [95% CI 1.2–4.0]; $P = 0.01$) and all-cause mortality (HR 3.3 [95% CI 1.8–6.5]; $P < 0.0001$) during follow-up.

CONCLUSIONS

Plasma copeptin is a predictor for the risk of ESRD, coronary heart disease, and all-cause mortality in people with type 1 diabetes. Results are consistent with data from experimental and epidemiological studies, suggesting that high circulating levels of vasopressin are deleterious to renal function.

Circulating levels of vasopressin (or antidiuretic hormone) are increased in people with type 1 or type 2 diabetes and in animal models with spontaneous or streptozotocin-induced diabetes (1). Vasopressin is cosecreted into the blood by the neurohypophysis in an equimolar amount with copeptin, the COOH-terminal portion of the preprovasopressin peptide. The main stimuli for the secretion of vasopressin are an increase in plasma osmolality and/or a decrease in arterial circulating

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volume. The causes of increased vasopressin and copeptin levels in diabetes are not fully elucidated. These high levels do not seem to result from an increase in plasma osmolality due to hyperglycemia but could result from a relative contraction of extracellular volume induced by glycosuria and/or from an increased sensitivity of hypothalamic osmoreceptor neurons to the plasma osmotic load (2). From an adaptive perspective, high levels of vasopressin may be beneficial in the short term by limiting the water loss in urine induced by the higher osmolar load due to glycosuria (1). However, in the long term, persistently high levels of vasopressin might aggravate hyperglycemia by increasing hepatic glucose production and inducing insulin resistance (3) and might be deleterious to renal function (4). Because vasopressin secretion can be modulated by water intake (5) and its actions by nonpeptide selective receptor antagonists (vaptans) (6), the vasopressin/hydration system could be a potential therapeutic target for the prevention and treatment of diabetic complications, notably diabetic nephropathy (4).

Circulating levels of vasopressin and copeptin correlate over a wide range of plasma osmolality (7,8), and copeptin, which is easier to assay, is an adequate surrogate marker of vasopressin and hydration status (7,8). Plasma copeptin is associated with hyperglycemia and type 2 diabetes as well as with other cardiovascular risk factors, such as arterial hypertension, abdominal obesity, insulin resistance, and the metabolic syndrome (9–14). Plasma copeptin also has been associated with the decline in renal function and albuminuria in population-based studies (4,15,16) and with the development and progression of diabetic nephropathy in people with type 2 diabetes (17,18). However, prospective data are lacking in people with type 1 diabetes. We assessed the association of plasma copeptin at baseline with the risk of subsequent kidney and coronary morbidity and all-cause mortality in people with long-standing type 1 diabetes. We tested the hypothesis that the associations with coronary morbidity and all-cause mortality could reflect the kidney morbidity associated with these outcomes.

RESEARCH DESIGN AND METHODS

Participants

Génétique de la Néphropathie Diabétique (GENEDIAB) and Genesis France-Belgium (GENESIS) are multicenter binational cohorts of people with long-standing type 1 diabetes that studied the vascular complications of diabetes. GENEDIAB included 494 participants selected on the basis of a diagnosis of type 1 diabetes before the age of 35 years and past or present diagnosis of severe diabetic retinopathy (19). GENESIS was a family-based study conducted in 578 first-degree relatives and 662 probands with type 1 diabetes selected on the basis of a diagnosis of diabetes before the age of 35 years and past or present diagnosis of diabetic retinopathy (20). Subsets of participants from GENEDIAB ($n = 260$) and GENESIS ($n = 550$) were included in a prospective observational study and followed until an end point was reached or until February 2007. The subsets comprised participants who attended outpatient clinics at least once during the follow-up period. Median (interquartile range) duration of follow-up was 10.2 (2.7) and 5.0 (1.6) years for GENEDIAB and GENESIS, respectively. In the present investigation, copeptin was effectively measured in 398 GENEDIAB participants and 588 GENESIS probands for whom plasma samples were available at baseline, including 218 GENEDIAB and 518 GENESIS participants seen at follow-up. The flowchart of participants is shown in Supplementary Fig. 1. Study protocols were approved by the ethics committee of the Angers University Hospital (Angers, France), and all participants gave written informed consent.

Definition of Clinical Parameters and Outcomes

An ad hoc event committee reviewed the case record of each patient to validate baseline data (19) and later the incidence of outcomes during follow-up. Diabetic nephropathy stages were defined according to the following criteria: 1) no nephropathy (urinary albumin excretion [UAE] <30 mg/24 h or <20 μ g/min or urinary albumin concentration [UAC] <20 mg/L and plasma creatinine <150 μ mol/L in at least two of three consecutive assessments and in the absence of antihypertension treatment), 2) incipient nephropathy (persistent microalbuminuria [UAE = 30–300 mg/24 h or

20–200 μ g/min or UAC = 20–200 mg/L] and plasma creatinine <150 μ mol/L), 3) established nephropathy (past or present macroalbuminuria [UAE >300 mg/24 h or >200 μ g/min or UAC >200 mg/L] and plasma creatinine <150 μ mol/L), and 4) advanced nephropathy (past or present macroalbuminuria and plasma creatinine >150 μ mol/L or history of end-stage renal disease [ESRD] defined as hemodialysis requirement or kidney transplantation). Diabetic retinopathy was staged according to Kohner's classification as nonproliferative, preproliferative, or proliferative (21) based on direct funduscopy and/or fluorescein angiography results. Systolic and diastolic blood pressure were measured in supine position with a mercury sphygmomanometer and are reported as the mean of two consecutive measurements (19). Myocardial infarction was diagnosed on the basis of at least two of the following three criteria: constrictive chest pain lasting ≥ 20 min, increased serum creatine phosphokinase activity and/or troponin concentration, or typical electrocardiographic changes. Cause of death was established from hospital records. Missing data were obtained by a phone interview with the patient's general practitioner and/or by consulting the death certificate national registry. We considered the incidence of three outcomes during follow-up: ESRD, a coronary event (defined as the occurrence of myocardial infarction and/or coronary revascularization procedure), and all-cause mortality. Characteristics of GENEDIAB and GENESIS participants at baseline by the incidence of ESRD and all-cause mortality during follow-up were published previously (22,23). Incidence rates and clinical data reported in the present study are those of the subsets of participants with an available plasma sample at baseline for copeptin assay.

Laboratory Procedure

Copeptin concentration was measured in 2014 in fasting plasma-EDTA samples collected at baseline and kept frozen at -80°C . An automated immunoluminometric assay (ultra-sensitive B.R.A.H.M.S Copeptin proAVP; Thermo Scientific, Hennigsdorf, Germany) on a KRYPTOR compact PLUS system was used (24). The limit of detection was 0.9 pmol/L. The intra-assay coefficient of variance reported by the manufacturer was $<15\%$ and $<8\%$ for concentrations ranging from 2.0 to

4.0 pmol/L and 4.0 to 15.0 pmol/L, respectively. The reported interassay coefficient of variance was <18% and <10%, respectively, for the lower and higher copeptin concentration range.

Computations and Statistical Analyses

Tertiles of plasma copeptin concentration were computed separately for women and men to take into account the well-known sex-related differences on copeptin levels (8,10–14,25). Estimated glomerular filtration rate (eGFR) was calculated by the MDRD equation (26). The annual 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. Results are expressed as mean \pm SD, except where stated otherwise. Differences between

groups were assessed by Pearson χ^2 test, Wilcoxon and Kruskal-Wallis test, ANOVA, or ANCOVA. Association of plasma copeptin with diabetic nephropathy at baseline was assessed by logistic regression analyses. Cox proportional hazards regression models were used to examine the effect of plasma copeptin at baseline on time-related survival (outcome-free) rates in prospective analyses. Adjustments for clinical and biological parameters were carried out by including these parameters as covariates in the regression models. Odds ratios (ORs) or hazard ratios (HRs) with their 95% CIs were computed in these analyses, respectively. For all outcomes in the Cox models, the validity of proportional hazards assumption was verified for all covariates by a test based on Schoenfeld

residuals (estat phtest, Stata 12.1 software; StataCorp, College Station, TX). Kaplan-Meier curves were used to plot the incidence of outcomes over time. To increase statistical power, all prospective analyses were performed in GENEDIAB and GENESIS pooled cohorts. Data were log-transformed for the analyses when the normality of the distribution was rejected by the Kolmogorov-Smirnov-Lilliefors goodness-of-fit test. Statistical analyses were performed with JMP (SAS Institute, Cary, NC) and Stata 12.1 software. $P < 0.05$ was considered significant.

RESULTS

Clinical Characteristics at Baseline

Participant characteristics at baseline by tertiles of plasma copeptin are shown in Table 1. Participants in the highest

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

	GENEDIAB study				GENESIS study			
	T1	T2	T3	<i>P</i> value	T1	T2	T3	<i>P</i> value
<i>n</i>	135	132	131		196	195	197	
Age (years)	46 \pm 12	44 \pm 13	44 \pm 12	0.21	43 \pm 10	41 \pm 11	42 \pm 11	0.61
Plasma copeptin (pmol/L)								
Women	1.9 (0.9–2.9)	4.5* (3.0–7.9)	14.6** (8.0–49.8)	<0.0001	2.2 (0.9–2.8)	3.9* (2.8–5.9)	13.8** (6.1–153.1)	<0.0001
Men	2.6 (0.9–3.9)	5.9* (4.0–9.1)	19.0** (9.3–158)	<0.0001	3.3 (0.9–4.7)	6.2* (5.0–8.7)	16.3** (8.8–210.6)	<0.0001
Age at diagnosis of diabetes (years)	18 \pm 9	15 \pm 9*	16 \pm 9	0.04	15 \pm 8	15 \pm 10	15 \pm 10	0.99
Duration of diabetes (years)	28 \pm 10	28 \pm 10	28 \pm 9	0.97	27 \pm 9	26 \pm 9	27 \pm 10	0.46
BMI (kg/m ²)	23.7 \pm 3.2	24.0 \pm 3.1	23.6 \pm 3.3	0.64	24.7 \pm 3.7	24.3 \pm 3.5	24.0 \pm 3.6	0.20
HbA _{1c} (%)	8.4 \pm 1.8	8.9 \pm 1.9	8.6 \pm 1.8	0.10	8.3 \pm 1.1	8.5 \pm 1.3	9.0 \pm 1.5*	0.001
mmol/mol	68 \pm 20	73 \pm 20	70 \pm 20		67 \pm 12	70 \pm 14	72 \pm 17	
Systolic BP (mmHg)	134 \pm 18	137 \pm 18	143 \pm 19*	0.0004	129 \pm 16	131 \pm 19	136 \pm 22*	0.01
Diastolic BP (mmHg)	77 \pm 13	79 \pm 10	81 \pm 12*	0.01	74 \pm 8	75 \pm 10	78 \pm 11**	0.002
Antihypertensive treatment (%)	46.7	48.0	69.8	0.0001	36.7	46.2	70.4	<0.0001
eGFR (mL/min/1.73 m ²)	79 (26)	78 (35)	49 (41)**	<0.0001	91 (36)	91 (43)	58 (59)**	<0.0001
UAC (mg/L)	8 (35)	26 (154)*	425 (1,398)**	<0.0001	10 (28)	13 (62)*	298 (1,183)**	<0.0001
Diabetic nephropathy stage (%)				<0.0001				<0.0001
Absent	48	40	14		69	59	20	
Incipient	27	27	11		20	23	18	
Established	18	24	24		9	12	26	
Advanced	7	9	51		2	6	36	
Use of diuretics (%)	10.1	13.2	33.3	<0.0001	5.1	7.2	32.7	<0.0001
Use of ACE-I or ARB (%)	37.8	41.3	50.0	0.13	28.6	36.4	61.7	<0.0001
Previous MI (%)	7.4	7.6	8.4	0.94	4.1	4.6	5.6	0.78

Data are mean \pm SD, median (range) (copeptin), and median (interquartile range) (eGFR and UAC), unless otherwise stated. Statistics for quantitative parameters are ANOVA with log-transformed data or Wilcoxon test (eGFR and UAC). Tukey Kramer honest significant difference test following ANOVA or Wilcoxon test were used for comparisons of each pair: *significantly different ($P < 0.05$) from T1; †significantly different ($P < 0.05$) from T2. Arterial hypertension was defined as the use of antihypertensive drugs and history of hypertension. ACE-I, ACE inhibitor; ARB, angiotensin receptor blocker; BP, blood pressure; MI, myocardial infarction.

tertile had higher HbA_{1c} levels (GENESIS study only) and systolic and diastolic blood pressures and were more likely to have a history of arterial hypertension. They had lower eGFR and higher UAC. Diabetic nephropathy at baseline was more frequent and more severe in participants in the highest tertile (T3) of plasma copeptin but was similar in participants in tertiles T1 and T2 (Supplementary Fig. 2). Regression analyses confirmed the associations of the highest tertile of plasma copeptin with the prevalence of established and advanced diabetic nephropathy at baseline in both cohorts. The ORs (95% CI) for established and advanced nephropathy in GENEDIAB were 3.43 (1.77–6.81; $P = 0.0002$) and 24.48 (11.97–53.72; $P < 0.0001$) for T3 versus T1 and T2, adjusted for sex, age, and duration of diabetes. The ORs were 7.91 (4.64–13.68; $P < 0.0001$) and 31.29 (16.64–62.38; $P < 0.0001$), respectively, in GENESIS.

Copeptin and Kidney and Coronary Outcomes During Follow-up

The mean \pm SEM annual decline in eGFR during follow-up in the GENESIS and GENEDIAB pooled study by tertiles of baseline plasma copeptin was -3.09 ± 0.37 (T1), -3.74 ± 0.36 (T2), and $-5.18 \pm$

0.46 (T3) mL/min/1.73 m² per year (ANCOVA $P = 0.002$), adjusted for age, duration of diabetes, HbA_{1c}, UAC (normo-, micro-, or macroalbuminuria), eGFR at baseline, sex, cohort membership, and duration of follow-up. Cumulative incidence of ESRD and coronary events during follow-up was 7.0% ($n = 47$) and 9.4% ($n = 67$), respectively. The incidence rates of these outcomes were 1.16 and 1.55 per 100 person-years, respectively. Characteristics of GENEDIAB and GENESIS participants at baseline by the incidence of outcomes during follow-up are shown in Supplementary Table 1. The incidence of ESRD and coronary events during follow-up in the pooled cohorts by tertiles of baseline plasma copeptin is shown in Table 2 and Fig. 1. Incidence of each outcome was significantly higher for participants in the upper tertile of plasma copeptin. Cox proportional hazards regression models confirmed a positive association of the upper tertile of plasma copeptin and log_e[copeptin] with the incidence of ESRD and coronary events in a minimally adjusted model, including as covariates cohort membership, sex, age, and duration of diabetes at baseline (Table 2, model 1). Associations with the incidence

of ESRD remained significant in sensitivity analyses when the cohorts were analyzed separately, whereas the association with the incidence of coronary events remained significant in the GENESIS cohort only (Supplementary Table 2).

Cox proportional hazards regression analyses were also performed in the pooled cohorts in a multiaadjusted model. Clinical and biological parameters at baseline with $P \leq 0.20$ at least in one of the cohorts in the comparison by tertiles of plasma copeptin (Table 1) were entered in the model as independent covariates, together with cohort membership, sex, age, duration of diabetes, and previous history of myocardial infarction (for the Cox of coronary events) at baseline. Plasma copeptin remained significantly associated with the incidence of ESRD in the multiaadjusted model (Table 2, model 2), although with an attenuated HR compared with the minimally adjusted model. The inclusion of baseline UAC and eGFR in the multiaadjusted model was responsible for most of the HR attenuation. UAC, eGFR, HbA_{1c}, BMI, diastolic blood pressure, and use of diuretic and antihypertensive medications at baseline also remained associated with the incidence of ESRD during follow-up (Supplementary Fig. 3). Only age, diastolic blood pressure, UAC, and a history of myocardial infarction at baseline remained associated with the incidence of coronary events in the multiaadjusted model (Table 2, model 2, and Supplementary Fig. 3). When UAC, eGFR, and systolic and diastolic blood pressure were excluded from the multiaadjusted model, copeptin became associated with the incidence of coronary events (HR for 1 unit of log_e[copeptin] 1.3 [95% CI 1.0–1.7]; $P = 0.04$), suggesting that these covariates accounted for the effect of copeptin on the outcome.

Table 2—Clinical outcomes during GENESIS and GENEDIAB follow-up by plasma copeptin at baseline

	Clinical outcome during follow-up					
	ESRD		Coronary event		All-cause mortality	
	No	Yes	No	Yes	No	Yes
T1	248 (99.2)	2 (0.8)	238 (92.6)	19 (7.4)	246 (94.6)	14 (5.4)
T2	228 (95.0)	12 (5.0)	224 (91.4)	21 (8.6)	230 (91.6)	21 (8.4)
T3	149 (81.9)	33 (18.1)	186 (87.3)	27 (12.7)	189 (85.5)	32 (14.5)
	HR (95% CI)	<i>P</i> value	HR (95% CI)	<i>P</i> value	HR (95% CI)	<i>P</i> value
Model 1						
T3 vs. T1	26.5 (8.0–163.3)	<0.0001	2.2 (1.2–4.0)	0.01	3.3 (1.8–6.5)	<0.0001
T3 vs. T2	4.4 (2.3–8.8)	<0.0001	1.7 (0.9–3.1)	0.07	2.0 (1.1–3.5)	0.02
T2 vs. T1	6.1 (1.7–39.1)	0.005	1.3 (0.7–2.4)	0.48	1.7 (0.9–3.5)	0.13
Log _e [copeptin]	4.3 (3.1–5.9)	<0.0001	1.4 (1.1–1.7)	0.009	1.7 (1.4–2.1)	<0.0001
Model 2						
T3 vs. T1	9.5 (1.8–174.71)	0.004	1.2 (0.6–2.5)	0.60	1.6 (0.7–3.4)	0.25
T3 vs. T2	1.5 (0.7–3.4)	0.33	1.0 (0.5–2.1)	0.98	1.3 (0.6–2.6)	0.52
T2 vs. T1	6.4 (1.1–118.4)	0.03	1.2 (0.6–2.4)	0.58	1.2 (0.6–2.5)	0.57
Log _e [copeptin]	1.7 (1.2–2.6)	0.005	1.1 (0.8–1.5)	0.58	1.3 (0.9–1.8)	0.13

Data are n (%) unless otherwise indicated. Analyses were performed in pooled cohorts. HRs were computed by Cox proportional hazards regression models for tertiles of plasma copeptin and for 1 unit of log_e[copeptin]. Model 1: adjusted for cohort membership, sex, age, and duration of diabetes at baseline. Model 2: model 1 plus adjustments for eGFR; UAC; systolic and diastolic blood pressure; BMI; HbA_{1c}; and use of ACE inhibitor or angiotensin receptor blocker, diuretic, and antihypertensive medications at baseline. Model 2 for coronary events and all-cause mortality were further adjusted for history of myocardial infarction at baseline. $P < 0.05$ is significant.

Copeptin and Mortality During Follow-up

The cumulative incidence of all-cause mortality during follow-up in the GENESIS and GENEDIAB pooled study was 9.2% ($n = 67$), and its incidence rate was 1.43 per 100 person-years. Characteristics of GENEDIAB and GENESIS participants at baseline by the incidence of all-cause mortality during follow-up are shown in the Supplementary Table 1. The incidence of all-cause mortality during follow-up was significantly higher for participants in the upper tertile of

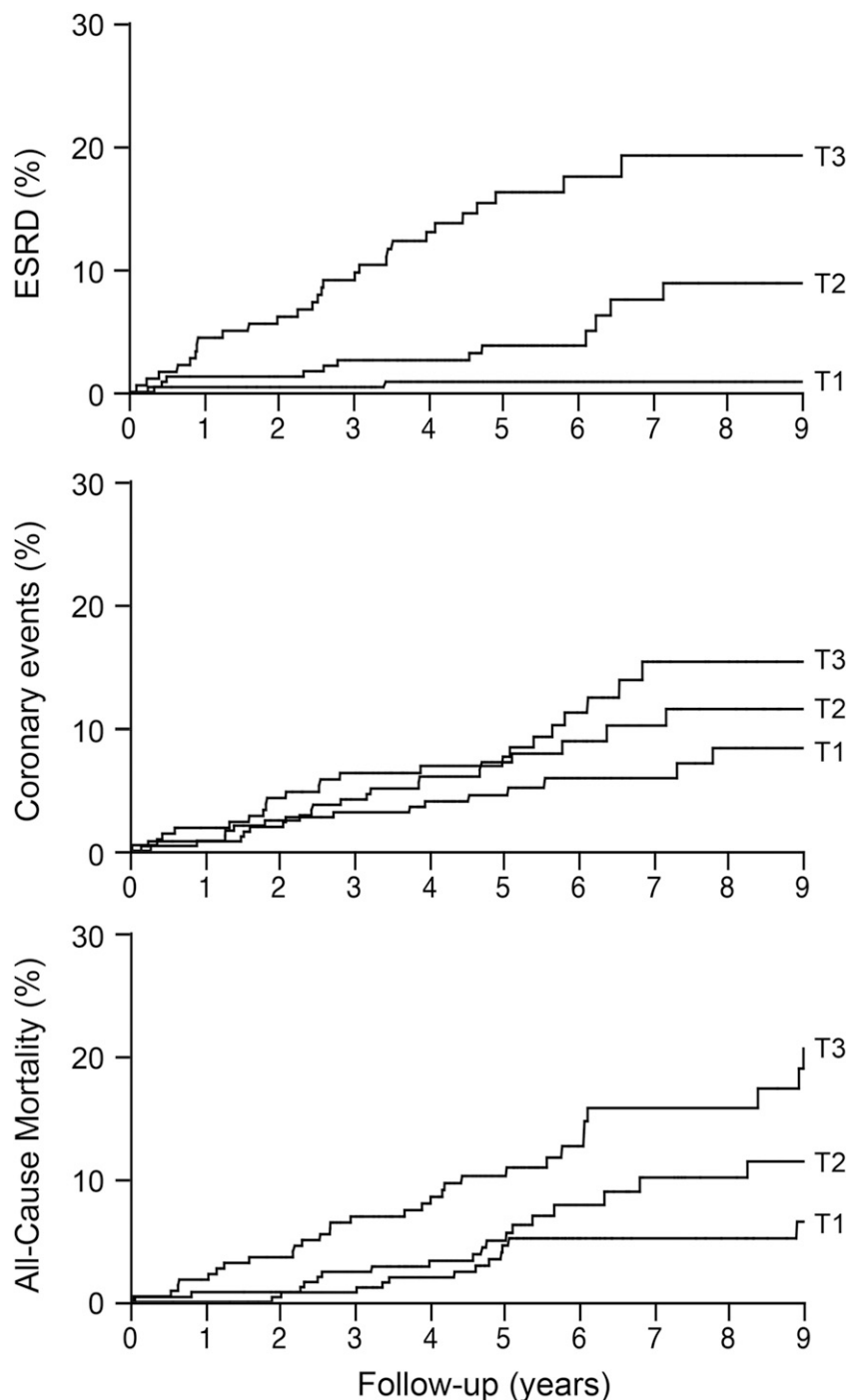


Figure 1—Kaplan-Meier curves for the cumulative incidence of ESRD, coronary events, and all-cause mortality during GENEDIAB and GENESIS follow-up by tertiles of baseline plasma copeptin. Median duration of follow-up (interquartile range) was 5.5 (4.6), 5.4 (4.7), and 5.5 (3.9) years ($P = 0.28$) for T1, T2, and T3, respectively. Data are from the pooled cohorts.

plasma copeptin at baseline (Table 2 and Fig. 1). Cox proportional hazards regression models confirmed a positive association of the upper tertile of plasma copeptin with all-cause mortality in the minimally adjusted model (Table 2). The association remained significant in sensitivity analyses when the cohorts were

analyzed separately (Supplementary Table 2). Population attributable risk of the upper tertile of plasma copeptin for all-cause mortality during follow-up in the pooled cohorts was 0.27. Cohort membership (GENEDIAB), HbA_{1c}, BMI, UAC, and a history of myocardial infarction at baseline remained associated with all-cause

mortality in the multiaadjusted model (Table 2, model 2, and Supplementary Fig. 3). When UAC and eGFR were excluded from the multiaadjusted model, copeptin became associated with all-cause mortality (HR for 1 unit of $\log_e[\text{copeptin}]$ 1.4 [95% CI 1.1–1.8]; $P = 0.01$), suggesting that these covariates accounted for the effect of copeptin on the outcome.

Causes of death during follow-up included cardiovascular complications (38.8%), infectious diseases (16.4%), cancer (10.4%), acute metabolic complications (9.0%), renal failure (3.0%), and other or undetermined etiologies (22.4%). We assessed associations of copeptin with cause-specific mortality grouped as cardiometabolic mortality (death caused by cardiovascular complications, acute metabolic complications, or renal failure) and all other causes of death. Cox proportional hazards regression models confirmed the association of high copeptin levels at baseline with cardiometabolic mortality (HR 2.9 [95% CI 1.3–7.0; $P = 0.009$] for T3 vs. T1; HR 1.6 [95% CI 1.2–2.2; $P = 0.002$] for 1 unit of $\log_e[\text{copeptin}]$ adjusted for cohort membership, sex, age, and duration of diabetes at baseline) and with other causes of death (HR 4.6 [95% CI 1.8–14.1; $P = 0.001$] for T3 vs. T1; HR 1.9 [95% CI 1.4–2.5; $P = 0.0001$] for 1 unit of $\log_e[\text{copeptin}]$, same adjustments as above). In the multiaadjusted model, plasma copeptin was not significantly associated with cardiometabolic mortality (HR for 1 unit of $\log_e[\text{copeptin}]$ 1.1 [95% CI 0.7–1.7]; $P = 0.74$), but remained associated with other causes of death (HR for 1 unit of $\log_e[\text{copeptin}]$ 1.8 [95% CI 1.1–2.8]; $P = 0.02$). When UAC, eGFR, and systolic and diastolic blood pressure were excluded from the multiaadjusted model, copeptin became associated with cardiometabolic mortality (HR for 1 unit of $\log_e[\text{copeptin}]$ 1.5 [95% CI 1.1–2.1]; $P = 0.02$).

CONCLUSIONS

In the present study, people with long-standing type 1 diabetes had high levels of plasma copeptin associated with the prevalence of established and advanced diabetic nephropathy at baseline and with an increased risk during follow-up of ESRD, coronary events, and all-cause mortality. The association with the incidence of ESRD during follow-up

remained significant after adjustment for relevant confounding factors at baseline, such as age, duration of diabetes, blood pressure, HbA_{1c}, eGFR, and UAC. On the other hand, the association of high plasma copeptin with increased risk of coronary events during follow-up could be accounted for by the higher blood pressure, eGFR, and UAC observed in participants with higher plasma copeptin. Finally, the association with increased mortality risk was not restricted to death from cardiometabolic causes but was significant when we considered other causes of death, including cancer and infectious diseases. The association of copeptin with other causes of death was independent from kidney phenotypes, but the association with cardiometabolic death could be accounted for by the higher UAC and lower eGFR observed in participants in the upper tertile of plasma copeptin. The population-attributable risk of the upper tertile of plasma copeptin at baseline (>6.1 pmol/L for women and >8.0 pmol/L for men) with regard to death from all causes was 27%. This figure corresponds to the reduction in mortality that could be observed if all participants had plasma copeptin below these levels.

To our knowledge, this prospective investigation is the first to evaluate copeptin as a risk factor for kidney and cardiovascular morbidity and mortality specifically in people with type 1 diabetes. In a recent cross-sectional study in 209 adults with type 1 diabetes, copeptin correlated positively with the urinary albumin-to-creatinine ratio and a coronary artery calcium score and negatively with eGFR (27). Other studies published so far concerned subjects from the general population or patients with kidney disease or type 2 diabetes. In the general population, plasma copeptin has been positively associated with renal function decline and progression to chronic kidney disease stage 3 or worse (16), the prevalence or the incidence of microalbuminuria (12,15,25), and the presence of renal cysts (15) and inversely associated with kidney length (15). In patients with autosomal-dominant polycystic kidney disease, copeptin was associated with markers of disease severity, including GFR and albuminuria (28,29). In addition, administration of

tolvaptan, a V₂-receptor antagonist, for 36 months was associated with slowed kidney growth and functional decline and with a reduced frequency of autosomal-dominant polycystic kidney disease-related complications (30). In renal transplant recipients, high copeptin level at baseline was associated with a faster decline in GFR during a 3- to 4-year follow-up (31). Plasma copeptin was associated with renal function decline in people with newly diagnosed type 2 diabetes (17) and with the risk of severe kidney outcomes (doubling of plasma creatinine concentration and/or ESRD) in people with type 2 diabetes and albuminuria (18). Other studies reported associations with cardiovascular disease and/or mortality. In the population-based Malmö Diet and Cancer Study–Cardiovascular cohort, plasma copeptin was associated with a combined end point comprising coronary heart disease, heart failure, and death in people with diabetes but not in people without diabetes (32). However, in older Swedish subjects, copeptin was associated with an increased risk of coronary heart disease and cardiovascular mortality in people with and without diabetes (33). Finally, in a study of patients with type 2 diabetes undergoing hemodialysis, high plasma copeptin was associated with increased risk for all-cause mortality (34).

Vasopressin binds to three different G-protein-coupled receptors. V_{1a}R is widely expressed, particularly in vascular smooth muscle cells, hepatocytes, platelets, and the central nervous system. V_{1b}R is expressed in the endocrine pancreas, in cells of the anterior pituitary, and throughout the brain. V₂R is predominantly expressed in the kidney collecting ducts and in the endothelium. Vasopressin stimulates hepatic gluconeogenesis and glycogenolysis through V_{1a}R (3,35) and the release of adrenocorticotrophic hormone and glucagon or insulin (depending on the extracellular glucose level) through V_{1b}R (35,36). Experimental evidence strongly supports a causal and direct role of vasopressin in the development and aggravation of chronic kidney disease through V₂R activation (37,38), but the mechanisms of these deleterious effects are not fully understood (4).

The risks for ischemic heart disease and cardiometabolic mortality in the current study were influenced by markers of nephropathy (ie, UAC, eGFR), and the association of copeptin with these outcomes depended on these markers and on arterial hypertension, mostly as a consequence of kidney disease, in people with type 1 diabetes. The results agree with data from the literature showing that the presence and severity of chronic kidney disease is the dominant contributor to the excess mortality associated with type 1 diabetes (39,40). Impaired kidney function may aggravate other cardiovascular risk factors, such as hypertension, oxidative stress, insulin resistance, dyslipidemia, inflammation, and arterial calcification. Thus, the association of copeptin with ischemic heart disease and cardiometabolic mortality observed in the present investigation may be partly accounted for by the deleterious effects of vasopressin on kidney function. However, vasopressin induces platelet aggregation and has a vasoconstrictor effect on vascular smooth muscle cells through V_{1a}R (35). To our knowledge, the role these vasopressin effects play in the pathophysiology of ischemic heart disease has not been evaluated.

There are limitations to this study. Because of the observational design, we could not establish a causal relationship between copeptin/vasopressin and the outcomes. Moreover, we used copeptin as a surrogate of vasopressin. Plasma concentrations of the peptides correlate over a wide range of plasma and/or urine osmolalities (7,8), but the ratio between their concentrations and the strength of their correlation varies with the GFR (8). Another limitation was the relatively small number of outcomes observed during follow-up, potentially reducing the statistical power to observe independent associations. Despite these limitations, the study has major strengths. It includes a cross-sectional analysis of ~1,000 patients with long-standing type 1 diabetes from two independent cohorts plus a longitudinal study over 5–10 years for >700 participants with comprehensive renal, cardiovascular, and survival outcomes, including cause-specific mortality data.

In conclusion, this study in people with long-standing type 1 diabetes,

together with a growing body of data from experimental and epidemiological studies, suggests that high levels of circulating vasopressin are deleterious to kidney function. Intervention studies are required to assess the potential benefit of reducing vasopressin secretion or action in the prevention of kidney disease. The study confirms in people with type 1 diabetes that copeptin is a risk marker for severe clinical outcomes, including ESRD, ischemic heart disease, and death.

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Author Contributions. G.V. and R.R. researched data and wrote the manuscript. R.E.B., G.L., and K.M. researched data, contributed to discussion, and reviewed and edited the manuscript. F.F., L.P., L.B., N.B., S.H., and M.M. contributed to discussion and reviewed and edited the manuscript. G.V. 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.

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References

- Bankir L, Bardoux P, Ahloulay M. Vasopressin and diabetes mellitus. *Nephron* 2001;87:8–18
- Zerbe RL, Vinicor F, Robertson GL. Regulation of plasma vasopressin in insulin-dependent diabetes mellitus. *Am J Physiol* 1985;249:E317–E325
- Taveau C, Chollet C, Waeckel L, et al. Vasopressin and hydration play a major role in the development of glucose intolerance and hepatic steatosis in obese rats. *Diabetologia* 2015;58:1081–1090
- 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
- Wang CJ, Grantham JJ, Wetmore JB. The medicinal use of water in renal disease. *Kidney Int* 2013;84:45–53
- Decaux G, Soupart A, Vassart G. Non-peptide arginine-vasopressin antagonists: the vaptans. *Lancet* 2008;371:1624–1632
- 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
- Roussel R, Fezeu L, Marre M, et al. Comparison between copeptin and vasopressin in a population from the community and in people with chronic kidney disease. *J Clin Endocrinol Metab* 2014;99:4656–4663
- Saleem U, Khaleghi M, Morgenthaler NG, et al. Plasma carboxy-terminal vasopressin (copeptin): a novel marker of insulin resistance and metabolic syndrome. *J Clin Endocrinol Metab* 2009;94:2558–2564
- Enhörning S, Wang TJ, Nilsson PM, et al. Plasma copeptin and the risk of diabetes mellitus. *Circulation* 2010;121:2102–2108
- Abbasi A, Corpeleijn E, Meijer E, et al. Sex differences in the association between plasma copeptin and incident type 2 diabetes: the Prevention of Renal and Vascular Endstage Disease (PREVEND) study. *Diabetologia* 2012;55:1963–1970
- Enhörning S, Bankir L, Bouby N, et al. Copeptin, a marker of vasopressin, in abdominal obesity, diabetes and microalbuminuria: the prospective Malmö Diet and Cancer Study cardiovascular cohort. *Int J Obes* 2013;37:598–603
- Then C, Kowall B, Lechner A, et al. Plasma copeptin is associated with type 2 diabetes in men but not in women in the population-based KORA F4 study. *Acta Diabetol* 2015;52:103–112
- Roussel R, El Boustany R, Bouby N, et al. Plasma copeptin, AVP gene variants, and incidence of type 2 diabetes in a cohort from the community. *J Clin Endocrinol Metab* 2016;101:2432–2439
- Ponte B, Pruijm M, Ackermann D, et al. Copeptin is associated with kidney length, renal function, and prevalence of simple cysts in a population-based study. *J Am Soc Nephrol* 2015;26:1415–1425
- Roussel R, Matallah N, Bouby N, et al. Plasma copeptin and decline in renal function in a cohort from the community: the prospective D.E.S.I.R. study. *Am J Nephrol* 2015;42:107–114
- Pikkemaat M, Melander O, Bengtsson Boström K. Association between copeptin and declining glomerular filtration rate in people with newly diagnosed diabetes. The Skaraborg Diabetes Register. *J Diabetes Complications* 2015;29:1062–1065
- Velho G, Bouby N, Hadjadj S, et al. Plasma copeptin and renal outcomes in patients with type 2 diabetes and albuminuria. *Diabetes Care* 2013;36:3639–3645
- Marre M, Jeunemaitre X, Gallois Y, et al. Contribution of genetic polymorphism in the renin-angiotensin system to the development of renal complications in insulin-dependent diabetes: Genetique de la Nephropathie Diabétique (GENEDIAB) study group. *J Clin Invest* 1997;99:1585–1595
- Hadjadj S, Pèan F, Gallois Y, et al.; Genesis France-Belgium Study. Different patterns of insulin resistance in relatives of type 1 diabetic patients with retinopathy or nephropathy: the Genesis France-Belgium Study. *Diabetes Care* 2004;27:2661–2668
- Kohner EM. The lesions and natural history of diabetic retinopathy. In *Textbook of Diabetes*. Pickup J, Williams G, Eds. Oxford, Blackwell Scientific, 1991, p. 575–588
- Patente TA, Mohammadi K, Bellili-Muñoz N, et al. Allelic variations in the CYBA gene of NADPH oxidase and risk of kidney complications in patients with type 1 diabetes. *Free Radic Biol Med* 2015;86:16–24
- Mohammadi K, Bellili-Muñoz N, Marklund SL, et al. Plasma extracellular superoxide dismutase concentration, allelic variations in the SOD3 gene and risk of myocardial infarction and all-cause mortality in people with type 1 and type 2 diabetes. *Cardiovasc Diabetol* 2015;14:845
- Fenske W, Störk S, Blechschmidt A, Maier SG, Morgenthaler NG, Allolio B. Copeptin in the differential diagnosis of hyponatremia. *J Clin Endocrinol Metab* 2009;94:123–129
- 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
- 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
- Bjornstad P, Maahs DM, Jensen T, et al. Elevated copeptin is associated with atherosclerosis and diabetic kidney disease in adults with type 1 diabetes. *J Diabetes Complications* 2016;30:1093–1096
- 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
- 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
- Torres VE, Chapman AB, Devuyst O, et al.; TEMPO 3:4 Trial Investigators. Tolvaptan in patients with autosomal dominant polycystic kidney disease. *N Engl J Med* 2012;367:2407–2418
- 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
- Enhörning S, Hedblad B, Nilsson PM, Engstrom G, Melander O. Copeptin is an independent predictor of diabetic heart disease and death. *Am Heart J* 2015;169:549–556
- Tasevska I, Enhörning S, Persson M, Nilsson PM, Melander O. Copeptin predicts coronary artery disease cardiovascular and total mortality. *Heart* 2016;102:127–132
- 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
35. Koshimizu TA, Nakamura K, Egashira N, Hiroyama M, Nonoguchi H, Tanoue A. Vasopressin V1a and V1b receptors: from molecules to physiological systems. *Physiol Rev* 2012;92:1813–1864
36. Abu-Basha EA, Yibchok-Anun S, Hsu WH. Glucose dependency of arginine vasopressin-induced insulin and glucagon release from the perfused rat pancreas. *Metabolism* 2002;51:1184–1190
37. 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
38. 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
39. Groop PH, Thomas MC, Moran JL, et al.; FinnDiane Study Group. The presence and severity of chronic kidney disease predicts all-cause mortality in type 1 diabetes. *Diabetes* 2009;58:1651–1658
40. Lind M, Svensson AM, Kosiborod M, et al. Glycemic control and excess mortality in type 1 diabetes. *N Engl J Med* 2014;371:1972–1982