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# Sex differences in the association between plasma copeptin and incident type 2 diabetes: the Prevention of Renal and Vascular Endstage Disease (PREVEND) study

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## Abstract

**Aims/hypothesis** Vasopressin plays a role in osmoregulation, glucose homeostasis and inflammation. Therefore, plasma copeptin, the stable C-terminal portion of the precursor of vasopressin, has strong potential as a biomarker for the cardiometabolic syndrome and diabetes. Previous results were contradictory, which may be explained by differences between men and women in responsiveness of the vasopressin system. The aim of this study was to evaluate the usefulness of copeptin for prediction of future type 2 diabetes in men and women separately.

**Methods** From the Prevention of Renal and Vascular Endstage Disease (PREVEND) study, 4,063 women and 3,909 men without diabetes at baseline were included. A total of 208 women and 288 men developed diabetes during a median follow-up of 7.7 years.

**Results** In multivariable-adjusted models, we observed a stronger association of copeptin with risk of future diabetes

in women (OR 1.49 [95% CI 1.24, 1.79]) than in men (OR 1.01 [95% CI 0.85, 1.19]) ( $p_{\text{interaction}} < 0.01$ ). The addition of copeptin to the Data from the Epidemiological Study on the Insulin Resistance Syndrome (DESIR) clinical model improved the discriminative value (C-statistic, +0.007,  $p = 0.02$ ) and reclassification (integrated discrimination improvement [IDI] = 0.004,  $p < 0.01$ ) in women. However, we observed no improvement in men. The additive value of copeptin in women was maintained when other independent predictors, such as glucose, high sensitivity C-reactive protein (hs-CRP) and 24 h urinary albumin excretion (UAE), were included in the model.

**Conclusions/interpretation** The association of plasma copeptin with the risk of developing diabetes was stronger in women than in men. Plasma copeptin alone, and along with existing biomarkers (glucose, hs-CRP and UAE), significantly improved the risk prediction for diabetes in women.

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**Keywords** Copeptin · Prediction · Sex · Type 2 diabetes · Vasopressin

## Abbreviations

AVP	Arginine vasopressin
DESIR	Data from the Epidemiological Study on the Insulin Resistance Syndrome
hs-CRP	High sensitivity C-reactive protein
IDI	Integrated discrimination improvement
IFG	Impaired fasting glucose
MDC	Malmö Diet and Cancer
PREVEND	Prevention of Renal and Vascular Endstage Disease
UAE	Urinary albumin excretion

## Introduction

The arginine vasopressin (AVP) stress-adaptation system has been shown to play a role in glucose homeostasis in both experimental and human studies [1, 2]. Epidemiological studies investigating the prospective association between plasma AVP levels and risk of type 2 diabetes are scarce. The main reason for this may be that reliable measurements of AVP are difficult in large collections of samples. AVP in blood is mainly bound to platelets in circulation and is unstable in isolated plasma [3, 4]. In addition, most AVP measurements have relatively limited sensitivity. Recently, an assay for copeptin, the C-terminal portion of the precursor of AVP, has been developed [5]. Copeptin is a reliable marker of AVP secretion and a surrogate for circulating AVP concentration [3, 5].

One recent study found that high baseline levels of copeptin are associated with increased risk for development of type 2 diabetes [6]. The link between the AVP stress-adaptation system and type 2 diabetes may lie in stimulatory effects of AVP on hepatic glucose production [7], effects on insulin release from the pancreas [8], stimulation of endogenous cortisol secretion [9] and adverse effects on whole-body insulin resistance [10]. Of note, there are marked differences in responsiveness of the AVP stress-adaptation system between men and women [11, 12].

We hypothesised that there could be a difference in the association of copeptin with type 2 diabetes between men and women. In a previous study in the population in which we planned to test this hypothesis, we found independent associations of high sensitivity C-reactive protein (hs-CRP) and 24 h urinary albumin excretion (UAE) with the risk of type 2 diabetes in the general population [13]. Associations of hs-CRP and UAE with the risk of type 2 diabetes have also been found in several other studies [14–17]. We aimed to test whether the association of copeptin with type 2 diabetes was independent of other covariates, including clinical variables and more established biomarkers such as glucose, hs-CRP and 24 h UAE. In addition, the present study evaluates the predictive ability of copeptin for the risk of developing type 2 diabetes in men and women separately. The predictive ability is evaluated by addition to an existing sex-specific prediction model. We then performed a comparison of the potential additive value of copeptin to glucose, hs-CRP and 24 h UAE.

## Methods

**Study population and design** The study population was obtained from the Prevention of Renal and Vascular End-stage Disease (PREVEND) study, a Dutch cohort drawn from the general population (age range between 28 and 75 years) of the city of Groningen, the Netherlands. Details on study design, recruitment, and procedures have been

published elsewhere [18]. Of 8,592 participants in the baseline cohort, we excluded 331 individuals with diabetes at baseline (self-reported physician diagnosis and screen-detected prevalent cases) and 289 with missing data on follow-up, leaving 4,063 non-diabetic women and 3,909 non-diabetic men for the post-hoc analysis. The PREVEND study was approved by the local medical ethics committee, University Medical Center Groningen, and was performed according to the principles outlined in the Declaration of Helsinki. All participants gave written informed consent.

**Clinical and biomarker measurements** The first screening took place in 1997–1998, the second in 2001–2003 and the third in 2003–2006. In each screening, the participants underwent two outpatient visits to assess medical history, anthropometry and cardiovascular and metabolic risk factors, and they collected two 24 h urine samples. Information on use of medication was completed and confirmed by using data from pharmacy registries of all community pharmacies in the city of Groningen [19]. In 89.9% of all participants, blood samples for measurement of copeptin were taken after overnight fasting. Total cholesterol and plasma glucose were measured by dry chemistry (Eastman Kodak, Rochester, NY, USA). HDL-cholesterol was measured using a homogeneous method (direct HDL, Aeroset TM System, Abbott Laboratories, Abbott Park, IL, USA). Hypertension was defined by self-reported physician diagnosis, use of antihypertensive medication, or blood pressure  $\geq 140/90$  mmHg. Triacylglycerol was measured enzymatically. hs-CRP was determined by nephelometry (BN II, Dade Behring, Marburg, Germany). UAE, given as the mean of the two 24 h urine excretions, was determined by nephelometry with a threshold of 2.3 mg/l and intra- and inter-assay coefficients of variation of less than 2.2% and less than 2.6%, respectively (Dade Behring Diagnostic, Marburg, Germany). Plasma copeptin level was measured using a new sandwich immunoassay (B.R.A.H.M.S GmbH/Thermo Fisher Scientific, Hennigsdorf/Berlin, Germany), which has been described previously [5, 20]. The lower detection limit was 0.4 pmol/l and the functional assay sensitivity (20% inter-assay coefficient of variation) was less than 1 pmol/l [5]. All the technicians were blinded to the participants' characteristics.

**Outcome definition** Incident cases of type 2 diabetes were ascertained if one or more of the following criteria were met: (1) fasting plasma glucose  $\geq 7.0$  mmol/l (126 mg/dl); (2) random sample plasma glucose  $\geq 11.1$  mmol/l (200 mg/dl); (3) self-report of a physician diagnosis; (4) use of glucose-lowering medications based on a central pharmacy registration [21]. We included cases from 3 months after the baseline screening visits (1997–1998) until January 2007.

**Statistical analyses** Continuous data were compared by using one-way ANOVA or a Kruskal–Wallis test, as applicable. A

$\chi^2$  test was used for the comparison of categorical variables to test differences across sex-specific quartiles of copeptin. Because of significant sex differences, we investigated the associations between baseline characteristics and plasma copeptin levels separately for women and men. We applied logistic regression analysis to examine the hypothesis that plasma copeptin is associated with the risk of developing type 2 diabetes in women and men. ORs with 95% CIs for type 2 diabetes were calculated according to base-2 logarithmically transformed copeptin measurements. In further analyses, these associations were tested across sex-specific quartiles of copeptin, with the lowest quartile considered as the reference. In model 1, basic adjustment was for age. In model 2, we further adjusted for alcohol use, smoking status and family history of diabetes as covariates that could confound the association between copeptin and risk of diabetes. In model 3, we further adjusted for covariates included in the metabolic syndrome, those are, waist circumference, hypertension, HDL-cholesterol, triacylglycerol and fasting glucose. In model 4, we further adjusted for hs-CRP and 24 h UAE.

We examined the added value of copeptin for the risk prediction of developing diabetes on top of the existing Data from the Epidemiological Study on the Insulin Resistance Syndrome (DESIR) clinical models. The DESIR models were chosen because they have separate prediction rules for women and men [22]. The DESIR models included data on family history of diabetes, waist circumference and hypertension in women; and data on smoking status, waist circumference and hypertension in men [22]. To evaluate the added value of copeptin, we compared the prediction of the DESIR models, as the reference, to that of the models including  $\log_2$  copeptin. Next, we added  $\log_2$  hs-CRP and  $\log_2$  UAE to the DESIR models and examined whether these two conventional cardiometabolic biomarkers could improve the risk prediction of diabetes. Thereafter, to evaluate the value of copeptin over existing biomarkers, we added  $\log_2$  copeptin along with  $\log_2$  hs-CRP and  $\log_2$  UAE to the DESIR models. Finally, we added glucose, a strong predictor for diabetes, to the DESIR models and examined whether copeptin, hs-CRP and 24 h UAE could improve prediction above the models incorporating glucose. We examined improvement of diabetes prediction in terms of discrimination and integrated discrimination improvement (IDI) [23, 24]. The discrimination performance denotes the extent to which the model distinguishes between individuals with and without incident diabetes; a value of 1 implies a perfect discrimination and a value of 0.5 implies performance no better than chance. Discrimination was examined by calculating the C-statistic with 95% CI. IDI, a continuous measure of reclassification, was calculated by subtracting the mean difference of predicted risk between the DESIR models and the models including biomarkers for those who developed diabetes from the corresponding risks for those who did not develop diabetes.

A significant  $p$  value of IDI represents an improved prediction [23, 24].

For most baseline variables, <1% were missing, whereas this was up to 8% for self-reported variables. A single imputation and predictive mean matching was applied for missing data. In the current analysis, a weighted method was performed to compensate for baseline enrichment of the PREVENT participants with high urinary albumin concentration (>10 mg/l).

Given the strong predictive value of glucose, we performed a sensitivity analysis with exclusion of individuals (women,  $n=305$ ; men,  $n=538$ ) with impaired fasting glucose (IFG) at baseline. IFG was defined by the ADA criteria of fasting glucose of 5.6–6.9 mmol/l [25]. Next, we repeated analyses after excluding those who used antihypertensive medications (women,  $n=569$ ; men,  $n=617$ ). In addition, we assessed whether the different components of the DESIR models might have affected the predictive value of copeptin. To do this, we fitted the model for women and examined the effect of adding copeptin in men.

A  $p$  value of 0.05 or less, two-sided, was considered statistically significant. All the statistical analyses were carried out using IBM SPSS Statistics 19 and R-2.13.1 for Windows (<http://cran.r-project.org/>).

## Results

**Baseline clinical characteristics** The associations between baseline clinical characteristics and plasma copeptin, stratified by sex, are summarised in Table 1. Median (interquartile range [IQR]) copeptin levels were higher in men, i.e. 6.2 (4.0–9.4) pmol/l in men and 3.6 (2.4–5.5) pmol/l in women ( $p<0.001$ ). For both men and women, across sex-specific quartiles of copeptin, a higher copeptin level was positively related to age, BMI, waist circumference, high blood pressure, fasting blood glucose and total cholesterol, but was not related to HDL-cholesterol. In addition, hs-CRP and UAE increased with higher copeptin levels. Women with higher copeptin levels were more likely to be smokers, whereas men with higher copeptin levels also had higher triacylglycerol.

**Plasma copeptin and type 2 diabetes** During median (IQR) follow-up for 7.7 (7.4–8.0) years, 208 (5.1%) women and 288 (7.4%) men developed type 2 diabetes. Table 2 depicts the association between copeptin and the risk of type 2 diabetes, calculated per doubling (per  $\log_2$ -unit increase) of copeptin levels and over sex-specific quartiles separately for women and men. In women, the crude OR (95% CI) for the risk of type 2 diabetes was 1.60 (1.37, 1.85) per doubling of copeptin levels. After adjustment for age (model 1), smoking, alcohol use, and family history of diabetes (model 2), and waist circumference, hypertension, fasting glucose, HDL-

**Table 1** Baseline clinical characteristics of participants in total and according to quartiles of plasma copeptin

Characteristic	Sex-specific quartiles					<i>p</i> value <sup>a</sup>
	Total	1	2	3	4	
<b>Women</b>						
No. of participants	4,063	1,001	1,025	1,019	1,018	–
Copeptin level (pg/ml)	3.6 (2.4–5.5)	1.8 (1.4–2.1)	2.9 (2.6–3.2)	4.4 (4.0–4.9)	7.6 (6.3–9.8)	–
Age (years)	47.7±12.2	46.7±12.2	46.8±11.8	47.8±12.2	49.6±12.3	<0.001
Family history of diabetes (%)	830 (20.4)	202 (20.2)	190 (18.5)	208 (20.4)	230 (22.6)	0.16
Current smoker (%)	1,374 (33.8)	275 (27.5)	313 (30.5)	366 (35.9)	420 (41.3)	<0.001
Ever use alcohol (%)	2,718 (67.2)	641 (64.2)	707 (69.2)	684 (67.5)	686 (67.9)	0.10
BMI (kg/m <sup>2</sup> )	25.8±4.6	25.4±4.1	25.6±4.5	26.0±4.7	26.2±5.2	<0.001
Waist circumference (cm)	82.9±12.4	81.2±11.1	82.5±12.3	83.4±12.3	84.3±13.6	<0.001
Systolic blood pressure (mmHg)	119.4±19.5	118.3±18.7	118.4±18.6	119.7±19.8	121.1±20.9	0.003
Diastolic blood pressure (mmHg)	68.7±9.0	68.3±8.9	68.5±8.8	68.7±9.2	69.4±9.0	0.03
Hypertension (%)	959 (23.6)	222 (22.2)	216 (21.1)	235 (23.1)	286 (28.1)	0.001
Glucose (mmol/l)	4.62±0.64	4.61±0.60	4.59±0.59	4.61±0.62	4.67±0.71	0.04
Total cholesterol (mmol/l)	5.60±1.14	5.56±1.15	5.51±1.12	5.61±1.15	5.71±1.15	0.001
HDL-cholesterol (mmol/l)	1.49±0.41	1.49±0.41	1.49±0.40	1.47±0.39	1.50±0.41	0.44
Triacylglycerol (mmol/l)	1.05 (0.78–1.46)	1.02 (0.76–1.43)	1.04 (0.77–1.45)	1.06 (0.78–1.48)	1.06 (0.80–1.50)	0.13
hs-CRP (mg/l)	1.31 (0.55–3.21)	1.20 (0.50–3.19)	1.26 (0.53–3.01)	1.35 (0.61–3.27)	1.46 (0.58–3.34)	0.03
UAE (mg/24 h)	10.4 (6.8–20.6)	7.3 (5.5–11.7)	8.2 (5.7–13.1)	8.3 (5.8–13.7)	9.8 (6.3–17.7)	<0.001
<b>Men</b>						
No. of participants	3,909	973	980	978	978	–
Copeptin level (pg/ml)	6.2 (4.0–9.4)	3.5 (3.0–2.3)	4.6 (5.1–5.6)	7.6 (6.9–8.5)	12.5 (10.5–15.5)	–
Age (years)	50.2±1.8	49.3±12.6	49.5±12.9	49.9±12.6	52.2±12.8	<0.001
Family history of diabetes (%)	753 (19.3)	187 (19.2)	169 (17.2)	206 (21.1)	191 (19.5)	0.20
Current smoker (%)	1,369 (35.0)	319 (32.8)	347 (35.4)	361 (36.9)	342 (35.0)	0.29
Ever use alcohol (%)	3,210 (82.5)	794 (81.8)	821 (84.3)	798 (81.9)	797 (81.9)	0.40
BMI (kg/m <sup>2</sup> )	26.2±3.6	25.8±3.4	26.1±3.5	26.4±3.6	26.5±3.8	<0.001
Waist circumference (cm)	93.6±10.9	92.6±10.4	92.7±10.9	94.0±10.8	94.9±11.4	<0.001
Systolic blood pressure (mmHg)	128.7±17.8	126.5±17.3	127.5±17.5	129.9±17.4	131.0±18.8	<0.001
Diastolic blood pressure (mmHg)	74.7±9.5	73.2±9.1	74.2±9.4	75.2±9.3	76.1±10.0	<0.001
Hypertension (%)	1,278 (32.7)	273 (28.1)	317 (32.3)	316 (32.3)	372 (38.0)	<0.001
Glucose (mmol/l)	4.86±0.66	4.82±0.66	4.80±0.61	4.86±0.65	4.95±0.70	<0.001
Total cholesterol (mmol/l)	5.68±1.10	5.60±1.04	5.64±1.10	5.66±1.09	5.81±1.17	<0.001
HDL-cholesterol (mmol/l)	1.16±0.31	1.17±0.31	1.16±.30	1.15±0.31	1.17±0.35	0.19
Triacylglycerol (mmol/l)	1.28 (0.91–1.88)	1.24 (0.89–1.75)	1.23 (0.90–1.80)	1.30 (0.93–1.90)	1.35 (0.95–2.00)	<0.001
hs-CRP (mg/l)	1.20 (2.64–0.55)	1.03 (0.47–2.28)	1.08 (0.52–2.50)	1.22 (0.58–2.74)	1.45 (0.70–3.11)	<0.001
UAE (mg/24 h)	8.3 (5.8–13.9)	9.3 (6.4–17.2)	9.7 (6.6–19.3)	10.7 (7.1–20.0)	11.9 (7.4–27.9)	<0.001

Data are given as mean ± SD or median (IQRs) for continuous variables, tested using ANOVA or the Kruskal–Wallis test, and numbers (percentage) for categorical variables, tested using the  $\chi^2$  test

<sup>a</sup> Univariate analyses were for comparison across sex-specific quartiles of plasma copeptin

cholesterol and triacylglycerol (model 3) this association (OR 1.50 [95% CI 1.25, 1.80]) remained statistically significant. In model 4, adjustment for hs-CRP and 24 h UAE did not further change the association of copeptin with type 2 diabetes (OR 1.49 [95% CI 1.24, 1.79]).

In men, crude OR (95% CI) for the risk of developing type 2 diabetes was 1.19 (1.03, 1.37) per doubling of copeptin levels.

Adjustment for the variables in model 2 did not materially change this association (OR 1.18 [95% CI 1.01, 1.37]). Further adjustments for waist circumference, hypertension, fasting glucose, HDL-cholesterol and triacylglycerol attenuated the association to non-significance ( $p=0.74$ ). Higher copeptin levels were a significantly stronger predictor of type 2 diabetes in women than in men ( $p<0.01$  for interaction) in both crude and



**Table 2** ORs (95% CI) for incident type 2 diabetes according to quartiles of plasma copeptin

Model	Sex-specific quartiles				OR (95% CI) per log <sub>2</sub> -unit increase <sup>a</sup>	p value <sup>a</sup>
	1	2	3	4		
<b>Women (n=4,063)</b>						
No. of cases (%)	31 (3.1)	42 (4.1)	59 (5.8)	76 (7.5)		
Crude analysis (95% CI)	1.00	1.33 (0.79, 2.26)	2.54 (1.58, 4.06)	3.82 (2.42, 6.03)	1.60 (1.37, 1.85)	<0.001
Model 1	1.00	1.42 (1.83, 2.41)	2.29 (1.42, 3.69)	3.24 (2.03, 5.16)	1.47 (1.26, 1.71)	<0.001
Model 2	1.00	1.47 (0.86, 2.52)	2.33 (1.43, 3.77)	3.22 (2.01, 5.15)	1.45 (1.24, 1.69)	<0.001
Model 3	1.00	1.66 (0.89, 3.11)	3.46 (1.95, 6.13)	3.57 (2.03, 6.27)	1.50 (1.25, 1.80)	<0.001
Model 4	1.00	1.66 (0.88, 3.10)	3.37 (1.90, 6.00)	3.54 (2.01, 6.24)	1.49 (1.24, 1.79)	<0.001
<b>Men (n=3,909)</b>						
No. of cases (%)	58 (6.0)	62 (6.3)	88 (9.0)	80 (8.2)		
Crude analysis (95% CI)	1.00	0.99 (0.65, 1.49)	1.30 (0.88, 1.93)	1.57 (1.07, 2.32)	1.19 (1.03, 1.37)	0.02
Model 1	1.00	1.00 (0.66, 1.52)	1.31 (0.88, 1.95)	1.46 (0.99, 2.16)	1.17 (1.01, 1.35)	0.04
Model 2	1.00	1.02 (0.67, 1.55)	1.28 (0.86, 1.91)	1.48 (0.99, 2.19)	1.18 (1.01, 1.37)	0.03
Model 3	1.00	1.18 (0.76, 1.84)	1.28 (0.83, 1.95)	1.03 (0.66, 1.59)	1.03 (0.87, 1.21)	0.74
Model 4	1.00	1.16 (0.74, 1.80)	1.25 (0.81, 1.91)	1.00 (0.63, 1.52)	1.01 (0.85, 1.19)	0.95

Model 1 is adjusted for age; model 2 is adjusted for age plus alcohol use, smoking status, and family history of diabetes; model 3 is adjusted for variables in model 3 plus waist circumference, hypertension, fasting glucose, HDL-cholesterol and triacylglycerol; model 4 is adjusted for variables in model 3 plus high sensitivity C-reactive protein and 24 h urine albumin excretion

<sup>a</sup>OR (95% CI) and p value expressed per unit increase in log<sub>2</sub>-transformed levels of plasma copeptin

multivariable-adjusted models. We also repeated the main analyses to examine the association between copeptin and the risk of diabetes in the entire sample. The age- and sex-adjusted OR (95% CI) for the risk of type 2 diabetes was 1.31 (1.18, 1.46) per doubling of copeptin levels. After multivariable adjustment, including age, sex and the other variables in model 4, the risk was attenuated to 1.21 (1.07, 1.37).

*Prognostic value of plasma copeptin* We examined the predictive value of copeptin for the risk of developing type 2 diabetes when added to the DESIR clinical models (Table 3) [22]. In women, the DESIR model, including data on family history of diabetes, waist circumference and hypertension as

predictors, showed a C-statistic of 0.822 (95% CI 0.795, 0.850). Addition of copeptin (log<sub>2</sub>) significantly improved the C-statistic (a change of +0.007; p=0.02) of the model, and led to an IDI of 0.004 (p<0.01). Addition of hs-CRP (log<sub>2</sub>) and 24 h UAE (log<sub>2</sub>) improved the C-statistic (a change of +0.009; p=0.09) and led to an IDI of 0.007 (p<0.02). After addition of copeptin along with hs-CRP and 24 h UAE, we observed a change of +0.013 for the C-statistic and an IDI of 0.010 (p=0.01). The DESIR model with the addition of glucose showed a C-statistic of 0.886 (95% CI 0.860, 0.911). Addition of copeptin significantly improved the C-statistic (a change of +0.005; p=0.01) of the DESIR model with glucose. When hs-CRP and 24 h UAE were included along with the DESIR

**Table 3** Added value of plasma copeptin above the DESIR model for the prediction risk of developing type 2 diabetes<sup>a</sup>

	Women	Men
C-statistic for the DESIR model (95% CI) <sup>a</sup>	0.822 (0.795, 0.850)	0.716 (0.686, 0.745)
C-statistic for the DESIR model plus copeptin (95% CI)	0.829 (0.803, 0.855)	0.714 (0.685, 0.744)
p value for change in C-statistic	0.02	0.40
IDI (p value)	0.004 (<0.01)	0.0005 (0.15)
C-statistic for the DESIR model plus hs-CRP and UAE (95% CI)	0.831 (0.805, 0.857)	0.729 (0.700, 0.757)
p value for change in C-statistic	0.09	0.01
IDI (p value)	0.007 (0.01)	0.006 (0.003)
C-statistic for the DESIR model plus copeptin, hs-CRP and UAE (95% CI)	0.835 (0.810, 0.860)	0.728 (0.700, 0.757)
p value for change in C-statistic	0.02	0.01
IDI (p value)	0.010 (0.01)	0.006 (0.002)

<sup>a</sup>The DESIR models include data on family history of diabetes, waist circumference and hypertension in women, and data on smoking status, waist circumference and hypertension in men. The DESIR model was considered as reference

model and glucose, we observed non-significant improvements (a change of C-statistic: +0.003;  $p=0.11$ ).

In men, the DESIR model including data on smoking status, waist circumference and hypertension as predictors showed a C-statistic of 0.716 (95% CI 0.681, 0.745), which was considerably lower than in women. The addition of copeptin ( $\log_2$ ) alone did not improve the prediction in terms of discrimination ( $p=0.40$ ) and reclassification (IDI of 0.0005;  $p=0.15$ ). Addition of hs-CRP ( $\log_2$ ) and 24 h UAE ( $\log_2$ ) significantly improved the C-statistic (a change of 0.011;  $p=0.01$ ) and led to an IDI of 0.006 ( $p=0.003$ ). The DESIR model with glucose showed a C-statistic of 0.835 (95% CI 0.811, 0.859). When hs-CRP and 24 h UAE were included along with the DESIR model with glucose, we observed borderline improvements (a change of C-statistic: +0.004;  $p=0.07$ ).

**Sensitivity analyses** First, when we excluded the individuals with IFG at baseline, the crude OR (95% CI) for the risk of diabetes was 1.93 (1.58, 2.36) per doubling of copeptin levels in women. The adjusted OR for model 4 was 1.81 (1.42, 2.30). In men, the crude and adjusted ORs were 1.21 (1.00, 1.47) and 1.03 (0.82, 1.28), respectively ( $p<0.01$  for interaction by sex in both the crude analyses and in model 4). The DESIR model combined with glucose showed a C-statistic of 0.801 (95% CI 0.756, 0.845) in women. Addition of copeptin significantly improved the C-statistic (a change of +0.016;  $p=0.05$ ) of the DESIR model combined with glucose. The DESIR model combined with glucose showed a C-statistic of 0.761 (95% CI 0.721, 0.801) in men. Addition of copeptin did not improve the C-statistic ( $p=0.63$ ).

Second, we calculated the risk of diabetes per doubling of copeptin in individuals who did not use antihypertensive medication. The crude OR and adjusted OR (95% CI) for model 4 in women were 1.75 (1.43, 2.14) and 1.65 (1.27, 2.13), respectively. The crude and adjusted ORs in men were 1.24 (1.05, 1.47) and 1.02 (0.84, 1.25), respectively.

Third, we fitted the model for women and calculated the C-statistic for predicting the risk of diabetes in men. In men, the model for prediction of diabetes risk in women, based on family history of diabetes, waist circumference and hypertension as predictors, showed a C-statistic of 0.740 (95% CI 0.711, 0.769). Addition of copeptin did not improve the C-statistic ( $p=0.94$ ) in men, which was similar to our finding when applying the DESIR model to men.

## Discussion

In this population-based cohort, we demonstrated that plasma copeptin, as a reliable surrogate marker for AVP, is of additive value in predicting future type 2 diabetes. Furthermore, we show that the association between copeptin and the risk of developing type 2 diabetes is modified by sex.

In women, the addition of copeptin to the DESIR model significantly improved the risk prediction for diabetes in terms of discrimination and reclassification. It is true that fasting glucose is a very good predictor of incident type 2 diabetes, because it is part of the diagnosis. Despite this, women in the fourth quartile for copeptin had a 3.5-times higher risk for developing type 2 diabetes compared with those in the first quartile for copeptin, when we adjusted for fasting glucose and other clinical variables. Of note, along with glucose and existing biomarkers for inflammation (hs-CRP) and renal function (24 h UAE), the addition of copeptin to the model further improved the risk prediction for diabetes. In men, we observed that the addition of copeptin did not improve the risk prediction for diabetes in terms of discrimination and reclassification. In addition, the association of copeptin with the risk of diabetes was strengthened in women when we further excluded the individuals with IFG at baseline. Thus, it is particularly important for the assessment of the value of novel biomarkers to take into account the possible effect of sex on the risk prediction of diabetes. There are several prediction models including data on demographics, anthropometric measures and lifestyle factors that have been developed to ascertain the risk of diabetes in the general population [26, 27]. In these models, sex has been incorporated as one of the most commonly used predictors for the risk of diabetes [27]. In our study, we used the DESIR clinical model, because the DESIR models were developed for men and women separately [22].

Another aspect regarding risk prediction is the clinical utility of novel biomarkers such as copeptin. A change in C-statistics is interpreted as showing whether the addition of biomarkers may improve the ability of the model to assign a higher probability of risk to cases compared with non-cases [24]. The C-statistic is considered to be one of the main commonly reported measures. However, it may be insensitive for small improvements in prediction [24, 28]. Alternatively, the IDI can be calculated as a measure of continuous reclassification. A significant IDI is interpreted as indicating that the addition of biomarkers to the model increases the difference in average predicted risk between cases and non-cases [23, 24]. It is difficult to judge whether the statistically significant improvements in the risk prediction of diabetes may be clinically relevant. In order to address this issue clinically, relevant definitions of risk strata are required so that the effect of movement of cases and non-cases between different risk strata using different predictive models can be described [24, 28]. However such clinically relevant definitions of risk strata from the DESIR models do not exist at present. Thus, further studies will need to replicate current findings in other settings and subsequently assess the clinical utility of novel biomarkers such as copeptin.

With regard to the differences in copeptin levels in men and women, the higher plasma copeptin level in men was consistently observed in our study and in previous studies

[4, 29–31]. Sex is one of the major determinants of plasma levels of copeptin. The range in copeptin levels is comparable between men and women, and the difference in absolute copeptin level is not likely to be the explanation for the difference in predictive ability for men and women. More generally, it is worth noting that most prediction models including data on common risk factors have shown a better performance in women compared with men [26]. Various known biomarkers, such as hs-CRP, insulin and endogenous sex hormone, improved the risk prediction for type 2 diabetes differently in women and in men [16, 32, 33].

We and others have shown before that higher plasma copeptin levels are positively associated with the metabolic syndrome, insulin resistance, the inflammatory marker hs-CRP and higher UAE in cross-sectional studies [4, 29, 30]. Likewise, all these conditions are known to be predictors for the risk of type 2 diabetes [13]. In an extension of these studies, two previous studies have investigated the association of copeptin with the risk of type 2 diabetes [6, 32]. The Malmö Diet and Cancer (MDC) study showed that copeptin, independently of a wide range of clinical risk factors, predicts the risk of type 2 diabetes in the general population [6]. However, the FINRISK97 study could not find an independent association [32]. The fact that we found a stronger association between copeptin and the risk of type 2 diabetes in women than in men might partly be explained by differences in population characteristics compared with other studies. For example, the MDC study from a Swedish population-based cohort included 4,472 participants with 174 incident cases [6] who were older (mean age of 58 years) and contained around 60% women. In the MDC study, including a higher number of women who had comparable copeptin levels to those in our study, a potential sex-related effect on the association of copeptin with diabetes risk was not addressed. The FINRISK97 study from a cohort of 7,827 participants with 417 incident cases included similar numbers of women and men [32]. In the FINRISK97 study, a higher but non-significant risk of type 2 diabetes per one SD increase in copeptin was found in the total and sex-stratified population [32]. In this latter study, the range of copeptin levels was smaller than in the MDC study and our study, for both women and men. Theoretically, this smaller range may also lead to overlap of copeptin levels between individuals with and without type 2 diabetes in the latter study, which limits the predictive value of copeptin above clinical risk factors [34].

The finding that the AVP system may provide promising biomarkers for the prediction of type 2 diabetes is in line with experimental data showing that the AVP system has various actions on underlying pathways involved in the pathogenesis of type 2 diabetes. AVP stimulates glycogenolysis and gluconeogenesis through the arginine vasopressin receptor 1 A (V1a) receptors in the liver [7]. In addition, AVP has been shown to induce glucagon and insulin release from the

pancreas, mediated by V1b receptors on islet cells [10]. Furthermore, AVP, via the same receptor (V1b), exerts stimulatory effects in maintaining basal secretion of ACTH and corticosterone, and in modulating hypothalamic–pituitary–adrenal axis (HPA) activity under stress conditions [9]. In another study, insulin sensitivity signalling was oppositely modulated by AVP effects via both V1a and V1b receptors in adipose tissue of mice [35].

Previous experimental and clinical data show differences between men and women in responsiveness to the vasopressin system. Both AVP V1a and V1b receptors have been shown to be more sensitive to some effects of AVP in women than in men [11, 36]. In addition, women have markedly lower AVP expression and lower AVP levels due to modulatory actions of oestrogen on the nuclear receptors in cells of the paraventricular nucleus [37]. One may assume that a lower tolerance to changes in AVP levels leads to a stronger effect in women than in men.

In conclusion, it is particularly important for the assessment of the value of novel biomarkers to take into account possible sex differences [38, 39]. We found a stronger association of plasma copeptin with the risk of type 2 diabetes in women than in men. In women, copeptin was an independent predictor for type 2 diabetes with added predictive value on top of the existing prediction model together with glucose and existing biomarkers for inflammation (hs-CRP) and renal function (UAE), whereas in men, copeptin showed no added predictive value.

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