Biomarkers and prediction models for type 2 diabetes and diabetes related outcomes

Abbasi, Ali

Chapter 8

Sex differences in the association between plasma copeptin and incident type 2 diabetes: the PREVEND study

Ali Abbasi[1,2]; Eva Corpeleijn[1]; Esther Meijer[2]; Douwe Postmus[1]; Ron T. Gansevoort[2]; Rijk O.B. Gans[2]; Joachim Struck[3]; Hans Hillege[1]; Ronald P. Stolk[1]; Gerjan Navis[2]; Stephan J.L. Bakker[2]

1 Department of Epidemiology, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands
2 Department of Internal Medicine, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands
3 Department of Research, BRAHMS AG (Part of Thermo Fisher Scientific), Hennigsdorf Germany

Diabetologia. 2012;55:1963-70

Abstract

Background 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 cardio-metabolic syndrome and diabetes. Previous results were contradictory, which may be explained by differences between men and women in responsiveness to the vasopressin system. The aim of this study was to evaluate the value of copeptin for prediction of future type 2 diabetes in men and women separately. **Methods** From the 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 future risk of diabetes in women (OR:1.49;95%CI:1.24,1.79) than in men (OR:1.01;95%CI:0.85,1.19) ($p_{interaction}<0.01$). Addition of copeptin to the DESIR (the Data from the Epidemiological Study on the Insulin Resistance Syndrome) clinical model improved the discriminative value of copeptin (C-statistic,+0.007, p=0.02) and reclassification (IDI=0.004, p<0.01) only in women. We, however, observed no improvement in men. The additive value of copeptin in □omen was maintained when other independent predictors for glucose homeostasis, renal function, and inflammation, such as glucose, hs-CRP and 24-hour urinary albumin excretion (UAE), □ere included in the model.

Conclusions 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 of diabetes in women.

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 may be that reliable measurements of AVP are difficult in large scale sample sizes. 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 can be 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 hypothesized 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 would like to test this hypothesis, we found independent associations of high sensitivity C-reactive protein (hs-CRP) and 24-hour urinary albumin excretion (UAE) with the risk of type 2 diabetes in the general population [13]. The 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 like glucose, hs-CRP and 24-hour 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 with addition to an existing sex specific prediction model. Next, we performed a comparison of the potential additive value of copeptin to glucose, hs-CRP and 24-hour UAE.

Methods

Study population and design

The study population was obtained from the Prevention of Renal and Vascular Endstage Disease (PREVEND) study, a Dutch cohort drawn from the general population (age ranged 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 men for the our 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 to 1998; the second in 2001 to 2003 and the third in 2003 to 2006. In each screening, the participants underwent two outpatient visits to assess medical history, anthropometry and cardiovascular and metabolic risk factors, and they had to collect two 24-hour 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, New York). High density lipoprotein (HDL) cholesterol was measured with a homogeneous method (direct HDL, Aeroset TM System, Abbott Laboratories, Abbott Park, Illinois). Hypertension was defined by self-reported physician diagnosis, use of antihypertensive medication, or blood pressure ≥ 140/90mmHg. 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 by a new sandwich immunoassay (B.R.A.H.M.S GmbH/Thermo Fisher Scientific, Hennigsdorf/Berlin, Germany), which was 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 ≥ 7.0 mmol/l (126 mg/dl); 2) random sample plasma glucose ≥ 11.1 mmol/l (200 mg/dl); 3) self-report of a physician diagnosis; 4) use of antidiabetic 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, when 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 analyses to examine the hypothesis that plasma copeptin is associated with the risk of developing type 2 diabetes in women and men. Odds ratios (ORs) with 95% confidence intervals (CIs) for type 2 diabetes were calculated according to base-two logarithmically transformed copeptin. In further analyses, these associations were tested across sex specific quartiles of copeptin while 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 which can be confounding of the association between copeptin and risk of diabetes. In model 3, we further adjusted for covariates included in the metabolic syndrome, i.e., waist circumference, hypertension, HDL-cholesterol, triacylglycerol and fasting glucose. In model 4, we further adjusted for hs-CRP and 24-hour UAE.

We examined the added value of copeptin for the risk prediction of developing diabetes on top of the existing DESIR clinical models. The DESIR models were chosen because it has prediction rules separately for women and men [22]. The DESIR models included data on family history of diabetes, waist circumference, 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 if these two conventional cardiometablic 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 to what extent 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 to that of 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% was 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 PREVEND 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 summarized in Table 1. Median (interquartile range [IQR]) copeptin levels were higher in men, i.e. 6.11 (3.97-9.25) pmol/l in men and 3.59 (2.39-5.47) pmol/l in women ($p<0.001$). For both men and women, across sex-specific quartiles of copeptin, higher copeptin was positively related to age, body mass index (BMI), waist circumference, high blood pressure, fasting blood glucose, and total cholesterol, but was not related to HDL-cholesterol. Also hs-CRP and UAE increased with higher copeptin levels. Women with higher copeptin were more likely to be smoker, whereas men with higher copeptin had higher triglycerides.

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 of 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-cholesterol and triglycerides (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-hour 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 triglycerides 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 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 for the risk of type 2 diabetes were 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 with the DESIR clinical models (Table 3) [22]. In women, the DESIR model, including data on family history of diabetes, waist circumference, hypertension as predictors, showed a C-statistic of 0.822 (0.795-0.850). Addition of copeptin (log_2) 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_2) and 24-hour UAE (log_2) improved the C-statistic (a change of +0.009; p=0.09) and led to IDI of 0.007 (p<0.02). After addition of copeptin along with hs-CRP and 24-hour UAE, we observed a change of +0.013 for the C-statistic and IDI of 0.010 (p=0.01). The DESIR model and glucose showed a C-statistic of 0.886 (0.860,0.911). Addition of copeptin significantly improved the C-statistic (a change of +0.005; p=0.01) of the DESIR model and glucose. When hs-CRP and 24-hour UAE were included along with the DESIR 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 (0.681,0.745), which was considerably lower than in the women. Addition of copeptin (log2) 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-hour UAE (log_2) significantly improved the C-statistic (a change of 0.011; p=0.01) and led to IDI of 0.006 (p=0.003). The DESIR model and glucose showed a C-statistic of 0.835 (0.811-0.859). When hs-CRP and 24-hour UAE were included along with the DESIR model and 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 (0.756,0.845) in women. Addition of copeptin

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

		Sex specific quartiles				p value*
Women	**Total**	**1**	**2**	**3**	**4**	
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- yr	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 - %	1374 (33.8)	275 (27.5)	313 (30.5)	366 (35.9)	420 (41.3)	<0.001
Ever alcohol use- %	2718 (67.2)	641 (64.2)	707 (69.2)	684 (67.5)	686 (67.9)	0.10
BMI- kg/m^2	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/24hour	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
Men						
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- yr	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 - %	1369 (35.0)	319 (32.8)	347 (35.4)	361 (36.9)	342 (35.0)	0.29
Ever alcohol use- %	3210 (82.5)	794 (81.8)	821 (84.3)	798 (81.9)	797 (81.9)	0.40
BMI- kg/m^2	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- %	1278 (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/24hour	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

BMI denotes body mass index, HDL high density lipoprotein, hs-CRP high sensitivity C-reactive protein and UAE urine albumin excretion.

Data were given as mean (SD) for continuous variables, tested using ANOVA or Kruskal-Wallis, and numbers (percentage) for categorical variables, tested using $\chi 2$ test.

* Univariate analyses were for comparison across sex-specific quartiles of plasma copeptin.

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

	Sex specific quartiles				OR (95% CI) per Log_2-unit increase *	p value *
	1	2	3	4		
Women (n=4,063)						
No. of cases (%)	31 (3.1)	42 (4.1)	59 (5.8)	76 (7.5)		
Crude analysis	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
Men (n=3,909)						
No. of cases (%)	58 (6.0)	62 (6.3)	88 (9.0)	80 (8.2)		
Crude analysis [b]	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

* OR (95% CI; p value) expressed per unit increase in log_2-transformed levels of plasma copeptin

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-hour urine albumin excretion.

Table 3. Added value of plasma copetin above the DESIR model for the prediction risk of developing type 2 diabetes*

	Women	Men
C,statistic for the DESIR model (95%CI) *	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 of 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 of 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 of C,statistic	0.02	0.01
IDI (p value)	0.010 (0.01)	0.006 (0.002)

DESIR denotes Data from the Epidemiological Study on the Insulin Resistance Syndrome (DESIR), IDI, integrated discrimination improvement, hs-CRP, high sensitivity C-reactive protein, and UAE, 24-hour urine albumin excretion.

* The DESIR models includes data on family history of diabetes, waist circumference, hypertension, in women, and data on smoking status, waist circumference and hypertension in men. The DESIR model was considered as reference.

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 (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 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, hypertension as predictors, showed a C-statistic of 0.740 (0.711,0.769). Addition of copeptin did not improve the C-statistic (p=0.94) in men similar to our finding for applying the DESIR model for men.

Discussion

In this population-based cohort, we demonstrated that plasma copeptin, as a reliable surrogate marker for AVP, is of additive value to predict 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, addition of copeptin to the DESIR model significantly improved the risk prediction of diabetes in terms of discrimination and reclassification. It is true that fasting glucose was a very good predictor of incident type 2 diabetes, because it is part of the diagnosis. Despite of this, women in the fourth quartile of copeptin had a 3.5-times higher risk for developing type 2 diabetes compared with those in the first quartile of copeptin when we adjusted for fasting glucose and other clinical variables. Of note, along with glucose and existing biomarkers for inflammation i.e. hs-CRP, and renal function i.e. 24-h UAE, the addition of copeptin to the model further improved the risk prediction of diabetes. In men, we observed that addition of copeptin did not improve the risk prediction of diabetes in terms of discrimination and reclassification. In addition, the association of copeptin with the risk of diabetes particularly 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 which have been developed for 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 like copeptin. The change of C-statistics is interpreted as whether

addition of biomarkers may improve the ability of model to assign a higher probability of risk to cases compared with non-cases [24]. The C-statistic is considered as one of main commonly reported measures. However, it may be insensitive for small improvements of prediction [24, 28]. Alternatively, the IDI can be calculated as a measure of continuous reclassification. A significant IDI is interpreted as that 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. To answer this question, one should first define the clinical relevant categories for the risk of diabetes and assign correct movements of cases and non-cases into risk strata [24, 28]. Currently, widely accepted cut points and the number of categories are lacking for the risk prediction of diabetes. Thus, further studies will need to replicate current findings in other settings and subsequently assess the clinical utility of novel biomarkers like copeptin.

With regard to the differences in copeptin level in men and women, the higher plasma copeptin level in men was consistently observed in our and 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 the explanation for the difference in predictive ability for men and women. More in general, it is worthy to note that most prediction models including data on common risk factors have shown a better performance in women when compared with men [26]. Various known biomarkers, like hs-CRP, insulin and endogenous sex hormone, improved the risk prediction of type 2 diabetes differently in women and in men [16, 32, 33].

We and others have shown before that higher plasma copeptin levels were positively associated with the metabolic syndrome, insulin resistance, inflammatory marker of hs-CRP and higher UAE in cross-sectional studies [4, 29, 30]. Likewise, all these conditions are known as predictors for the risk of type 2 diabetes [13]. In extension of these studies, two previous studies investigated the association of copeptin with the risk of type 2 diabetes [6, 32]. 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 of copeptin with the risk of type 2 diabetes in women than in men might partly be explained by differences in population characteristics compared to 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 numbers of women who had comparable copeptin levels to that of our study, a potential sex-related effect on the association of copeptin with the 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 of copeptin was found

in total and sex-stratified population [32]. In this latter study, the range of copeptin levels was smaller than 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 AVP system has various actions on underlying pathways involved in the pathogenesis of type 2 diabetes. AVP stimulates glycogenolysis and gluconeogenesis through the V1a receptors in the liver [7]. In addition, AVP have been shown to induce glucagon and insulin release from pancreas which is mediated via V1b receptors of islet cells [10]. Furthermore, AVP, via the same receptor (V1b), exerts stimulatory effects in maintaining basal secretion of ACTH and corticosterone, and in modulating HPA activity under stress conditions [9]. In another aspect, insulin sensitivity signalling was oppositely modulated by AVP effects via both V1a and V1b 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 another aspect, women have markedly lower AVP expression and lower AVP levels due to modulatory actions of estrogen on the nuclear receptors in cells of the paraventricular nucleus [37]. One may assume that a lower tolerance to changes in AVP levels has 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 the 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 the added predictive value on top of the existing prediction model along with glucose and existing biomarkers for inflammation (hs-CRP) and renal function (UAE), whereas in men, copeptin showed no added predictive value.

Acknowledgments

This work was supported by the Netherlands Heart Foundation, Dutch Diabetes Research Foundation and Dutch Kidney Foundation. This research was performed within the framework of CTMM, the Center for Translational Molecular Medicine (www.ctmm.nl); project PREDICCt (grant 01C-104-07). We thank Prof. dr. L.T.W. de Jong-van den Berg and dr. S.T. Visser from the Department of Social Pharmacy, Pharmacoepidemiology and Pharmacotherapy, Groningen University Institute for Drug Exploration, University Medical Center Groningen and University of Groningen, The Netherlands for providing the data on pharmacy-registered use of antidiabetic medication.

Duality of interest: Dr Struck is an employee of B.R.A.H.M.S GmbH/Thermo Fisher Scientific, a company, which holds patent rights on and manufactures the copeptin assay. The present study was not financed by B.R.A.H.M.S GmbH. No other author has anything to potential conflicts of interest relevant to this article. None of the study sponsors had a role in study design; in data collection, analysis, or interpretation; in writing the report; or in the decision to submit for publication.

References

1. Aoyagi T, Birumachi J, Hiroyama M, et al. Alteration of glucose homeostasis in V1a vasopressin receptor-deficient mice. Endocrinology 2007;148(5):2075-84.
2. Zerbe RL, Vinicor F, Robertson GL. Plasma vasopressin in uncontrolled diabetes mellitus. Diabetes 1979;28(5):503-8.
3. Struck J, Morgenthaler NG, Bergmann A. Copeptin, a stable peptide derived from the vasopressin precursor, is elevated in serum of sepsis patients. Peptides 2005;26(12):2500-4.
4. 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.
5. 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.
6. Enhorning S, Wang TJ, Nilsson PM, et al. Plasma copeptin and the risk of diabetes mellitus. Circulation 2010 121(19):2102-8.
7. Kirk CJ, Rodrigues LM, Hems DA. The influence of vasopressin and related peptides on glycogen phosphorylase activity and phosphatidylinositol metabolism in hepatocytes. Biochem J 1979;178(2):493-6.
8. Oshikawa S, Tanoue A, Koshimizu TA, Kitagawa Y, Tsujimoto G. Vasopressin stimulates insulin release from islet cells through V1b receptors: a combined pharmacological/knockout approach. Mol Pharmacol 2004;65(3):623-9.
9. Tanoue A, Ito S, Honda K, et al. The vasopressin V1b receptor critically regulates hypothalamic-pituitary-adrenal axis activity under both stress and resting conditions. J Clin Invest 2004;113(2):302-9.
10. Fujiwara Y, Hiroyama M, Sanbe A, et al. Insulin hypersensitivity in mice lacking the V1b vasopressin receptor. J Physiol 2007;584(Pt 1):235-44.
11. Stachenfeld NS, Splenser AE, Calzone WL, Taylor MP, Keefe DL. Sex differences in osmotic regulation of AVP and renal sodium handling. J Appl Physiol 2001;91(4):1893-901.
12. Simmler LD, Hysek CM, Liechti ME. Sex differences in the effects of MDMA (ecstasy) on plasma copeptin in healthy subjects. J Clin Endocrinol Metab 2011;96(9):2844-50.
13. Brantsma AH, Bakker SJ, Hillege HL, de Zeeuw D, de Jong PE, Gansevoort RT. Urinary albumin excretion and its relation with C-reactive protein and the metabolic syndrome in the prediction of type 2 diabetes. Diabetes Care 2005;28(10):2525-30.
14. Halimi JM, Bonnet F, Lange C, Balkau B, Tichet J, Marre M. Urinary albumin excretion is a risk factor for diabetes mellitus in men, independently of initial metabolic profile and development of insulin resistance. The DESIR Study. J Hypertens 2008;26(11):2198-206.
15. Thorand B, Lowel H, Schneider A, et al. C-reactive protein as a predictor for incident diabetes mellitus among middle-aged men: results from the MONICA Augsburg cohort study, 1984-1998. Arch Intern Med 2003;163(1):93-9.
16. Thorand B, Baumert J, Kolb H, et al. Sex differences in the prediction of type 2 diabetes by inflammatory markers: results from the MONICA/KORA Augsburg case-cohort study, 1984-2002. Diabetes Care 2007;30(4):854-60.
17. Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. JAMA 2001;286(3):327-34.
18. Lambers Heerspink HJ, Brantsma AH, de Zeeuw D, Bakker SJ, de Jong PE, Gansevoort RT. Albuminuria assessed from first-morning-void urine samples versus 24-hour urine collections as a predictor of cardiovascular morbidity and mortality. Am J Epidemiol 2008;168(8):897-905.
19. Monster TB, Janssen WM, de Jong PE, de Jong-van den Berg LT. Pharmacy data in epidemiological studies: an easy to obtain and reliable tool. Pharmacoepidemiol Drug Saf 2002;11(5):379-84.

20. 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.
21. Abbasi A, Corpeleijn E, Postmus D, et al. Plasma procalcitonin and risk of type 2 diabetes in the general population. Diabetologia 2011;54(9):2463-5.
22. Balkau B, Lange C, Fezeu L, et al. Predicting diabetes: clinical, biological, and genetic approaches: data from the Epidemiological Study on the Insulin Resistance Syndrome (DESIR). Diabetes Care 2008;31(10):2056-61.
23. Pencina MJ, D'Agostino RB, Sr., Steyerberg EW. Extensions of net reclassification improvement calculations to measure usefulness of new biomarkers. Stat Med 2011;30(1):11-21.
24. Cook NR, Ridker PM. Advances in measuring the effect of individual predictors of cardiovascular risk: the role of reclassification measures. Ann Intern Med 2009;150(11):795-802.
25. American Diabetes Association. Standards of medical care in diabetes--2007. Diabetes Care 2007;30 Suppl 1:S4-S41.
26. Buijsse B, Simmons RK, Griffin SJ, Schulze MB. Risk assessment tools for identifying individuals at risk of developing type 2 diabetes. Epidemiol Rev 2011;33(1):46-62.
27. Collins GS, Mallett S, Omar O, Yu LM. Developing risk prediction models for type 2 diabetes: a systematic review of methodology and reporting. BMC Med 2011;9:103.
28. McGeechan K, Macaskill P, Irwig L, Liew G, Wong TY. Assessing new biomarkers and predictive models for use in clinical practice: a clinician's guide. Arch Intern Med 2008;168(21):2304-10.
29. 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.
30. Saleem U, Khaleghi M, Morgenthaler NG, et al. Plasma carboxy-terminal provasopressin (copeptin): a novel marker of insulin resistance and metabolic syndrome. J Clin Endocrinol Metab 2009;94(7):2558-64.
31. Bhandari SS, Loke I, Davies JE, Squire IB, Struck J, Ng LL. Gender and renal function influence plasma levels of copeptin in healthy individuals. Clin Sci (Lond) 2009;116(3):257-63.
32. Salomaa V, Havulinna A, Saarela O, et al. Thirty-one novel biomarkers as predictors for clinically incident diabetes. PLoS One 2010;5(4):e10100.
33. Ding EL, Song Y, Malik VS, Liu S. Sex differences of endogenous sex hormones and risk of type 2 diabetes: a systematic review and meta-analysis. JAMA 2006;295(11):1288-99.
34. Herder C, Karakas M, Koenig W. Biomarkers for the prediction of type 2 diabetes and cardiovascular disease. Clin Pharmacol Ther 2011;90(1):52-66.
35. Nakamura K, Aoyagi T, Hiroyama M, et al. Both V(1A) and V(1B) vasopressin receptors deficiency result in impaired glucose tolerance. Eur J Pharmacol 2009;613(1-3):182-8.
36. Kajantie E, Phillips DI. The effects of sex and hormonal status on the physiological response to acute psychosocial stress. Psychoneuroendocrinology 2006;31(2):151-78.
37. Rhodes ME, Rubin RT. Functional sex differences ('sexual diergism') of central nervous system cholinergic systems, vasopressin, and hypothalamic-pituitary-adrenal axis activity in mammals: a selective review. Brain Res Brain Res Rev 1999;30(2):135-52.
38. Taking sex into account in medicine. Lancet 2011;378(9806):1826.
39. Oertelt-Prigione S, Parol R, Krohn S, Preissner R, Regitz-Zagrosek V. Analysis of sex and gender-specific research reveals a common increase in publications and marked differences between disciplines. BMC Med 2010;8:70.