Diet-sensitive prognostic markers for cardiovascular and renal disease
Riphagen, Ineke Jowanna

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2016

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
Riphagen, I. J. (2016). Diet-sensitive prognostic markers for cardiovascular and renal disease. Rijksuniversiteit Groningen.

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Download date: 10-01-2021
Chapter 4

Copeptin, a Surrogate Marker for Arginine Vasopressin, is Associated with Cardiovascular and All-Cause Mortality in Patients with Type 2 Diabetes (ZODIAC-31)

Ineke J. Riphagen
Wendy E. Boertien
Alaa Alkhalaf
Nanne Kleeistra
Ron T. Gansevoort
Klaas H. Groenier
Kornelis J.J. van Hateren
Joachim Struck
Gerjan Navis
Henk J.G. Bilo
Stephan J.L. Bakker

Diabetes Care 2013; 36(10): 3201-7
Abstract

Objective. Copeptin, a surrogate marker for vasopressin, has been associated with cardiovascular (CV) events and mortality in patients with type 2 diabetes complicated by end-stage renal disease or acute myocardial infarction. For stable outpatients, these associations are unknown. Our aim was to investigate whether copeptin is associated with CV and all-cause mortality in patients with type 2 diabetes treated in primary care.

Research Design and Methods. Patients with type 2 diabetes participating in the observational ZODIAC study were included. Cox regression analyses with age as time scale were used to assess the relation of baseline copeptin with CV and all-cause mortality.

Results. We included 1,195 patients (age 67 ± 12 years, 44% male). Median baseline copeptin concentration was 5.4 [IQR 3.1-9.6] pmol/L. After a median follow-up of 5.9 [3.2-10.1] years, 345 patients died (29%), with 148 CV deaths (12%). Log2 copeptin was associated with CV (HR 1.17 [95% CI 0.99-1.39], P=0.068) and all-cause mortality (1.22 [1.09-1.36], P=0.001) after adjustment for age, gender, BMI, smoking, systolic blood pressure, cholesterol-HDL ratio, duration of diabetes, HbA1c, ACEi/ARB, history of CV diseases, log serum creatinine, and log ACR. However, copeptin did not substantially improve risk prediction for CV (IDI 0.14 [-0.27-0.55]%) and all-cause mortality (IDI 0.77 [0.17-1.37]%) beyond currently used clinical markers.

Conclusions. We found copeptin to be associated with CV and all-cause mortality in patients with type 2 diabetes treated in primary care. Intervention studies should show whether the high CV risk in type 2 diabetes can be reduced by suppression of vasopressin, e.g. by reducing salt intake.
Introduction

The prevalence of type 2 diabetes and its complications are increasing worldwide (1). One of the major complications in type 2 diabetes is cardiovascular disease (CVD), which is the main cause of morbidity and mortality in this patient group (2).

Arginine vasopressin (AVP), also known as antidiuretic hormone (ADH), is a hormone exerting cardiovascular and renal effects (3). Several studies have reported that AVP levels are elevated in animals and patients with diabetes mellitus (4-7). Increased levels of AVP may have long-term deleterious effects. AVP acts through three different vasopressin receptors, namely the $V_{1a}$, $V_2$, and $V_3$ (or $V_{1b}$) receptors, which mediate vasoconstriction, stimulate water retention, and facilitate secretion of adrenocorticotropic hormone (ACTH) respectively (3). High concentrations of plasma AVP are known to preferably stimulate $V_{1a}$ receptors (8), which may contribute to the cardiovascular complications in type 2 diabetes.

Despite the pivotal role of AVP in cardiovascular disease, technical difficulties due to AVP’s small size, short plasma half-life time, and association of circulating AVP with platelets have hindered the large-scale clinical use of AVP as a biomarker (3,9,10). Vasopressin is synthesized via a polypeptide precursor which contains AVP, neurophysin II, and copeptin (3). Copeptin, or C-terminal proarginine vasopressin (CT-proAVP), is released in equimolar amounts to AVP during precursor processing and was found to be a stable and sensitive surrogate marker for AVP (11,12).

A recent study of Fenske et al. (8) showed that copeptin levels were strongly associated with cardiovascular events and mortality in patients with type 2 diabetes and end-stage renal disease (ESRD). Copeptin was also found to be associated with cardiovascular events in patients with acute myocardial infarction and type 2 diabetes (13).

However, to our knowledge, these associations have not been demonstrated for stable ambulatory patients with type 2 diabetes. This is of particular interest, since it would point to a new modifiable system for treatment and prevention of cardiovascular events and mortality in type 2 diabetes (8,14). Our primary objective was to assess the association of baseline plasma copeptin levels with cardiovascular and all-cause mortality in a population of patients with type 2 diabetes treated in primary care. Our secondary aim was to investigate the additional predictive value of copeptin for risk prediction of cardiovascular and all-cause mortality in patients with type 2 diabetes.
Research Design and Methods

Study group
The Zwolle Outpatient Diabetes Project Integrating Available Care (ZODIAC) study was initiated in 1998, in the Zwolle region of the Netherlands. The design and details of this study have been published elsewhere (15,16). In this study, general practitioners were assisted by hospital-based diabetes specialist nurses in their care of patients with type 2 diabetes. In the first year, 1,143 patients with type 2 diabetes were included in this prospective cohort study. In 2001, 546 patients with type 2 diabetes enrolled in addition, leaving a combined cohort of 1,689 patients (17). Baseline plasma copeptin values were measured in 1,257 (74%) patients. In this study, we included 1,195 (95%) patients with complete data. The ZODIAC study was approved by the local medical ethics committee, and all patients provided informed consent.

Data collection and measurements
Baseline data were collected in 1998 and 2001, consisting of a full medical history including a history of cardiovascular diseases, use of medication, and tobacco consumption. Patients were considered to have a history of CVD if they had a history of angina pectoris, myocardial infarction, percutaneous transluminal coronary angioplasty, coronary artery bypass grafting, stroke or transient ischemic attack. Laboratory and physical assessment data were collected annually and included non-fasting lipid profile, glycated hemoglobin (HbA\textsubscript{1c}), serum creatinine (SCr), urinary albumin-to-creatinine ratio (ACR), and blood pressure. SCr was measured by a kinetic colorimetric Jaffe method (Modular P Analyzer, Roche Almere, the Netherlands), ACR was measured using immunonephelometry (Behring Nephelometer; Mannheim, Germany), and blood pressure was measured twice with a Welch Allyn sphygmomanometer in the supine position after at least 5 minutes of rest. The creatinine-based CKD-EPI equation was used to estimate glomerular filtration rate (eGFR) (18).

Copeptin was measured in plasma samples collected at baseline and kept frozen at -80° Celsius until analysis in 2010. Morgenthaler et al. (12) showed that prolonged frozen storage and repeated freeze-thaw cycles have no effect on copeptin values. Plasma copeptin was measured using a sandwich immunoassay (B.R.A.H.M.S. GmbH, Hennigsdorf/Berlin, Germany) based on the assay described by Morgenthaler et al. (12). Measurement of copeptin was performed in batches. The lower detection limit was 0.4 pmol/L, the interassay coefficient of variation (CV) was <6% for copeptin concentrations >6 pmol/L, and the functional assay sensitivity (20% interassay CV) was less than 1 pmol/L.
Clinical end points
In this study, we examined the association between baseline copeptin concentration and two co-primary clinical endpoints: cardiovascular mortality and all-cause mortality. In 2009, vital status and cause of death were retrieved from records maintained by the hospital and the general practitioners for the first 1,143 patients. For the additional 546 patients, vital status and cause of death were retrieved in 2005. Causes of death were coded according to the International Classification of Diseases, 9th revision (ICD-9). Cardiovascular death was defined as death in which the principal cause of death was cardiovascular in nature, using ICD-9 codes 390-459.

Statistical analyses
Statistical analyses were performed using SPSS version 18.0 for Windows (SPSS Inc., Chicago, Illinois, USA) and STATA version 11 (StataCorp., College Station, TX: StataCorp LP). Results were expressed as mean ± standard deviation (SD) or median [interquartile range] for normally distributed and non-normally distributed data, respectively. Nominal data were presented as the number of patients (percentage). A two-sided P-value <0.05 was considered to indicate statistical significance.

For illustrative purposes, the study population was subdivided in tertiles of baseline copeptin concentration, and data were presented accordingly in order to visualize associations with copeptin. Since copeptin concentrations are significantly higher in men than in women (19), tertiles were gender-stratified. P-values for differences in copeptin tertiles were assessed using ANOVA for normally distributed continuous data, the Kruskal-Wallis test for non-normally distributed data, and the \( \chi^2 \)-test for nominal data. Multivariable linear regression analyses were used to investigate whether baseline copeptin concentrations were associated with clinical parameters. Since copeptin values were non-normally distributed, logarithmic transformation (base 2) was applied to fulfill the criteria for linear regression analyses.

We investigated whether there were differences in baseline characteristics of patients with and without copeptin measurement. P-values for differences between patients with and without copeptin measurement were assessed using the independent sample t-test for normally distributed continuous data, the Mann-Whitney U-test for non-normally distributed data, and the \( \chi^2 \)-test for nominal data.

Cox regression analyses were used to test whether there were interactions between copeptin and clinical parameters including age, gender, history of CVD, and duration of diabetes.

We used Cox regression analyses with age as the primary time scale in which we accounted for left truncation (delayed entry) to analyze the risk of cardiovascular and...
all-cause mortality during follow-up. We applied log₂ transformation of copeptin values so the hazard ratios derived from Cox regression analyses were expressed as an increase in risk per doubling of baseline copeptin values. Various models were built to adjust for possible confounders. First, the univariable association of log₂ copeptin with cardiovascular and all-cause mortality was investigated. Second, the model was adjusted for age and sex. Finally, the model was additionally adjusted for cardiovascular risk factors and medication which could potentially influence copeptin secretion (i.e., BMI, smoking, systolic blood pressure, cholesterol-HDL ratio, duration of diabetes, HbA₁c, history of CVD, use of angiotensin-converting enzyme inhibitors (ACEi)/angiotensin receptor blockers (ARB), log SCr, and log ACR). The assumption of proportional hazards for baseline predictors was investigated by inspecting the Schoenfeld residuals. As sensitivity analyses, we repeated the Cox regression analyses with follow-up time as time scale.

Furthermore, we investigated the effect of inclusion of time-dependent covariates (i.e., age, systolic blood pressure, cholesterol-HDL ratio, duration of diabetes, HbA₁c, SCr, and ACR) in Cox regression analyses.

In addition, Cox regression analyses were used to test whether an association existed between the presence or absence of a copeptin measurement and cardiovascular and all-cause mortality in the combined cohort of 1,689 patients.

Discrimination, a measure to evaluate how well a model distinguishes between patients who died and those who survived while taking follow-up time into account, was assessed using the Harrell’s C statistic (20). The additional value of copeptin for the risk prediction of cardiovascular and all-cause mortality was assessed in terms of integrated discrimination improvement (IDI) and net reclassification improvement (NRI) (21). The IDI can be interpreted as the difference between model-based probabilities for events and non-events for the models with and without copeptin. The NRI is calculated by assessing the net improvement in risk classification (<10, 10-20, 20-30, and >30%) for events and non-events separately. Calibration, a measure to evaluate how well predicted probabilities agree with observed risks, was assessed using the Grønnessby and Borgan ‘goodness-of-fit’ likelihood-ratio test (22).
Results

Patient characteristics
A total of 1,195 patients with type 2 diabetes were included in this study. Median age of the study population was 67 ± 12 years, and 524 patients (44%) were male. Median copeptin concentration was 5.4 [3.1-9.6] pmol/L. Copeptin concentrations were significantly higher in men than in women, 7.4 [4.5-11.5] versus 4.1 [2.6-7.3] pmol/L, \( P < 0.001 \). Baseline patient characteristics are presented as gender-stratified tertiles in Table 1. Variables that were significantly different between tertiles of copeptin concentrations were age, history of CVD, BMI, cholesterol-HDL ratio, duration of diabetes, HbA\(_1c\), ACR, Scr, and eGFR (Table 1). Multivariable linear regression analyses showed that gender (b = -0.57, \( P < 0.001 \)), age (b = 0.01, \( P < 0.001 \)), BMI (b = 0.03, \( P < 0.001 \)), HbA\(_1c\) (b = 0.11, \( P < 0.001 \)), systolic blood pressure (b = -0.004, \( P < 0.001 \)), log ACR (b = 0.21, \( P < 0.001 \)), and log SCr (b = 3.69, \( P < 0.001 \)) were associated with baseline copeptin concentrations.

In addition, we investigated whether there were differences in baseline patient characteristics of patients with and without copeptin measurement (Supplementary Table S1). Only baseline serum creatinine values were slightly higher in patients without copeptin measurement (98 ± 23 µmol/L) compared to patients with copeptin measurement (95 ± 22 µmol/L, \( P = 0.04 \)). We found no other significant differences between baseline characteristics of patients with and without copeptin measurement.

Copeptin as a predictor for mortality
After a follow-up period of 10 years for the patients entering the study in 1998 and 3 years for those included in 2001, 345 out of 1,195 included patients had died (29%), with 148 deaths (12%) attributable to cardiovascular causes. There was no larger number of deaths (152 males [29%] vs. 193 females [29%], \( P = 0.9 \)) or cardiovascular deaths (64 males [12%] vs. 84 females [13%], \( P = 0.9 \)) in male subjects compared to female subjects. Furthermore, there was no difference in follow-up time for male and female subjects (5.5 [3.1-10.1] years vs. 6.2 [3.2-10.1] years, respectively, \( P = 0.4 \)). The median copeptin level of patients who had died of cardiovascular causes (7.9 [4.1-12.9] pmol/L) as well as the median copeptin level of all patients who had died during follow-up (7.5 [3.7-12.6] pmol/L) were significantly higher than the median baseline copeptin concentration for survivors (4.9 [3.0-8.5] pmol/L), both \( P \)-values <0.001.

We found no significant interactions between copeptin and clinical parameters including age, gender, history of CVD, and duration of diabetes.
Table 1. Baseline patient characteristics of the study population presented as gender-stratified tertiles of copeptin concentration.

| Copeptin (pmol/L) | Tertile 1 | Tertile 2 | Tertile 3 | P-value |
|-------------------|-----------|-----------|-----------|---------|
| M                 | <5.2      | 5.2-9.9   | >9.9      |         |
| F                 | <3.1      | 3.1-5.9   | >5.9      |         |
| N                 | 175       | 174       | 175       |         |

Demographics
- Age (years)
  - M: 65 ± 10
  - F: 66 ± 12
  - N: 69 ± 12
  - P-value: <0.001
- Smoking (n, %)
  - M: 79 (19.8)
  - F: 73 (18.2)
  - N: 61 (15.5)
  - P-value: 0.3
- History of CVD (n, %)
  - M: 114 (28.5)
  - F: 129 (32.2)
  - N: 167 (42.4)
  - P-value: <0.001

Body composition
- BMI (kg/m²)
  - M: 28.5 ± 4.2
  - F: 29.3 ± 4.8
  - N: 29.9 ± 5.2
  - P-value: <0.001

Blood pressure
- Systolic blood pressure (mmHg)
  - M: 153 ± 24
  - F: 153 ± 25
  - N: 151 ± 24
  - P-value: 0.3
- Diastolic blood pressure (mmHg)
  - M: 84 ± 10
  - F: 83 ± 10
  - N: 84 ± 10
  - P-value: 0.8
- Use of ACEi/ARB (n, %)
  - M: 102 (25.5)
  - F: 102 (25.4)
  - N: 117 (29.7)
  - P-value: 0.3

Lipids
- Total cholesterol (mmol/L)
  - M: 5.4 ± 1.0
  - F: 5.6 ± 1.1
  - N: 5.6 ± 1.1
  - P-value: 0.1
- HDL cholesterol (mmol/L)
  - M: 1.2 ± 0.4
  - F: 1.2 ± 0.3
  - N: 1.2 ± 0.6
  - P-value: 0.5
- Triglycerides (mmol/L)
  - M: 2.0 [1.5-2.8]
  - F: 2.1 [1.5-3.1]
  - N: 2.1 [1.5-3.2]
  - P-value: 0.2
- Cholesterol-HDL ratio
  - M: 4.7 ± 1.5
  - F: 5.0 ± 1.5
  - N: 5.0 ± 1.4
  - P-value: 0.04
- Use of lipid lowering drugs (n, %)
  - M: 61 (15.3)
  - F: 62 (15.5)
  - N: 50 (12.7)
  - P-value: 0.5

Glucose homeostasis
- Duration diabetes (years)
  - M: 4.0 [1.8-9.0]
  - F: 4.0 [1.8-8.9]
  - N: 5.0 [2.0-10.0]
  - P-value: 0.002
- HbA₁c (%)
  - M: 6.8 [6.2-7.8]
  - F: 7.0 [6.3-8.2]
  - N: 7.3 [6.5-8.4]
  - P-value: <0.001
- HbA₁c (mmol/mol)
  - M: 51 [44-62]
  - F: 53 [45-66]
  - N: 56 [48-68]
  - P-value: <0.001

Renal function
- ACR (mg/mmol)
  - M: 1.6 [0.9-4.0]
  - F: 1.7 [0.8-6.1]
  - N: 2.7 [1.0-8.2]
  - P-value: <0.001
- Serum creatinine (µmol/L)
  - M: 90 ± 14
  - F: 91 ± 16
  - N: 103 ± 29
  - P-value: <0.001
- eGFR (mL/min/1.73m²)
  - M: 68 ± 14
  - F: 67 ± 15
  - N: 59 ± 18
  - P-value: <0.001

Abbreviations: ACEi, angiotensin-converting enzyme inhibitor; ACR, urinary albumin-to-creatinine ratio; ARB, angiotensin receptor blocker; CVD, cardiovascular diseases; eGFR, estimated glomerular filtration rate; F, females; HDL, high-density lipoprotein; M, males.
Copeptin and Mortality in Type 2 Diabetes

In univariable Cox regression analyses with age as time scale, log₂ copeptin was significantly associated with cardiovascular mortality (HR 1.55 [95% CI 1.34-1.80], P<0.001) and all-cause mortality (1.39 [1.26-1.53], P<0.001). The association of copeptin with all-cause mortality remained significant after adjustment for the different confounders (Table 2). The Schoenfeld residuals showed no substantial deviations, supporting the assumption of proportional hazards.

As sensitivity analyses, we repeated the Cox regression analyses with follow-up time as time scale (Table 3). The hazard ratios and 95% confidence intervals of the adjusted models in the sensitivity analyses were not materially different from the analyses with age as time scale. In the Cox regression models with follow-up time as time scale, the associations of copeptin with cardiovascular and all-cause mortality remained significant after adjustment for the different confounders (Table 3).

We also investigated the influence of time-dependent covariates in Cox regression analyses (Supplementary Table S2). In the fully adjusted models, log₂ copeptin was significantly associated with cardiovascular mortality (1.21 [1.03-1.42], P=0.02) and all-cause mortality (1.23 [1.10-1.36], P<0.001).

Furthermore, we tested whether an association existed between the presence or absence of a copeptin measurement and cardiovascular and all-cause mortality in the combined cohort of 1,689 patients. In univariable and multivariable Cox regression analyses we found no association of the presence or absence of a copeptin measurement with cardiovascular and all-cause mortality.

### Table 2. Associations of baseline log₂ copeptin concentrations with cardiovascular and all-cause mortality in Cox regression analyses with age as time scale.

|                | Cardiovascular Mortality | All-Cause Mortality |
|----------------|--------------------------|---------------------|
|                | HR (95% CI)              | P-value             | HR (95% CI)              | P-value             |
| Model 1        | 1.55 (1.34-1.80)         | <0.001              | 1.39 (1.26-1.53)         | <0.001              |
| Model 2        | 1.51 (1.30-1.77)         | <0.001              | 1.36 (1.22-1.51)         | <0.001              |
| Model 3        | 1.17 (0.99-1.39)         | 0.068               | 1.22 (1.09-1.36)         | 0.001               |

Abbreviations: ACEi, angiotensin-converting enzyme inhibitor; ACR, urinary albumin-to-creatinine ratio; ARB, angiotensin receptor blocker; CVD, cardiovascular diseases; HDL, high-density lipoprotein; SCr, serum creatinine.

Model 1: crude.
Model 2: adjusted for age and sex.
Model 3: adjusted for age, sex, BMI, smoking, SBP, cholesterol-HDL ratio, duration of diabetes, HbA₁c, history of CVD, use of ACEi/ARB, log SCr, and log ACR.
Chapter 4

Predictive value of copeptin

The additional value of copeptin for risk prediction of cardiovascular and all-cause mortality was assessed in terms of discrimination (Harrell’s C-statistic), NRI, and IDI (Table 4). Harrell’s C statistics for models 2 and 3 without copeptin predicting cardiovascular mortality were 0.75 (0.71-0.78) and 0.80 (0.77-0.84) respectively. Harrell’s C statistics for models 2 and 3 without copeptin predicting all-cause mortality were 0.77 (0.75-0.80) and 0.79 (0.77-0.82) respectively. Harrell’s C statistics in Table 4 show that the more confounders we adjusted for, the better the model predicted cardiovascular and all-cause mortality. The Grønnesby and Borgan P-values in Table 4 indicate that predicted probabilities correspond well with observed risks (except for model 1 predicting all-cause mortality), so the models were well calibrated. The IDI and NRI values for model 2 predicting cardiovascular mortality were 2.68% and 16.93% respectively, indicating that copeptin had additional value on top of age and gender for risk prediction of cardiovascular mortality. However, in the fully adjusted models, the IDI and NRI values appeared <2%, indicating that copeptin did not substantially improve risk prediction for cardiovascular and all-cause mortality beyond currently used clinical markers.

|                                | Cardiovascular Mortality | All-Cause Mortality |
|--------------------------------|--------------------------|---------------------|
|                                | HR (95% CI) P-value      | HR (95% CI) P-value |
| Model 1                        | 1.74 (1.49-2.04) <0.001  | 1.57 (1.42-1.74) <0.001 |
| Model 2                        | 1.54 (1.32-1.80) <0.001  | 1.37 (1.24-1.52) <0.001 |
| Model 3                        | 1.19 (1.00-1.41) 0.04    | 1.23 (1.10-1.37) <0.001 |

Abbreviations: ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; CVD, cardiovascular diseases; HDL, high-density lipoprotein; SCr, serum creatinine.

Model 1: crude.
Model 2: adjusted for age and sex.
Model 3: adjusted for age, sex, BMI, smoking, SBP, cholesterol-HDL ratio, duration of diabetes, HbA1c, history of CVD, use of ACEi/ARB, log SCr, and log ACR.

Table 3. Associations of baseline log$_2$ copeptin concentrations with cardiovascular and all-cause mortality in Cox regression analyses with follow-up time as time scale.
Table 4. Additional value of baseline log₂ copeptin concentrations in risk prediction compared with established cardiovascular risk markers.

|                      | Harrell’s C (95% CI) | IDI (%) (95% CI) | NRI (%) (95% CI) | Grønnessby and Borgen |
|----------------------|----------------------|------------------|------------------|-----------------------|
| **CV Mortality**     |                      |                  |                  |                       |
| Model 1              | 0.63 (0.59, 0.68)    | NA               | NA               | 0.10                  |
| Model 2              | 0.76 (0.72, 0.80)    | 2.68 (1.43, 3.93)| 16.93 (6.53, 27.33)| 0.98                  |
| Model 3              | 0.81 (0.77, 0.84)    | 0.14 (-0.27, 0.55)| 1.82 (-4.11, 7.76)| 0.15                  |
| **All-Cause Mortality** |                     |                  |                  |                       |
| Model 1              | 0.63 (0.59, 0.66)    | NA               | NA               | 0.01                  |
| Model 2              | 0.78 (0.75, 0.80)    | 2.47 (1.45, 3.50)| 2.14 (-2.99, 7.27)| 0.84                  |
| Model 3              | 0.80 (0.77, 0.82)    | 0.77 (0.17, 1.37)| 0.55 (-3.36, 4.46)| 0.53                  |

Abbreviations: ACEi, angiotensin-converting enzyme inhibitor; ACR, urinary albumin-to-creatinine ratio; ARB, angiotensin receptor blocker; CVD, cardiovascular diseases; HDL, high-density lipoprotein; NA, not applicable; SCr, serum creatinine.

Model 1: crude.
Model 2: adjusted for age and sex.
Model 3: adjusted for age, sex, BMI, smoking, SBP, cholesterol-HDL ratio, duration of diabetes, HbA₁c, history of CVD, use of ACEi/ARB, log SCr, and log ACR.

Discussion

In this prospective cohort of 1,195 patients with type 2 diabetes treated in primary care, we found copeptin to be associated with cardiovascular and all-cause mortality. After adjustment for established cardiovascular risk factors, we observed a trend between copeptin and cardiovascular mortality, while the association of baseline plasma copeptin with all-cause mortality remained significant. Our findings are of particular interest since the AVP system is a potentially modifiable system through pharmacological and non-pharmacological interventions and could provide a possible target for treatment and prevention of cardiovascular events and mortality in type 2 diabetes.

Several studies have reported that plasma vasopressin levels are elevated in animals and patients with diabetes mellitus (4-7). Vasopressin promotes water reabsorption through stimulation of V₂ receptors, and it is suggested that increased levels of vasopressin limit glucose-induced water loss in patients with diabetes (23).
However, increased levels of vasopressin may have long-term deleterious renal and cardiovascular effects. In experimental animal studies, as well as in humans, it is shown that vasopressin contributes to hyperfiltration, albuminuria, and renal hypertrophy in diabetes mellitus (24,25). This notion is supported by the renal protective effects of vasopressin inhibition by drinking water or chronic treatment with a V2 receptor antagonist in rats with renal failure (26,27) and diabetes (28).

High concentrations of plasma AVP are known to preferably stimulate V1a receptors (8), that results in (coronary) vasoconstriction (29), which increases afterload, ventricular stress, and cardiac hypertrophy (8,30,31). Several studies have reported that copeptin, a surrogate for vasopressin, is associated with cardiovascular events and mortality in patients with cardiovascular diseases (i.e., acute myocardial infarction, heart failure, and stroke) (3). A recent study of Fenske et al. (19) showed that copeptin is associated with cardiovascular events, sudden death, and all-cause mortality in patients with type 2 diabetes and ESRD.

Stimulation of V3 (V1b) receptors through AVP results in the release of ACTH, which stimulates cortisol release from the adrenal gland (3). AVP-induced ACTH release, in contrast to corticotropin-releasing hormone (CRH) induced ACTH release, is reported to be less sensitive to feedback inhibition by glucocorticoids than that of CRH (32), which might worsen multiple aspects of the metabolic syndrome.

Median copeptin concentration of this study group was 5.4 pmol/L [range: 0.9-85.7 pmol/L], which is higher than the median copeptin level of 4.2 pmol/L [range: 1-13.8 pmol/L] measured in healthy subjects (33). In line with previous studies, we found baseline plasma copeptin to be associated with renal function and albuminuria (34,35).

In addition, we found that baseline copeptin concentration was associated with cardiovascular and all-cause mortality. However, we found no differences in the number of deaths, cardiovascular deaths or follow-up time between male and female subjects. This lack of difference is consistent with the literature on cardiovascular risk in diabetes, which indicates that women with diabetes have a higher relative risk for cardiovascular events compared to men with diabetes (36,37). Furthermore, copeptin values are consistently shown to be higher in males than in females, even in healthy subjects (12,19). Therefore, risk categories based on copeptin level or reference values for copeptin concentrations should be gender-specific.

During the past years, several studies have shown that copeptin measurement has diagnostic and prognostic value in patients with acute cardiovascular diseases (3). Since copeptin was found to be associated with cardiovascular and all-cause mortality in patients with type 2 diabetes, we investigated whether copeptin had additional value for risk prediction of cardiovascular and all-cause mortality. In this study
population, copeptin did not substantially improve risk prediction for cardiovascular and all-cause mortality beyond currently used clinical markers. However, copeptin was found to be associated with several cardiovascular risk factors (i.e., BMI, HbA1c, systolic blood pressure, SCr, and ACR), and copeptin substantially improved risk prediction for cardiovascular mortality beyond age and gender. Thus, copeptin might be a unified marker for these known causes of CVD and might be useful to identify patients who would benefit for intensification of therapy (38).

We acknowledge that this study has several limitations. First, given the observational nature of this study, it is impossible to draw a definite conclusion about the causality of the association of copeptin with cardiovascular and all-cause mortality. Second, selection bias may have occurred because patients whose copeptin had not been measured were excluded from statistical analysis. However, in additional Cox regression analyses, we found no significant association of the presence or absence of a copeptin measurement with cardiovascular and all-cause mortality. Third, blood samples were taken without restriction on food or water intake, which could have influenced plasma osmolarity and consequently copeptin concentration. Furthermore, measured plasma osmolarity, data required to calculate plasma osmolarity, and data on the use of diuretics were not available in this study group, which is a limitation of the present study since plasma osmolality and plasma volume are determinants of AVP secretion. In addition, no data on plasma albumin levels and total plasma protein were available in the present study. It could have been interesting to include these measures since plasma albumin and protein might influence plasma osmolality and subsequently levels of AVP. Finally, the number of cardiovascular deaths in this study population is relatively small, which limits the number of covariates used in Cox regression analyses. It has been suggested that for each variable included in the model at least 10 events are required (20). With inclusion of 13 variables in the final Cox regression models, we are approaching the maximum number of variables allowed by the number of 148 cardiovascular deaths.

Strengths of this study are that this is the first study to investigate the association of copeptin with cardiovascular and all-cause mortality in patients with type 2 diabetes. In addition, this study included a relatively large observational cohort of patients with type 2 diabetes with a relatively long follow-up period (i.e., 10 years) and a reasonable number of events (i.e., all-cause mortality), therefore we could prospectively investigate the association of baseline plasma copeptin levels with cardiovascular and all-cause mortality.

In conclusion, in this cohort of patients with type 2 diabetes, plasma copeptin, a surrogate marker for vasopressin, was associated with cardiovascular and all-cause
mortality. These findings suggest that vasopressin may play a role in cardiovascular complications of type 2 diabetes and that interventions towards lowering vasopressin e.g. by limiting sodium intake, improving glycemic control, and improving/preventing nephropathy-associated albuminuria may be beneficial for the prevention of cardiovascular complications in type 2 diabetes. Water supplementation in patients without low serum albumin, edema, and not at risk for hyponatremia, would also be a possibility to lower AVP secretion. Furthermore, copeptin might be a unified marker for known causes of cardiovascular diseases and might be useful to identify patients who would benefit for intensification of therapy (38).

Acknowledgements

Sources of Funding: JR and SJLB received support from the Netherlands Heart Foundation, Dutch Diabetes Research Foundation, and Dutch Kidney Foundation, together participating in the framework of the Center for Translational Molecular Medicine (www.ctmm.nl), project PREDICCT (grant 01C-104). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.
Copeptin and Mortality in Type 2 Diabetes

References

1. Lam DW, LeRoith D. The worldwide diabetes epidemic. *Curr Opin Endocrinol Diabetes Obes* 2012; 19(2): 93-6.
2. American Diabetes Association. Standards of medical care in diabetes-2011. *Diabetes Care* 2011; 34 Suppl 1: S11-61.
3. Morgenthaler NG. Copeptin: a biomarker of cardiovascular and renal function. *Congest Heart Fail* 2010; 16 Suppl 1: S37-44.
4. Bankir L, Bardoux P, Ahloulay M. Vasopressin and diabetes mellitus. *Nephron* 2001; 87(1): 8-18.
5. Zerbe RL, Vinicor F, Robertson GL. Plasma vasopressin in uncontrolled diabetes mellitus. *Diabetes* 1979; 28(5): 503-8.
6. Kamoi K, Ishibashi M, Yamaji T. Thirst and plasma levels of vasopressin, angiotensin II and atrial natriuretic peptide in patients with non-insulin-dependent diabetes mellitus. *Diabetes Res Clin Pract* 1991; 11(3): 195-202.
7. Iwasaki Y, Kondo K, Murase T, Hasegawa H, Oiso Y. Osmoregulation of plasma vasopressin in diabetes mellitus with sustained hyperglycemia. *J Neuroendocrinol* 1996; 8(10): 755-60.
8. Fenske W, Wanner C, Alloio B, Drechsler C, Blouin K, Lilenthal J, et al. Copeptin levels associate with cardiovascular events in patients with ESRD and type 2 diabetes mellitus. *J Am Soc Nephrol* 2011; 22(4): 782-90.
9. Voors AA, von Haehling S, Anker SD, Hillege HL, Struck J, Hartmann O, et al. C-terminal provasopressin (copeptin) is a strong prognostic marker in patients with heart failure after an acute myocardial infarction: results from the OPTIMAAL study. *Eur Heart J* 2009; 30(10): 1187-94.
10. Preibisz JJ, Sealey JE, Laragh JH, Cody RJ, Weksler BB. Plasma and platelet vasopressin in essential hypertension and congestive heart failure. *Hypertension* 1983; 5(2 Pt 2): I129-38.
11. Bolignano D, Zoccali C. Vasopressin beyond water: implications for renal diseases. *Curr Opin Nephrol Hypertens* 2010; 19(5): 499-504.
12. 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(1): 112-9.
13. Mellbin LG, Ryden L, Brismar K, Morgenthaler NG, Ohvrik J, Catrina SB. Copeptin, IGFBP-1, and cardiovascular prognosis in patients with type 2 diabetes and acute myocardial infarction: a report from the DIGAMI 2 trial. *Diabetes Care* 2010; 33(7): 1604-6.
14. Enhorning S, Bankir L, Bouby N, Struck J, Hedblad B, Persson M, 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)* 2012; .
15. Ubink-Veltmaat LJ, Blio HJ, Groenier KH, Rischen RO, Meyboom-de Jong B. Shared care with task delegation to nurses for type 2 diabetes: prospective observational study. *Neth J Med* 2005; 63(3): 103-10.
16. Drion I, Kleefstra N, Landman GW, Alikhalaf A, Struck J, Groenier KH, et al. Plasma COOH-Terminal Proendothelin-1: A marker of fatal cardiovascular events, all-cause mortality, and new-onset albuminuria in type 2 diabetes? (ZODIAC-29). *Diabetes Care* 2012; .
17. Lutgerts HL, Gerrits EG, Sluiter WJ, Ubink-Veltmaat LJ, Landman GW, Links TP, et al. Life expectancy in a large cohort of type 2 diabetes patients treated in primary care (ZODIAC-10). *PLoS One* 2009; 4(8): e6817.
18. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, 3rd, Feldman HI, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med* 2009; 150(9): 604-12.
19. Fenske W, Stork S, Blechschmidt A, Maier SG, Morgenthaler NG, Allolio B. Copeptin in the differential diagnosis of hyponatremia. *J Clin Endocrinol Metab* 2009; 94(1): 123-9.
20. Harrell FE, Jr, Lee KL, Mark DB. Multivariable prognostic models: issues in developing models, evaluating assumptions and adequacy, and measuring and reducing errors. *Stat Med* 1996; 15(4): 361-87.
21. Steyerberg EW, Vickers AJ, Cook NR, Gerds T, Gonen M, Obuchowski N, et al. Assessing the performance of prediction models: a framework for traditional and novel measures. *Epidemiology* 2010; 21(1): 128-38.
22. May S, Hosmer DW. A simplified method of calculating an overall goodness-of-fit test for the Cox proportional hazards model. *Lifetime Data Anal* 1998, 4(2): 109-20.
23. Bankir L, Fernandes S, Bardoux P, Bouby N, Bichet DG. Vasopressin-V2 receptor stimulation reduces sodium excretion in healthy humans. *J Am Soc Nephrol* 2005; 16(7): 1920-8.
24. Bardoux P, Martin H, Ahloulay M, Schmitt F, Bouby N, Trinh-Trang-Tan MM, et al. Vasopressin contributes to hyperfiltration, albuminuria, and renal hypertrophy in diabetes mellitus: study in vasopressin-deficient Brattleboro rats. *Proc Natl Acad Sci U S A* 1999, 96(18): 10397-402.
25. Bardoux P, Bichet DG, Martin H, Gallois Y, Marre M, Arthus MF, 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(3): 497-506.
26. 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(4 Pt 2): F973-9.
27. Sugiuira T, Yamauchi A, Kitamura H, Matsuoka Y, Horio M, Imai E, et al. High water intake ameliorates tubulointerstitial injury in rats with subtotal nephrectomy: possible role of TGF-beta. *Kidney Int* 1999; 55(5): 1800-10.
28. 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(9): 1755-63.
29. Maturi MF, Martin SE, Markle D, Maxwell M, Burrell CR, Speir E, et al. Coronary vasoconstriction induced by vasopressin. Production of myocardial ischemia in dogs by constriction of nondiseased small vessels. *Circulation* 1991; 83(6): 2111-21.
30. Goldsmith SR. Vasopressin as vasopressor. *Am J Med* 1987; 82(6): 1213-9.
31. Fukuzawa J, Haneda T, Kikuchi K. Arginine vasopressin increases the rate of protein synthesis in isolated perfused adult rat heart via the V1 receptor. *Mol Cell Biochem* 1999; 195(1-2): 93-8.
32. Rababab-Diehl C, Aguilera G. Glucocorticoids increase vasopressin V1b receptor coupling to phospholipase C. *Endocrinology* 1998, 139(7): 3220-6.
33. Morgenthaler NG, Struck J, Jochberger S, Dunser MW. Copeptin: clinical use of a new biomarker. *Trends Endocrinol Metab* 2008, 19(2): 43-9.
34. 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(1): 29-36.
35. Meijer E, Bakker SJ, de Jong PE, Homan van der Heide JJ, van Son WJ, Struck J, et al. Copeptin, a surrogate marker of vasopressin, is associated with accelerated renal function decline in renal transplant recipients. *Transplantation* 2009, 88(4): 561-7.
36. Kanaya AM, Grady D, Barnett-Connor E. Explaining the sex difference in coronary heart disease mortality among patients with type 2 diabetes mellitus: a meta-analysis. *Arch Intern Med* 2002, 162(15): 1737-45.
37. Rivellese AA, Riccardi G, Vaccaro O. Cardiovascular risk in women with diabetes. *Nutr Metab Cardiovasc Dis* 2010, 20(6): 474-80.
38. Enhorning S, Struck J, Wirfalt E, Hedblad B, Morgenthaler NG, Melander O. Plasma copeptin, a unifying factor behind the metabolic syndrome. *J Clin Endocrinol Metab* 2011; 96(7): E1065-72.
### Supplementary Table S1: Baseline patient characteristics of patients with and without copeptin measurement.

| Demographics                        | Patients with copeptin measurement | Patients without copeptin measurement | P-value |
|-------------------------------------|-----------------------------------|--------------------------------------|---------|
| Male gender (n, %)                  | 558 (44.4)                        | 192 (44.4)                           | 0.9     |
| Age (years)                         | 67 ± 12                           | 67 ± 12                              | 0.9     |
| Smoking (n, %)                      | 215 (17.1)                        | 85 (19.7)                            | 0.3     |
| History of CVD (n, %)               | 436 (34.7)                        | 157 (36.3)                           | 0.5     |
| **Body composition**                |                                   |                                      |         |
| BMI (kg/m²)                         | 29.2 ± 4.8                        | 29.0 ± 4.8                           | 0.4     |
| **Blood pressure**                  |                                   |                                      |         |
| Systolic blood pressure (mmHg)      | 152 ± 24                          | 150 ± 24                             | 0.2     |
| Diastolic blood pressure (mmHg)     | 84 ± 10                           | 83 ± 10                              | 0.5     |
| Use of ACEi/ARB (n, %)              | 322 (25.6)                        | 113 (26.2)                           | 0.7     |
| **Lipids**                          |                                   |                                      |         |
| Total cholesterol (mmol/L)          | 5.6 ± 1.1                         | 5.5 ± 1.1                            | 0.2     |
| HDL cholesterol (mmol/L)            | 1.2 ± 0.4                         | 1.2 ± 0.9                            | 0.2     |
| Triglycerides (mmol/L)              | 2.1 [1.5-3.1]                     | 2.1 [1.4-3.1]                        | 0.6     |
| Cholesterol-HDL ratio               | 5.0 ± 1.5                         | 5.1 ± 1.7                            | 0.3     |
| Use of lipid lowering drugs (n, %)  | 186 (14.8)                        | 82 (19.0)                            | 0.1     |
| **Glucose homeostasis**             |                                   |                                      |         |
| Duration diabetes (years)           | 4.0 [2.0-9.0]                     | 4.0 [2.0-8.0]                        | 0.2     |
| HbA₁c (%)                           | 7.0 [6.3-8.1]                     | 6.9 [6.2-8.0]                        | 0.1     |
| HbA₁c (mmol/mol)                    | 53 [45-65]                        | 52 [44-64]                           | 0.1     |
| **Renal function**                  |                                   |                                      |         |
| ACR (mg/mmol)                       | 2.0 [0.9-7.1]                     | 2.0 [0.9-8.1]                        | 0.8     |
| Serum creatinine (µmol/L)           | 95 ± 22                           | 98 ± 23                              | 0.04    |
| eGFR (mL/min/1.73m²)                | 64 ± 16                           | 63 ± 16                              | 0.05    |

Abbreviations: ACEi, angiotensin-converting enzyme inhibitor; ACR, urinary albumin-to-creatinine ratio; ARB, angiotensin receptor blocker; CVD, cardiovascular diseases; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein.
**Supplementary Table S2.** Associations of baseline log₂ copeptin concentrations with cardiovascular and all-cause mortality in Cox regression analyses with time-dependent covariates.

|               | Cardiovascular Mortality |               | All-Cause Mortality |
|---------------|--------------------------|---------------|--------------------|
|               | HR (95% CI)              | P-value       | HR (95% CI)        | P-value |
| Model 1       | 1.73 (1.48-2.03)         | <0.001        | 1.57 (1.42-1.74)   | <0.001  |
| Model 2       | 1.60 (1.37-1.87)         | <0.001        | 1.44 (1.30-1.59)   | <0.001  |
| Model 3       | 1.21 (1.03-1.42)         | 0.02          | 1.23 (1.10-1.36)   | <0.001  |

Abbreviations: ACEi, angiotensin-converting enzyme inhibitor; ACR, urinary albumin-to-creatinine ratio; ARB, angiotensin receptor blocker; CVD, cardiovascular diseases; HDL, high-density lipoprotein; SBP, systolic blood pressure; SCr, serum creatinine.

Model 1: crude.
Model 2: adjusted for age and sex.
Model 3: adjusted for age, sex, BMI (baseline), smoking (baseline), SBP, cholesterol-HDL ratio, duration of diabetes, HbA₁c, history of CVD (baseline), use of ACEi/ARB (baseline), log SCr, and log ACR.
