Plasma Copeptin and Risk of Lower-Extremity Amputation in Type 1 and Type 2 Diabetes

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OBJECTIVE

Diabetes is the leading cause of nontraumatic lower-extremity amputations (LEAs). Identification of patients with foot ulcers at risk for amputation remains clinically challenging. Plasma copeptin, a surrogate marker of vasopressin, is associated with the risk of cardiovascular and renal complications in diabetes.

RESEARCH DESIGN AND METHODS

We assessed the association between baseline plasma copeptin and risk of LEA during follow-up in four cohorts of people with type 1 (GENESIS, $n = 503$, and GENEDIAB, $n = 207$) or type 2 diabetes (DIABHYCAR, $n = 3{,}101$, and SURDIAGENE, $n = 1{,}452$) with a median duration of follow-up between 5 and 10 years. Copeptin concentration was measured in baseline plasma samples by an immunoluminometric assay.

RESULTS

In the pooled cohorts with type 1 diabetes ($n = 710$), the cumulative incidence of LEA during follow-up by increasing tertiles (tertile 1 [TER1], TER2, and TER3) of baseline plasma copeptin was 3.9% (TER1), 3.3% (TER2), and 10.0% (TER3) ($P = 0.002$). Cox regression analyses confirmed the association of copeptin with LEA: hazard ratio (HR) for 1 SD increment of log[copeptin] was 1.89 (95% CI 1.28–2.82), $P = 0.002$. In the pooled cohorts of type 2 diabetes ($n = 4{,}553$), the cumulative incidence of LEA was 1.1% (TER1), 2.9% (TER2), and 3.6% (TER3) ($P < 0.0001$). In Cox regression analyses, baseline plasma copeptin was significantly associated with LEA: HR for 1 SD increment of log[copeptin] was 1.42 (1.15–1.74), $P = 0.001$. Similar results were observed in the cohort with type 2 diabetes for lower-limb revascularization (HR 1.20 [95% CI 1.03–1.39], $P = 0.02$).

CONCLUSIONS

Baseline plasma copeptin is associated with cumulative incidence of LEA in cohorts of people with both type 1 and type 2 diabetes and may help to identify patients at risk for LEA.

Diabetes is the leading cause of nontraumatic lower-extremity amputations (LEAs). LEA is a major complication of diabetes and is associated with low quality of life and higher risk of mortality (1). The high prevalence of LEA in people with diabetes is mainly related to the presence of foot ulcers, and this complication is driven by a range of factors including peripheral arterial disease (PAD), diabetic neuropathy, impaired wound healing, and susceptibility to infection (2). However, despite these well-known causal factors, biomarkers able to predict the risk of LEA are lacking. We have recently

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reported in a prospective cohort of people with type 2 diabetes that the use of diuretics was associated with a higher risk of amputation (3). That work was driven by the assumption that diuretic-induced hypovolemia would worsen hypoperfusion of distal lower extremities, triggering ischemia and necrosis, eventually leading to amputation.

More recent work has identified copeptin as a marker of circulating volume status. Copeptin is the COOH-terminal portion of the preprovasopressin peptide and is cosecreted into the blood by the neurohypophysis in an equimolar amount with vasopressin. The main stimuli for the secretion of vasopressin are an increase in plasma osmolality and/or a decrease in arterial circulating volume. Plasma copeptin has been involved in a wide range of pathophysiological processes, especially in patients with diabetes, including the development and progression of diabetic kidney disease and cardiovascular morbidity and mortality (4–9).

In line with this hypothesis, in the present investigation, we assessed the relationship between copeptin, a surrogate of vasopressin and therefore hydration status and LEA in people with diabetes. Specifically, in this analysis, we assessed the association between plasma copeptin at baseline with the risk of subsequent LEA in independent cohorts with type 1 and type 2 diabetes.

RESEARCH DESIGN AND METHODS

Study Population

Cohorts With Type 1 Diabetes

Génétique de la Néphropathie Diabétique (GENEDIAB) and Genesis France-Belgium (GENESIS) are two multicenter binational cohorts of people with long-standing type 1 diabetes designed to study the vascular complications of diabetes. GENEDIAB participants were 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 (10). GENESIS was a family-based study conducted in first-degree relatives and 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 (11). 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 were composed of participants who attended outpatient clinics at least once during the follow-up period. Median duration of follow-up was 10.2 years (interquartile range 2.7) and 5.0 years (1.6) for GENEDIAB and GENESIS, respectively. In the present investigation, we studied 207 GENEDIAB and 503 GENESIS participants for whom plasma copeptin at baseline and LEA information during follow-up were available. Study protocols were approved by the ethics committee of the Angers University Hospital (Angers, France), and all participants gave written informed consent.

Cohorts With Type 2 Diabetes

DIABHYCAR was a multinational, multicentric clinical trial conducted in people with type 2 diabetes selected on the basis of persistent microalbuminuria (urinary albumin concentration [UAC] 20–200 mg/L) or macroalbuminuria (UAC >200 mg/L) without renal failure (plasma creatinine <150 μmol/L) at baseline. The trial tested the effect of a low dose of ramipril, an ACE inhibitor, on the incidence of cardiovascular and/or renal events. The median duration of follow-up was 5 years. Results were negative regarding the drug effect and have previously were published (12). SURDIAGENE is an ongoing prospective monocentric study aiming to identify the genetic and environmental determinants of vascular complications in type 2 diabetes (13). Patients have been recruited and followed regularly since 2002 at the Diabetes Department of the University Hospital of Poitiers, France. Living status and cardiovascular and kidney end points were determined from patients' hospital records and interviews with general practitioners and recorded every other year since 2007. Median duration of follow-up was 7 years. A detailed description of study population, outcome criteria, and adjudication procedure was previously published for both cohorts (12). In the present investigation, we studied 3,101 and 1,452 participants with type 2 diabetes from the DIABHYCAR and SURDIAGENE cohorts, respectively, for whom plasma copeptin at baseline and LEA information during follow-up were available. Participants from both cohorts provided written informed consent, and study protocols were approved by the ethics committee of Angers University Hospital (DIABHYCAR) and the Comités de Protection des Personnes (CPP) Ouest III, Poitiers, France (SURDIAGENE).

Definition of Clinical Parameters and Outcomes

In both type 1 and type 2 diabetes cohorts, an ad hoc event committee reviewed the case record of each patient to validate the baseline data and, later, the incidence of outcomes during follow-up (10,11). Estimated glomerular filtration rate (eGFR) was calculated using the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) study equation for serum creatinine (14). Microalbuminuria was defined as UAC 30–300 mg/24 h, 20–200 μg/min, or 20–200 mg/L and macroalbuminuria as UAC >300 mg/24 h, >200 μg/min, or >200 mg/L. The primary outcome was the first occurrence of LEA during follow-up, as defined as a nontraumatic amputation at or above the metatarsophalangeal joint. The requirement of lower-extremity revascularization (angioplasty or bypass surgery) during follow-up was considered a secondary outcome for sensitivity analyses (data available only in the cohorts with type 2 diabetes). History of diabetic foot ulcer at baseline and incident diabetic foot ulcer during follow-up was not reported in any of the cohorts.

Laboratory Procedures

Copeptin concentration was measured in fasting plasma–EDTA samples, collected at baseline and kept frozen at −80°C. Copeptin measurements were performed by Thermo Fisher Scientific using their automated immunoluminometric assay (ultrasensitive Copeptin proAVP; Thermo Fisher Scientific, Hennigsdorf, Germany) (15). The limit of detection was 0.9 pmol/L. Intra-assay coefficient of variation reported by the manufacturer was <15% and <8% for concentration ranges of 2.0–4.0 pmol/L and 4.0–15.0 pmol/L, respectively. Interassay coefficient of variation was <18% and <10%, respectively, for the lower and higher copeptin concentration range.

Computations and Statistical Analyses

Results are expressed as mean ± SD, except copeptin, AER, and triglycerides, which are expressed as median (interquartile range). Differences between groups were assessed by Student *t* test, Wilcoxon test, and Fischer exact test. Kaplan-Meier curves were used to plot

the incidence of the outcome over time. The copeptin concentrations were log transformed to adjust for positive skewness. Difference of incidence between groups was assessed by log-rank tests. Cox proportional hazards survival regression analyses were used to examine the effect of plasma copeptin at baseline on the outcomes during follow-up and to evaluate the independence of this association from other relevant covariates. Hazard ratios (HRs) with 95% CICIs were computed in these analyses for 1 SD of log[copeptin], and two regression models were tested. Model 1 included as independent covariates cohort membership (see below), sex, use of diuretics, and baseline parameters with $P < 0.05$ in the comparison between incident cases and participants who did not present the outcome (data from Table 1 and Supplementary Table 1), except UAC and eGFR, while model 2 also included these markers of kidney function. As death could compete with the occurrence of the primary outcome, we have also performed competing-risk regression analyses according to the Fine and Gray method (16) with death from all causes during follow-up as a competing risk (model 3). Subhazard ratios (sHR) with 95% CI were computed for 1 SD of log[copeptin]. Since we showed that risk of LEA is higher in patients using diuretics and that diuretics can modify volume homeostasis and so plasma copeptin, we also compared copeptin-related risk of LEA in subgroups of patients according to diuretic use at baseline (3). We also assessed the interaction between diuretics and copeptin in LEA risk. To increase sample size and the number of events during follow-up, and thus the statistical power of the analyses, data from GENESIS and GENEDIAB, and from SURDIAGENE and DIABHYCAR, were pooled for the analyses in cohorts with type 1 and type 2 diabetes, respectively. Cohort membership was always included as a covariate in the regression models to take into account cohort-related differences. Statistics were performed with JMP (SAS Institute, Cary, NC) and with R statistical packages. $P < 0.05$ was considered significant.

RESULTS

Copeptin and Risk of LEA in Cohorts With Type 1 Diabetes

The cumulative incidence of LEA during follow-up in GENESIS and GENEDIAB pooled study was 5.5% ($n = 39$), and its incidence rate was 0.89 per 100 person-years. Characteristics of participants at baseline according to occurrence of LEA during follow-up are shown in Table 1. Briefly, incident LEA case subjects, compared with participants not presenting the outcome, were older; had a longer duration of diabetes; had higher systolic blood pressure; had higher concentrations of copeptin, HbA_{1c}, and UAC; had lower eGFR; and were more likely to take antihypertensive drugs. Previous myocardial infarction and LEA at baseline were more frequent in incident LEA case subjects.

The incidence of LEA during follow-up by tertiles of baseline plasma copeptin was 3.9% (tertile 1 [TER1]), 3.3% (TER2), and 10.0% (TER3 [log-rank test $\chi^2 = 12.7$, $P = 0.002$]) (Fig. 1*A*). Cox proportional hazards survival regression analyses were performed with LEA as outcome and the clinical and biological parameters mentioned above plus sex, cohort membership, and use of diuretics at baseline as independent covariates. Baseline plasma copeptin was significantly and positively associated with incidence of LEA during follow-up in all regression models that were tested (Table 2). HbA_{1c} and a previous history of LEA at baseline also remained significantly and positively associated with the outcome (Supplementary Fig. 1).

The incidence of LEA was higher in users of diuretics at baseline than in nonusers (14.2% vs. 3.7%, respectively, $P < 0.0001$). Copeptin was also higher in users of diuretics than in nonusers (median 12.5 [interquartile range 25.6] vs. 4.5 [5.2] pmol/L, respectively; Wilcoxon test, $P < 0.0001$). No difference was observed in copeptin-related risk of LEA during follow-up between users and nonusers of diuretics (fully adjusted model 2): median 2.14 (interquartile range 1.06–4.78) for 1 SD of log[copeptin], $P = 0.03$ in diuretic users and 1.97 (1.10–3.58), $P = 0.02$ in nonusers. No interaction between diuretics and copeptin was observed for the association with LEA.

Copeptin and Risk of LEA in Cohorts With Type 2 Diabetes

The cumulative incidence of LEA during follow-up in DIABHYCAR and SURDIAGENE pooled study was 2.5% ($n = 115$), and its incidence rate was 0.47 per 100 person-years. Characteristics of participants at baseline according the occurrence of LEA during follow-up are shown in Table 1. Briefly, incident LEA case subjects, compared with participants not presenting the outcome, were more likely to be men; were older; had a longer duration of diabetes; had higher concentrations of copeptin, total cholesterol, and UAC; had lower eGFR and HDL cholesterol; and were more likely to be taking renin-angiotensin system blockers, diuretics, antihypertensive drugs, antiplatelet or anticoagulation drugs, lipid-lowering drugs, and insulin. Previous LEAs at baseline were more frequent in incident LEA case subjects. The incidence of LEA during follow-up by tertiles of baseline plasma copeptin was 1.1% (TER1), 2.9% (TER2), and 3.6% (TER3) (log-rank test $\chi^2 = 26.6$, $P < 0.0001$) (Fig. 1*B*). In Cox proportional hazards survival regression analyses, baseline plasma copeptin was significantly and positively associated with incidence of LEA during follow-up in all regression models that were tested (Table 2). Sex (male), UAC, and a previous history of LEA at baseline also remained significantly and positively associated with the outcome (Supplementary Fig. 1).

The incidence of LEA was higher in users of diuretics at baseline than in nonusers (4.2% vs. 1.8%, respectively; $P < 0.0001$). Copeptin was also higher in users of diuretics than in nonusers (median 7.9 [interquartile range 8.9] vs. 6.9 [6.6] pmol/L, respectively; Wilcoxon test, $P < 0.0001$). Copeptin-related risk of LEA (fully adjusted model 2) during follow-up was median 1.25 (interquartile range 0.93–1.68) for 1 SD of log[copeptin], $P = 0.14$ in users and 1.58 (1.17–2.11), $P = 0.003$ in nonusers. No interaction between diuretics and copeptin was observed in the association with LEA.

Sensitivity Analysis: Baseline Copeptin and Lower-Extremity Revascularization During Follow-up in Cohorts With Type 2 Diabetes

Lower-extremity revascularization was performed in 115 (2.5%) participants from the cohorts with type 2 diabetes. Characteristics of participants who had a revascularization compared with those who had not are shown in Supplementary Table 1. The incidence of the outcome during follow-up by tertiles of baseline plasma copeptin was 3.2% (TER1), 4.3% (TER2), and 6.1% (TER3 [log-rank test

Table 1—Characteristics of participants at baseline by the incidence of LEA during follow-up

	Cohorts with type 1 diabetes			Cohorts with type 2 diabetes		
	No LEA	Incident LEA	*P*	No LEA	Incident LEA	*P*
n (%)	671 (94.5)	39 (5.5)	—	4,438 (97.5)	115 (2.5)	—
GENESIS cohort, *n* (%)	489 (97.2)	14 (2.8)	<0.0001	—	—	—
GENEDIAB cohort, *n* (%)	182 (87.9)	25 (12.1)		—	—	—
DIABHYCAR cohort, *n* (%)	—	—	—	3,064 (98.8)	37 (1.2)	<0.0001
SURDIAGENE cohort, *n* (%)	—	—	—	1,374 (94.6)	78 (5.4)	
Male sex, *n* (%)	366 (55)	24 (62)	0.41	3,008 (68)	102 (89)	<0.0001
Age, years	43 ± 11	48 ± 11	0.006	65 ± 9	67 ± 9	0.10
Duration of diabetes, years	27 ± 9	30 ± 8	0.04	12 ± 9	15 ± 10	<0.0001
BMI, kg/m^2	24.3 ± 3.5	24.0 ± 3.7	0.58	30.0 ± 5.3	30.1 ± 4.4	0.80
Systolic BP, mmHg	133 ± 19	143 ± 19	0.003	141 ± 16	143 ± 16	0.16
Diastolic BP, mmHg	77 ± 11	78 ± 9	0.57	79 ± 10	78 ± 11	0.20
Arterial hypertension, *n* (%)	336 (50)	31 (79)	0.0004	2,847 (64)	96 (83)	<0.0001
Current tobacco smoking, *n* (%)	191 (29)	8 (22)	0.45	578 (13)	18 (16)	0.32
Previous MI, *n* (%)	30 (5)	7 (18)	0.003	381 (8.6)	14 (12.2)	0.18
Previous stroke, *n* (%)	21 (3)	3 (8)	0.13	195 (4.4)	8 (7.0)	0.17
Previous LEA, *n* (%)	34 (5)	19 (49)	<0.0001	48 (1.1)	24 (20.9)	<0.0001
Copeptin, pmol/L	4.8 [6.3]	9.6 [23.7]	0.0003	7.0 [7.1]	9.1 [12.1]	<0.0001
HbA_{1c}, %	8.5 ± 1.4	9.2 ± 2.0	0.007	7.8 ± 1.7	8.0 ± 1.8	0.38
HbA_{1c}, mmol/mol	70 ± 16	77 ± 21	0.007	62 ± 18	64 ± 20	0.38
Total cholesterol, mmol/L*	5.8 ± 1.5	5.6 ± 1.7	0.55	5.5 ± 1.2	5.2 ± 1.3	0.02
HDL cholesterol, mmol/L	NA	NA	—	1.3 ± 0.4	1.2 ± 0.4	0.0007
Triglycerides, mmol/L*	1.1 [0.9]	1.4 [1.2]	0.51	1.8 [1.3]	1.7 [1.1]	0.56
Plasma creatinine, μmol/L	84 [25]	104 [126]	0.0002	87 [29]	97 [40]	<0.0001
eGFR, mL/min/1.73 m^2	84 ± 31	61 ± 34	<0.0001	74 ± 20	65 ± 26	<0.0001
UAC, mg/L	19 [175]	105 [1,086]	0.004	60 [128]	168 [453]	<0.0001
UAC stages, *n* (%)						
Normoalbuminuria	333 (50)	8 (20.5)	0.0002	637 (14)	17 (15)	<0.0001
Microalbuminuria	142 (21)	8 (20.5)		2,801 (63)	45 (39)	
Macroalbuminuria	196 (29)	23 (59)		993 (23)	53 (46)	
Use of antiplatelet or anticoagulation drugs, *n* (%)	NA	NA	—	1,277 (29)	63 (55)	<0.0001
Use of lipid-lowering drugs, *n* (%)	55 (8)	6 (15)	0.14	1,195 (27)	15 (13)	0.0006
Use of BP-lowering drugs, *n* (%)	336 (50)	31 (79)	0.0004	2,846 (64)	95 (83)	<0.0001
Use of ACE-I or ARB, *n* (%)	279 (42)	21 (54)	0.18	1,034 (23)	65 (57)	<0.0001
Use of diuretics, *n* (%)	280 (42)	21 (54)	0.18	1,302 (29)	57 (50)	<0.0001
Use of insulin, *n* (%)	671 (100)	39 (100)	0.99	821 (19)	53 (46)	<0.0001

Data are expressed as means ± SD or median [interquartile range] unless otherwise indicated. Differences between groups are assessed by Student *t* test, Wilcoxon test, or Fischer exact test. eGFR computed by CKD-EPI formula. ACE-I, ACE inhibitor; ARB, angiotensin receptor blocker; BP, blood pressure; MI, myocardial infarction; NA, no available data. *Data from cohorts with type 1 diabetes available only for GENEDIAB participants. $P < 0.05$ was significant.

$\chi^2 = 24.4$, $P < 0.0001$]) (Supplementary Fig. 2). In Cox proportional hazards survival regression analyses, baseline plasma copeptin was significantly and positively associated with the requirement of lower-extremity revascularization during follow-up in all regression models that were tested (Table 3). Cohort (DIABHYCAR), sex (male), active tobacco smoking, total cholesterol, UAC, use of antiplatelet or anticoagulation drugs, and a previous history of LEA at baseline also remained significantly and positively associated and BMI and HDL cholesterol inversely associated with the outcome (data not shown).

Death as a Competing Risk of LEA

During follow-up, death occurred in 59 participants (8.3%) in cohorts with type 1 diabetes, including 15 incident cases of LEA, and in 991 participants (21.8%) in cohorts with type 2 diabetes, including 67 incident cases of LEA. The association between baseline copeptin and the incidence of LEA evaluated with the Cox model might be biased if many patients died before achieving the LEA end point. Consequently, we performed competing risk regression analyses to estimate sHR for risk of LEA according to baseline plasma copeptin with all cause death as a competing risk. In both cohorts with type 1 and cohorts with type 2 diabetes, copeptin remained significantly associated with LEA (Table 2) (model 3), indicating that death was not a significant competing risk.

CONCLUSIONS

In this study, we showed that high plasma copeptin at baseline is associated with

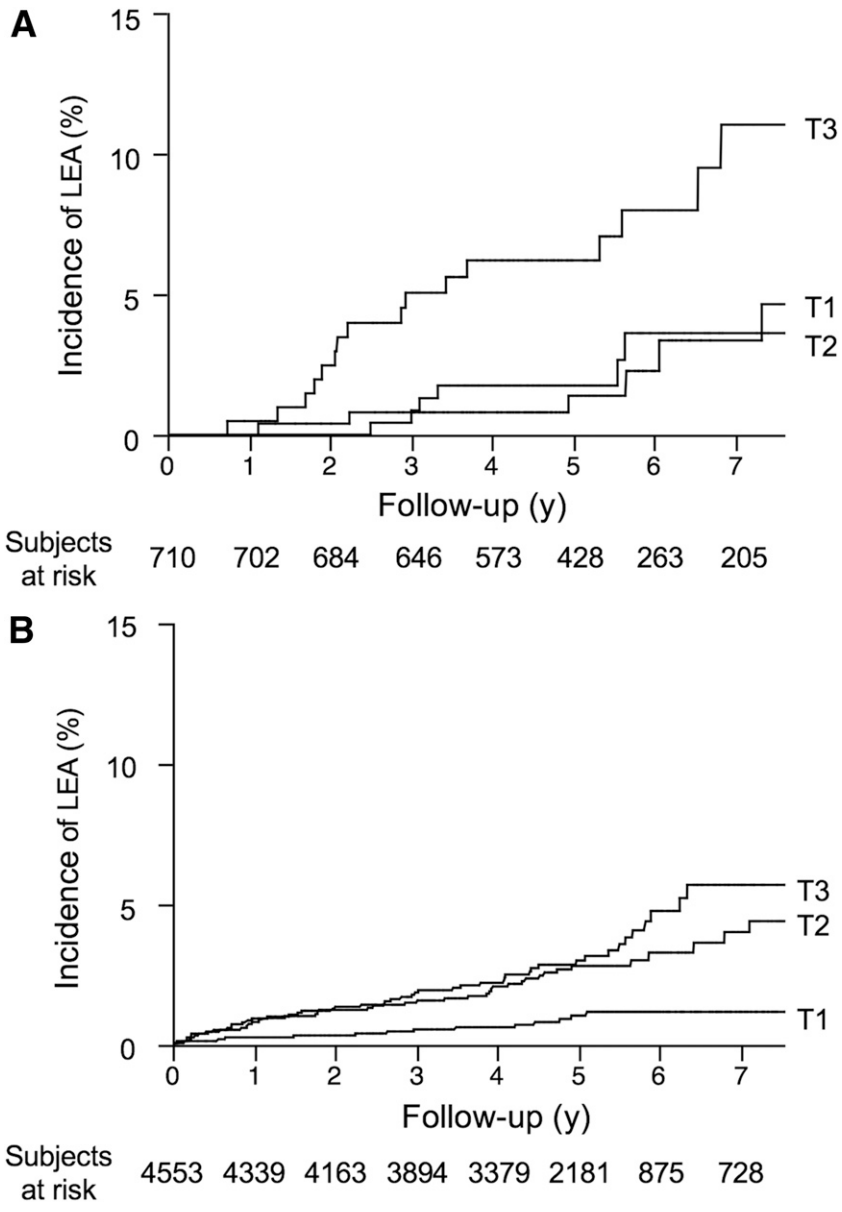

Figure 1—Kaplan-Meier curves for the cumulative incidence of LEA during follow-up by tertiles (T) of baseline plasma copeptin. *A*: Cohorts with type 1 diabetes, log-rank test $\chi^2 = 12.7$, $P = 0.002$. *B*: Cohorts with type 2 diabetes cohorts, log-rank test $\chi^2 = 26.6$, $P < 0.001$. y, years.

increased cumulated incidence of LEA over a 5- to 10-year follow-up in cohorts of people with diabetes. The associations were independent from other relevant risk factors for LEA such as dyslipidemia, arterial hypertension, the severity and duration of diabetes, markers of kidney disease, and a previous history of cardiovascular disease and LEA at baseline. Moreover, our results were consistent across cohorts of both people with type 1 diabetes and people with type 2 diabetes.

We and others have previously shown that plasma copeptin is strongly associated with a higher risk of chronic kidney disease and cardiovascular disease in people with diabetes (4–9,17). However, to our knowledge, this study is the first to investigate the association between copeptin and risk of LEA in prospective cohorts of both people with type 1 and people with type 2 diabetes.

The pathophysiological mechanisms behind the association of copeptin with LEA are likely complex. Vasopressin acts through three different G-protein–coupled receptors, which are widely distributed across tissues. Vasopressin has been involved in a wide range of pathological processes such as chronic kidney disease, diabetes and metabolic disorders, hypertension, and aging (18). Similarly, in diabetes, LEA is the consequence of several intricately linked factors such as foot deformations, diabetic neuropathy, peripheral artery disease, skin microangiopathy with impairment of skin blood flow, coagulation disorders, and infectious diseases (19).

There is a growing body of evidence that copeptin is a biomarker of atherosclerotic disease in people with diabetes (8,9). Indeed, we previously showed that copeptin was positively associated with coronary heart disease. Since PAD, a manifestation of atherosclerosis, is a major trigger of LEA in people with diabetes, the relationship between copeptin and cardiovascular disease could explain, at least in part, the increased risk of LEA associated with copeptin. Consistent with this, two previous studies reported an association between PAD and copeptin. Bar-Shalom et al. (20) showed that plasma copeptin was significantly associated with surrogate markers of PAD (ankle brachial index and toe systolic pressure index) in 302 patients with type 2 diabetes without known or suspected cardiovascular disease. Similarly, Ozkaramanli Gur et al. (21) showed a significant increase of plasma copeptin as ankle brachial index decreased in 180 participants with previous multivessel coronary artery bypass grafting surgery (half of them had diabetes). Furthermore, vasopressin is involved in vascular function and may play a pathophysiological role in thrombosis function. The vasopressin receptor V1a subtype is expressed in vascular smooth muscle cells and platelet membrane. It has been shown that vasopressin has a vasoconstrictor effect in lower-limb arteries and induces platelet aggregation via V1aR activation (22,23). On the other hand, the V2 receptor is expressed in endothelium and have been shown to increase the circulating levels of coagulation factor VIII, von Willebrand factor, and tissue plasminogen activator (24,25).

A large body of data supports a direct role for vasopressin, through the activation of V2 receptors, in the development and progression of CKD, including diabetic kidney disease (5,8,17,18,26–28). Impaired kidney function may aggravate other risk factors for LEA such as hypertension, oxidative stress, dyslipidemia, inflammation, and arterial calcification

Table 2—Baseline plasma copeptin and risk for LEA during follow-up

	Cohorts with type 1 diabetes			Cohorts with type 2 diabetes		
	HR or sHR	95% CI	*P*	HR or sHR	95% CI	*P*
Crude	1.91	1.47–2.45	<0.0001	1.79	1.52–2.09	<0.0001
Model 1	1.80	1.31–2.47	0.0003	1.53	1.28–1.81	<0.0001
Model 2	1.89	1.28–2.82	0.002	1.42	1.15–1.74	0.001
Model 3	1.82	1.28–2.61	0.001	1.64	1.38–1.94	0.001

HR and sHR computed for 1 SD of log[copeptin] by Cox proportional hazards survival regression analysis (HR) or competing risk regression analyses (sHR). Model 1 for cohorts with type 1 diabetes: adjustment for cohort membership, sex, age, duration of diabetes, systolic blood pressure, HbA_{1c}, use of diuretics and of blood pressure–lowering drugs, and previous history of myocardial infarction and LEA at baseline. Model 1 for cohorts with type 2 diabetes: adjustment for cohort membership; sex; age; duration of diabetes; arterial hypertension; HbA_{1c}; total cholesterol; HDL cholesterol; use of insulin, ACE inhibitors, angiotensin receptor blockers, diuretics, blood pressure–lowering drugs, antiplatelet or anticoagulation drugs, or lipid-lowering drugs; and previous history of LEA at baseline. Model 2 for all cohorts: model 1 adjustments plus adjustment for eGFR and UAC at baseline. Model 3 for all cohorts: death from all causes as a competing risk over model 2. $P < 0.05$ is significant.

(29). The presence and severity of CKD are associated with a dramatic increased risk of diabetic foot ulcers, PAD, and LEA in patients with diabetes (30). Therefore, the association of copeptin with LEA observed in the present investigation may be partly accounted for by the deleterious effects of vasopressin on kidney function. However, in our study, in both of the cohorts, with type 1 and with type 2 diabetes, association between plasma copeptin and LEA remained significant after adjustment for markers of kidney disease (UAC and eGFR), suggesting that this relationship was mainly independent of the effect of vasopressin on renal function.

Table 3—Baseline plasma copeptin and requirement of lower-extremity revascularization during follow-up in the cohorts with type 2 diabetes

	HR	95% CI	*P*
Crude	1.44	1.26–1.63	<0.0001
Model 1	1.30	1.13–1.49	0.0002
Model 2	1.20	1.03–1.39	0.02

HR computed by Cox proportional hazards survival regression analysis for 1 SD of log[copeptin]. Model 1: adjustment for cohort membership; sex; age; duration of diabetes; BMI; arterial hypertension; diastolic blood pressure; total cholesterol; HDL cholesterol; current tobacco smoking; use of insulin, ACE inhibitors, angiotensin receptor blockers, diuretics, blood pressure–lowering drugs, or antiplatelet or anticoagulation drugs; and previous history of myocardial infarction, stroke, or LEA at baseline. Model 2: model 1 adjustments plus adjustment for eGFR and UAC at baseline. $P < 0.05$ is significant.

The primary function of vasopressin is to adapt water excretion by the kidney to maintain body fluid balance and plasma osmolality within narrow limits (31). Dehydration and a modest elevation of plasma osmolality are major stimuli for vasopressin secretion by the neurohypophysis. Thus, plasma copeptin is a surrogate marker of blood volume, and it has been shown that volume depletion led to a significant increase of copeptin (32). The latter could further decrease peripheral perfusion in patients with PAD, which would favor decompensation and eventually LEA. There is evidence from case reports that extracellular volume depletion could lead to lower-limb or mesenteric ischemia (33,34). Use of diuretics is associated with an increased risk of hypovolemia, and in the present analysis, patients taking diuretics at baseline indeed had a higher copeptin concentration in both cohorts. This is consistent with our recent report of an association between diuretic use and a higher risk of LEA in people with type 2 diabetes (3). Furthermore, an unexplained association between sodium–glucose cotransporter 2 (SGLT2) inhibitors and amputation risk was recently observed in a cardiovascular safety trial, and in some observational studies (35–37). In light of our results, this unexpected safety signal could be seen as plausible due to the osmotic diuretic effect of this new class (38). Indeed, volume-depletion adverse events have been reported with SGLT2 inhibitors in randomized control trials (39). Moreover, plasma copeptin concentrations increase in response to 8 weeks of SGLT2 inhibition in patients with type 1 diabetes (P.B., D.Z.C., personal communication). Therefore, medications that induce a contraction of plasma volume, both traditional and novel agents with a diuretic mode of action, appear to raise circulating copeptin levels. Further work is required to better understand the interaction between circulating volume and copeptin stimulation and the potential for tissue ischemia. Specifically, since the risk of LEA has only been reported to be increased with a single agent in one of three available cardiovascular outcome trials, it will be important to determine whether there is a threshold for copeptin as a biomarker of limb ischemia either with SGLT2 inhibitors or with traditional diuretics. At a more general level, future work should determine whether LEA risk could be predicted by increased concentrations of copeptin, regardless of the underlying cause.

Our study has several limitations. First, due to the observational design, we could not ascertain a causal relationship between copeptin/vasopressin and LEA. Moreover, we used copeptin as a surrogate of vasopressin. However, plasma concentrations of both peptides correlate over a wide range of plasma and/or urine osmolalities, and the correlation seems relatively stable for eGFR >28 mL/min/1.73 m^2 (40). Only a small number of participants in the four cohorts had eGFR below this threshold, and their exclusion had no impact on the results (data not shown). Another limitation was the relatively small number of LEAs observed during follow-up, potentially reducing the statistical power to observe independent associations. Finally, since plasma copeptin was only measured at baseline, we were not able to assess the effect of copeptin changes on LEA. Despite these limitations, the study has major strengths. It includes a longitudinal study over 5–10 years for >700 participants with long-standing type 1 diabetes and ~4,500 participants with type 2 diabetes and diabetic kidney disease. Moreover, our results were consistent across cohorts and replicated with lower-limb revascularization in cohorts with type 2 diabetes.

In conclusion, this study show for the first time a positive and significant association between concentrations of plasma copeptin at baseline and risk of

LEA in observational and independent cohorts of type 1 and type 2 diabetes. Plasma copeptin could possibly help to identify patients with diabetes and high risk of LEA, due to the relationship between copeptin and hydration status. Our results raise the hypothesis that optimization of hydration through lifestyle recommendations (increased water drinking) could alleviate this risk (41). Intervention studies are required to assess the causality of this suggested association between blood volume and LEA risk and to test any preventive recommendation related to hydration.

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References

1. Hoffstad O, Mitra N, Walsh J, Margolis DJ. Diabetes, lower-extremity amputation, and death. Diabetes Care 2015;38:1852–1857
2. Armstrong DG, Boulton AJM, Bus SA. Diabetic foot ulcers and their recurrence. N Engl J Med 2017;376:2367–2375
3. Potier L, Roussel R, Velho G, et al. Lower limb events in individuals with type 2 diabetes: evidence for an increased risk associated with diuretic use. Diabetologia 2019;62:939–947
4. 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
5. 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
6. Boertien WE, Riphagen IJ, Drion I, et al. Copeptin, a surrogate marker for arginine vasopressin, is associated with declining glomerular filtration in patients with diabetes mellitus (ZODIAC-33). Diabetologia 2013;56:1680–1688
7. Wannamethee SG, Welsh P, Lennon L, Papacosta O, Whincup PH, Sattar N. Copeptin and the risk of incident stroke, CHD and cardiovascular mortality in older men with and without diabetes: the British Regional Heart Study. Diabetologia 2016;59:1904–1912
8. Velho G, El Boustany R, Lefèvre G, et al. Plasma copeptin, kidney outcomes, ischemic heart disease, and all-cause mortality in people with longstanding type 1 diabetes. Diabetes Care 2016;39:2288–2295
9. Velho G, Ragot S, El Boustany R, et al. Plasma copeptin, kidney disease, and risk for cardiovascular morbidity and mortality in two cohorts of type 2 diabetes. Cardiovasc Diabetol 2018;17:110
10. 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 Diabetique (GENEDIAB) study group. J Clin Invest 1997;99:1585–1595
11. Hadjadj S, Péan 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
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. Hadjadj S, Fumeron F, Roussel R, et al.; DIABHYCAR Study Group; DIAB2NEPHROGENE Study Group; SURDIAGENE Study Group. Prognostic value of the insertion/deletion polymorphism of the ACE gene in type 2 diabetic subjects: results from the Non-insulin-dependent Diabetes, Hypertension, Microalbuminuria or Proteinuria, Cardiovascular Events, and Ramipril (DIABHYCAR), Diabete de type 2, Nephropathie et Genetique (DIAB2NEPHROGENE), and Survie, Diabete de type 2 et Genetique (SURDIAGENE) studies. Diabetes Care 2008;31:1847–1852
14. Levey AS, Stevens LA, Schmid CH, et al.; CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration). A new equation to estimate glomerular filtration rate. Ann Intern Med 2009;150:604–612
15. 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
16. Fine JP. Regression modeling of competing crude failure probabilities. Biostatistics 2001;2:85–97
17. El Boustany R, Tasevska I, Meijer E, et al. Plasma copeptin and chronic kidney disease risk in 3 European cohorts from the general population. JCI Insight 2018;3:e121479
18. 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
19. Khan F, Elhadd TA, Greene SA, Belch JJ. Impaired skin microvascular function in children, adolescents, and young adults with type 1 diabetes. Diabetes Care 2000;23:215–220
20. Bar-Shalom D, Poulsen MK, Rasmussen LM, et al. Plasma copeptin as marker of cardiovascular disease in asymptomatic type 2 diabetes patients. Diab Vasc Dis Res 2014;11:448–450
21. Ozkaramanli Gur D, Gur O, Guzel S, et al. Inflammatory mediators across the spectrum of ankle-brachial index. J Atheroscler Thromb 2019;26:351–361
22. McClure JM, Rossi NF, Chen H, O'Leary DS, Scislo TJ. Vasopressin is a major vasoconstrictor involved in hindlimb vascular responses to stimulation of adenosine A(1) receptors in the nucleus of the solitary tract. Am J Physiol Heart Circ Physiol 2009;297:H1661–H1672

23. Liu ZW, Gu H, Zhang BF, et al. Rapidly increased vasopressin promotes acute platelet aggregation and early brain injury after experimental subarachnoid hemorrhage in a rat model. Brain Res 2016;1639:108–119
24. Kaufmann JE, Oksche A, Wollheim CB, Günther G, Rosenthal W, Vischer UM. Vasopressin-induced von Willebrand factor secretion from endothelial cells involves V2 receptors and cAMP. J Clin Invest 2000;106:107–116
25. Juul KV, Bichet DG, Nielsen S, Nørgaard JP. The physiological and pathophysiological functions of renal and extrarenal vasopressin V2 receptors. Am J Physiol Renal Physiol 2014; 306:F931–F940
26. 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
27. El Boustany R, Taveau C, Chollet C, et al. Antagonism of vasopressin V2 receptor improves albuminuria at the early stage of diabetic nephropathy in a mouse model of type 2 diabetes. J Diabetes Complications 2017;31:929–932
28. Roussel R, Velho G, Bankir L. Vasopressin and diabetic nephropathy. Curr Opin Nephrol Hypertens 2017;26:311–318
29. Gansevoort RT, Correa-Rotter R, Hemmelgarn BR, et al. Chronic kidney disease and cardiovascular risk: epidemiology, mechanisms, and prevention. Lancet 2013;382:339–352
30. Matsushita K, Ballew SH, Coresh J, et al.; Chronic Kidney Disease Prognosis Consortium. Measures of chronic kidney disease and risk of incident peripheral artery disease: a collaborative meta-analysis of individual participant data. Lancet Diabetes Endocrinol 2017;5:718–728
31. Bankir L, Bichet DG, Morgenthaler NG. Vasopressin: physiology, assessment and osmosensation. J Intern Med 2017;282:284–297
32. 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
33. Sharefkin JB, Silen W. Diuretic agents: inciting factor in nonocclusive mesenteric infarction? JAMA 1974;229:1451–1453
34. O'Rourke DA, Hede JE. Reversible leg ischaemia due to diuretics. BMJ 1978;1:1114
35. Neal B, Perkovic V, Mahaffey KW, et al.; CANVAS Program Collaborative Group. Canagliflozin and cardiovascular and renal events in type 2 diabetes. N Engl J Med 2017;377:644–657
36. Ueda P, Svanström H, Melbye M, et al. Sodium glucose cotransporter 2 inhibitors and risk of serious adverse events: nationwide register based cohort study. BMJ 2018;363: k4365
37. Chang HY, Singh S, Mansour O, Baksh S, Alexander GC. Association between sodium-glucose cotransporter 2 inhibitors and lower extremity amputation among patients with type 2 diabetes. JAMA Intern Med 2018;178:1190–1198
38. Lambers Heerspink HJ, de Zeeuw D, Wie L, Leslie B, List J. Dapagliflozin a glucose-regulating drug with diuretic properties in subjects with type 2 diabetes. Diabetes Obes Metab 2013;15: 853–862
39. Rådholm K, Wu JH, Wong MG, et al. Effects of sodium-glucose cotransporter-2 inhibitors on cardiovascular disease, death and safety outcomes in type 2 diabetes - a systematic review. Diabetes Res Clin Pract 2018;140:118–128
40. 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
41. Sontrop JM, Huang SH, Garg AX, et al. Effect of increased water intake on plasma copeptin in patients with chronic kidney disease: results from a pilot randomised controlled trial. BMJ Open 2015;5:e008634