ORIGINAL ARTICLE

Copeptin, a marker of vasopressin, in abdominal obesity, diabetes and microalbuminuria: the prospective Malmö Diet and Cancer Study cardiovascular cohort

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BACKGROUND: High plasma copeptin (copeptin), the C-terminal fragment of arginine vasopressin pro-hormone, has been associated with the metabolic syndrome (MetS), diabetes mellitus (DM) development and nephropathy. Here we tested whether elevated copeptin level is associated with later development of the MetS, its individual components and microalbuminuria.

METHODS: We analysed copeptin at baseline (1991–1994) in the population-based Malmö Diet and Cancer Study cardiovascular cohort and re-examined 2064 subjects 15.8 years later (mean age 72.8 years, 59% women) with oral glucose tolerance test and measurement of MetS and its individual components.

RESULTS: After age and sex adjustment, increasing quartiles of copeptin at baseline (the lowest quartile as reference) were associated with MetS (P for trend = 0.008), incident abdominal obesity (P for trend = 0.002), DM (P for trend = 0.001) and microalbuminuria (P for trend = 0.002). After additional adjustment for all the MetS components at baseline, increasing copeptin quartiles predicted incident abdominal obesity (odds ratios 1.55, 1.30 and 1.59; P for trend = 0.04), DM (odds ratios 1.18, 1.32 and 1.46; P for trend = 0.04) and microalbuminuria (odds ratios 1.05, 1.08 and 1.65; P for trend = 0.02) but not MetS (P for trend = 0.19) at the reexamination. Further, the relationship between copeptin and microalbuminuria was independent of baseline C-reactive protein, incident DM and incident hypertension.

CONCLUSION: Copeptin independently predicts DM and abdominal obesity but not the cluster of MetS. Apart from predicting DM and abdominal obesity, elevated copeptin signals increased risk of microalbuminuria. Interestingly, the association between copeptin and later microalbuminuria was independent of both prevalent and incident DM and hypertension. Our findings suggest a relationship between a dysregulated vasopressin system and cardiometabolic risk, which could have implications for risk assessment and novel preventive treatments.

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INTRODUCTION

Arginine vasopressin (AVP), which is also called antidiuretic hormone, is released from the neurohypophysis as a response to increased plasma osmolality and decreased blood volume. AVP exerts an antidiuretic effect in the kidney and a vasoconstrictive and blood platelet-aggregating effect in the vessels. In addition, animal studies have shown effects of AVP on glucose metabolism. AVP influences gluconeogenesis and glycogenolysis in the liver,^{1,2} insulin and glucagon release by the Langerhans islets of the pancreas³ and adrenocorticotrophic hormone release from the anterior hypophysis.⁴ Vasopressin is a short-lived peptide and most assays have relatively limited sensitivity. An assay has been developed to measure plasma copeptin (copeptin), the C-terminal portion of the precursor of AVP. Copeptin is considered to be a reliable and clinically useful surrogate marker for AVP.⁵

In a Swedish population-based sample, we recently found elevated copeptin to be associated with incident diabetes mellitus (DM) independently of all clinically used DM confounders including plasma glucose and insulin.⁶ Further, in the same population,

we found elevated copeptin to be cross-sectionally associated with the metabolic syndrome (MetS), obesity, hypertension, abdominal obesity and high C-reactive protein (CRP), suggesting that the AVP system is an underlying factor behind the MetS. However, we did not find any association between copeptin and triglycerides (TGs) or high-density lipoprotein (HDL) levels.⁷

Microalbuminuria, a common and early sign of target-organ damage in DM, hypertension and MetS, is associated with vascular dysfunction and is an independent potent risk factor for cardiovascular disease.^{8–10} To improve cardiovascular prognosis and reduce morbidity and mortality in individuals prone to DM development and in patients with established DM, it is thus important to identify mechanisms leading to microalbuminuria and other signs of target-organ damage. Previous studies in humans and animals suggest a role for AVP in diabetic nephropathy, microalbuminuria and renal failure,^{11–15} and recent studies show that elevated copeptin is associated with renal function decline in transplant recipients¹⁶ and cross-sectionally associated with microalbuminuria in humans.¹⁷ Further, copeptin

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is associated with cardiovascular events among patients with end-stage renal disease and type 2 DM.¹⁸

In the current study, we now aim to expand our previous findings and evaluate if elevated copeptin is related not only cross-sectionally to MetS, obesity, hypertension and abdominal obesity⁷ but also longitudinally to future development of these conditions. Further, we aim to validate our previous finding that copeptin is associated to future development of DM by sharpening the DM end point, previously ascertained through registers, by a reexamination including an oral glucose tolerance test (OGTT). Finally, to further explore the possible involvement of AVP in target-organ damage, we test whether copeptin predicts development of microalbuminuria.

MATERIALS AND METHODS

Subjects

The Malmö Diet and Cancer Study (MDC) is a population-based prospective cohort consisting of 28 449 persons surveyed in 1991–1996.¹⁹ From this cohort, 6103 persons were randomly selected to be studied for the epidemiology of carotid artery disease, and this sample is referred to as the MDC cardiovascular cohort. Fasting plasma samples were obtained in 5405 subjects in the MDC cardiovascular cohort.²⁰ Of those, complete data on covariates, including components of the MetS, potential confounders and copeptin, were available in 5131 individuals.

Baseline examination. Baseline measurements in plasma and whole blood were performed in overnight fasting samples. Analyses of fasting plasma lipids and whole-blood glucose were carried out at the time of baseline and follow-up examination at the Department of Clinical Chemistry, Skane University Hospital in Malmö, which is attached to a national standardization and quality control system. CRP was measured by a high-sensitivity assay (Tina-quant CRP; Roche Diagnostics, Basel, Switzerland). Cystatin C was measured using a particle-enhanced immuno-nephelometric assay (N Latex Cystatin C; Dade Behring, Deerfield, IL, USA). Copeptin was measured in fasting plasma samples using a commercially available assay in the chemiluminescence/coated tube format (B.R.A.H.M.S AG, Hennigsdorf, Germany) as described previously.²¹ Mid-regional atrial natriuretic peptide was measured using an immunoluminometric sandwich assay targeted against amino acids in the mid-region of the peptide (B.R.A.H.M.S AG).²² The motif for measuring cystatin C is that cystatin C is a good marker of glomerular filtration rate. Adjusting for cystatin C may thus be important in order to minimize bias related to variation of glomerular filtration rate as copeptin is largely cleared by glomerular filtration.

According to the National Cholesterol Education Program-Adult Treatment Panel III (NCEP ATP-III) criteria,²³ we classified subjects as having the MetS if they had three or more of the following characteristics: waist circumference >102 cm in men and >88 cm in women; fasting whole-blood glucose ≥ 5.4 mmol l⁻¹ (corresponding to fasting plasma glucose concentration of ≥ 6.1 mmol l⁻¹) or treatment for diabetes; HDL <1.0 mmol l⁻¹ in men and <1.3 mmol l⁻¹ in women; TGs ≥ 1.7 mmol l⁻¹; and systolic blood pressure (BP) ≥ 130 mm Hg and/or diastolic BP ≥ 85 mm Hg or use of anti-hypertensive medication (AHT). DM at baseline was defined as self-report of a physician diagnosis or use of diabetes medication or fasting whole blood glucose ≥ 6.1 mmol l⁻¹ (corresponding to fasting plasma glucose concentration of ≥ 7.0 mmol l⁻¹). Abdominal obesity was defined as waist circumference >102 cm in men and >88 cm in women. Obesity was defined as body mass index ≥ 30 kg m⁻². BP was measured using a mercury-column sphygmomanometer after 10 min of rest in the supine position. Hypertension at baseline was defined as baseline systolic BP ≥ 140 mm Hg, diastolic BP ≥ 90 mm Hg or use of AHT according to a baseline questionnaire or a 7-day menu book.

Reinvestigation and end points. Of the 5131 individuals at baseline, 2064 individuals had been reinvestigated from January 2007 to March 2010 (participation rate 70% of those invited), resulting in a mean follow-up time of 15.8 years. At the reinvestigation, we used a protocol similar to that applied at the baseline examination but with additional measurement of urine albumin excretion and an OGTT (75 g glucose) after an overnight fast, with measurement of plasma glucose at time 0 (before) and 120 min after glucose ingestion.

In the analyses of incidence of MetS, abdominal obesity, obesity, hypertension and DM, we excluded subjects with baseline prevalence of the corresponding outcome variable (Tables 1 and 2).

Table 1. Population description—baseline characteristics (n = 2064)

Age (years)	57.0 $\pm$ 5.7
Sex (% men)	40.9
Waist circumference (cm)	83.0 $\pm$ 12.6
Abdominal obesity	265 (12.8)
BMI (kg m ⁻²)	25.6 $\pm$ 3.7
Obesity	232 (11.2)
HDL (mmol l ⁻¹)	1.39 $\pm$ 0.37
Triglycerides (mmol l ⁻¹) ^a	1.14 (0.84–1.59)
Glucose (mmol l ⁻¹) ^a	4.9 (4.6–5.2)
Diabetes mellitus	136 (6.6)
Systolic BP (mm Hg)	140 $\pm$ 18
Diastolic BP (mm Hg)	86 $\pm$ 9.1
Hypertension	1263 (61.2)
AHT	333 (16.1)
Metabolic syndrome	411 (19.9)
Cystatin C (mg l ⁻¹)	0.77 $\pm$ 0.13
CRP (mg l ⁻¹) ^a	1.2 (0.6–2.5)
Copeptin (pmol l ⁻¹) ^a	5.08 (3.19–8.09)

Abbreviations: AHT, anti-hypertensive treatment; BMI, body mass index; BP, blood pressure; CRP, C-reactive protein; HDL, high-density lipoprotein. Values are presented as mean $\pm$ s.d. or n (%) (if not otherwise specified).
^aExpressed as median (interquartile range).

DM at reinvestigation was defined as self-report of a physician diagnosis or use of DM medication after the baseline examination, or fasting plasma glucose of ≥ 7.0 mmol l⁻¹ or 120-min value post OGTT plasma glucose >11.0 mmol l⁻¹.

The prevalence of hypertension at baseline was as high as 61.2% (Table 1). Instead of excluding 61.2% of the population in the incidence analysis of hypertension, we only excluded subjects on AHT at baseline and used initiation of AHT during follow-up as the end point, assuming that initiation of AHT is a more valid indicator of a diagnosis of hypertension. However, as a secondary analysis, we excluded all the subjects with prevalent hypertension at baseline and used the broader definition in the incidence analysis of hypertension (systolic BP ≥ 140 mm Hg, diastolic BP ≥ 90 mm Hg or use of AHT at the reinvestigation). Albumin and creatinine were measured in morning urine samples with methods described earlier²⁴ and microalbuminuria at reinvestigation was defined according to the Swedish upper (95%) reference limits of ≥ 3.0 g albumin per mol creatinine in a morning urine sample.²⁴ The definitions of abdominal obesity, obesity and MetS at reinvestigation were the same as those at the baseline examination.

Statistics

SPSS statistical software (version 17.0, SPSS Inc., Chicago, IL, USA) was used for all analyses. Skewed variables were logarithmically transformed before analysis. We used sex-specific quartiles of copeptin, which were pooled according to sex in all the analyses, as copeptin is known to be significantly higher in men. Multivariate-adjusted logistic and linear regression models were used to test the relationship between quartiles of copeptin and the different outcome variables. A two-sided P-value <0.05 was considered statistically significant.

RESULTS

The average follow-up time was 15.8 years. During the follow-up, 433 subjects (26.2%) developed MetS, 236 (12.9%) developed obesity, 533 (29.6%) developed abdominal obesity, 730 (42.2%) started AHT treatment, 458 (57.2%) developed hypertension and 308 (16.0%) developed new-onset DM. The prevalence of microalbuminuria at baseline investigation was not measured, but the prevalence at reinvestigation was 191 (9.3%) among subjects with DM at baseline and 159 (8.2%) among subjects without DM at baseline. Medians (interquartile range) of copeptin (in pmol l⁻¹) in quartiles 1–4 were 3.16 (2.21–3.80), 5.56 (4.86–6.27), 8.44 (7.64–9.53) and 13.5 (11.50–16.60), respectively, in men, and 1.86 (1.36–2.34), 3.41 (3.04–3.77), 5.14 (4.70–5.76) and 8.41 (7.22–10.45), respectively, in women.

Table 2. Baseline plasma copeptin quartiles (Q1–Q4) in relation to incidence of components of the metabolic syndrome and microalbuminuria at reinvestigation after 15.8 years of follow-up

	Model 1		Model 2	
	OR (95% CI) ^a	P trend	OR (95% CI) ^b	P trend
<i>Abdominal obesity, subjects without abdominal obesity at baseline (n = 1799)</i>				
Q1	1.00 (ref)	0.003	1.00 (ref)	0.04
Q2	1.30 (0.97–1.74)		1.55 (1.08–2.21)	
Q3	1.37 (1.02–1.83)		1.30 (0.91–1.84)	
Q4	1.58 (1.18–2.12)		1.59 (1.11–2.28)	
<i>Obesity, subjects without obesity at baseline (n = 1832)</i>				
Q1	1.00 (ref)	0.21	1.00 (ref)	0.86
Q2	1.26 (0.85–1.87)		1.17 (0.76–1.80)	
Q3	1.24 (0.83–1.83)		1.13 (0.74–1.73)	
Q4	1.31 (0.88–1.95)		1.06 (0.69–1.63)	
<i>Incident AHT, subjects without AHT at baseline (n = 1731)</i>				
Q1	1.00 (ref)	0.62	1.00 (ref)	0.45
Q2	1.11 (0.84–1.46)		1.06 (0.79–1.43)	
Q3	1.15 (0.88–1.51)		1.00 (0.74–1.34)	
Q4	1.06 (0.80–1.39)		0.90 (0.66–1.22)	
<i>Diabetes mellitus, subjects without diabetes at baseline (n = 1928)</i>				
Q1	1.00 (ref)	0.001	1.00 (ref)	0.04
Q2	1.42 (0.98–2.06)		1.18 (0.79–1.76)	
Q3	1.49 (1.03–2.15)		1.32 (0.89–1.96)	
Q4	1.87 (1.30–2.67)		1.46 (0.99–2.16)	
<i>Metabolic syndrome, subjects without metabolic syndrome at baseline (n = 1653)</i>				
Q1	1.00 (ref)	0.008	1.00 (ref)	0.19
Q2	1.25 (0.91–1.72)		1.21 (0.85–1.72)	
Q3	1.23 (0.90–1.68)		1.05 (0.74–1.49)	
Q4	1.57 (1.15–2.14)		1.34 (0.95–1.91)	
<i>Microalbuminuria, subjects with and without diabetes at baseline (n = 2064)</i>				
Q1	1.00 (ref)	0.002	1.00 (ref)	0.02
Q2	1.19 (0.74–1.91)		1.05 (0.65–1.69)	
Q3	1.25 (0.79–2.00)		1.08 (0.67–1.73)	
Q4	1.97 (1.27–3.04)		1.65 (1.06–2.59)	
<i>Microalbuminuria, subjects without diabetes at baseline (n = 1928)</i>				
Q1	1.00 (ref)	0.005	1.00 (ref)	0.02
Q2	1.26 (0.76–2.09)		1.14 (0.69–1.91)	
Q3	1.14 (0.68–1.90)		1.01 (0.60–1.70)	
Q4	2.01 (1.26–3.22)		1.78 (1.11–2.88)	

Abbreviations: AHT, anti-hypertensive treatment; CI, confidence interval; OR, odds ratio. Subjects with baseline prevalence of the outcome variable in question (shown in Table 1) were excluded from the analyses. Medians (interquartile range) of copeptin (in pmol l⁻¹) in quartiles 1–4 were 3.16 (2.21–3.80), 5.56 (4.86–6.27), 8.44 (7.64–9.53) and 13.5 (11.50–16.60), respectively, in men and 1.86 (1.36–2.34), 3.41 (3.04–3.77), 5.14 (4.70–5.76) and 8.41 (7.22–10.45), respectively, in women. ^aAdjusted for follow-up time, age and sex. ^bAdjusted for follow-up time, age, sex, cystatin C, hypertension (systolic blood pressure and diastolic blood pressure in analyses with incident AHT as outcome), glucose, triglycerides, high-density lipoprotein and waist circumference.

Increasing quartiles of baseline copeptin predicted incident MetS in a model adjusted for baseline age, sex and follow-up time (model 1), but the association did not remain significant when adjusted further for baseline hypertension, glucose, TGs, HDL, waist, cystatin C and follow-up time (model 2) (Table 2, Figure 1).

We then analysed the longitudinal relationship between baseline copeptin and incident abdominal obesity, DM and incident AHT, respectively, as these individual MetS components were

previously found to be cross-sectionally associated with elevated copeptin.⁷ Increasing quartile of copeptin predicted both incident abdominal obesity and incident DM when adjusted for model 1 covariates, as well as after full adjustment for model 2 covariates (baseline age, sex, hypertension, glucose, TG, HDL, waist, cystatin C and follow-up time) (Table 2, Figure 1). When the prospective relationship between baseline copeptin and incident DM was additionally adjusted for incident abdominal obesity on top of model 2 covariates, it remained significant (odds ratio (95% confidence interval (CI)) in quartiles 1–4: 1.00 (reference), 1.17 (0.78–1.74), 1.31 (0.88–1.94) and 1.45 (0.98–2.14), respectively; *P* for trend = 0.049). Similarly, we adjusted the relationship between baseline copeptin and incident abdominal obesity for incident DM on top of model 2 covariates, and this relationship remained significant also (odds ratio (95% CI): 1.00 (reference), 1.55 (1.08–2.21), 1.30 (0.91–1.85) and 1.60 (1.12–2.29), respectively; *P* for trend = 0.03). We did not find any longitudinal relationship between baseline copeptin and incident AHT (Table 2). Neither was there any significant relationship between baseline copeptin and incident hypertension (*P* = 0.65). Copeptin was previously found to be borderline significantly and cross-sectionally associated with obesity,⁷ but we did not find any longitudinal relationship between baseline copeptin and incident obesity (Table 2).

Increasing quartiles of copeptin was associated with Δ HDL, Δ LDL and Δ TG (all expressed as mm change per year). In a model adjusted for age and sex, increasing quartiles of copeptin was associated with Δ HDL (beta coefficient (95% CI) –0.001 (–0.002 to –0.0002); *P* = 0.007) and Δ LDL (–0.005 (–0.008 to –0.002); *P* = 0.002), whereas there was no association with Δ TG (–0.0002 (–0.002 to 0.001); *P* = 0.75). When age, sex, cystatin C, hypertension, glucose, TG, HDL and waist circumference (and LDL in analysis with Δ LDL as outcome) were added to the model, only Δ HDL remained negatively associated with increasing copeptin quartile (–0.001 (–0.002 to –0.0003); *P* = 0.002), whereas Δ LDL and Δ TG did not (–0.001 (–0.004 to 0.001); *P* = 0.28 and 0.001 (–0.001 to 0.002); *P* = 0.39, respectively).

Increasing quartiles of copeptin at baseline was associated with microalbuminuria at reinvestigation after model 1 adjustments, as well as after model 2 adjustments, both before and after exclusion of prevalent DM from the cohort (Table 2, Figure 1). As there is a strong link between CRP and microalbuminuria,²⁵ we further adjusted for baseline CRP on top of model 2 and found that the association across increasing copeptin quartiles among subjects free from diabetes at baseline was not affected (odds ratio (95% CI): 1.0 (reference), 1.14 (0.68–1.91), 1.03 (0.61–1.73) and 1.91 (1.18–3.09); *P* for trend = 0.01). Finally, on top of model 2 and CRP, we additionally adjusted for incident hypertension and DM and found that the association across increasing quartiles of copeptin remained significant (odds ratio (95% CI): 1.00 (reference), 1.09 (0.65–1.83), 0.97 (0.57–1.66) and 1.82 (1.12–2.96); *P* for trend = 0.02).

As we recently reported that mid-regional atrial natriuretic peptide predicts the development of DM in our study population,²⁶ we additionally adjusted for mid-regional atrial natriuretic peptide in analyses, relating copeptin to incidence of DM, abdominal obesity and microalbuminuria. When adding mid-regional atrial natriuretic peptide as a covariate on top of model 2 (Table 2), copeptin remained significantly associated with DM (*P* = 0.04), abdominal obesity (*P* = 0.04) and microalbuminuria (*P* = 0.02) (Supplementary Table S1).

DISCUSSION

The key finding of this study is that an elevated copeptin at baseline predicts incident DM, abdominal obesity and microalbuminuria during a long-term follow-up (15.8 years on average). These results extend our previous cross-sectional findings that

Figure 1. Baseline plasma copeptin quartiles (Q1–Q4) in relation to incidence of components of the MetS (**a–c**) and microalbuminuria (**d**) at reinvestigation in model adjusted for follow-up time, age and sex. Model adjusted for follow-up time, age, sex, cystatin C, hypertension, glucose, TGs, HDL and waist circumference.

copeptin is associated with abdominal obesity,⁷ by showing that this association is also valid prospectively. Furthermore, in the current results, incident DM was based on a reinvestigation including an OGTT. Thus, they extend and strengthen our previous finding that copeptin independently predicted DM where the DM end point was based only on register data.⁶

Copeptin and MetS

Interestingly, despite of the prospective relationship between copeptin and two core components of the MetS (abdominal obesity and DM) and our previously observed cross-sectional association between copeptin and MetS,⁷ in the current study, copeptin was not an independent predictor of the cluster of MetS. Our previous finding of cross-sectional relationship between copeptin and MetS was probably driven by the association between copeptin and both DM and abdominal obesity, respectively.

Copeptin, abdominal obesity and DM

The fact that copeptin was elevated many years before the development of overt DM and abdominal obesity, and remained significantly associated independently of a broad range of potential confounders, suggests a primary role for the AVP system in the pathophysiology of DM and abdominal obesity. Assuming that there is such a primary role of AVP, one wonders whether AVP induces development of abdominal obesity and DM independently of each other or whether development of the two closely related end points is dependent upon each other. As the association between baseline copeptin and incident DM was independent of incident abdominal obesity and vice versa, it is possible that AVP triggers two different pathways leading to DM and abdominal obesity, independently. However, as we do not have repeated measurements of waist circumference and glycaemia during the follow-up period, it is possible that we have missed temporal trends occurring during that time frame. Thus, we cannot exclude the possibility that a primary elevation of AVP leads to DM development by increasing abdominal fat depositions.

Even though metabolic effects of AVP may be expected, it is not yet elucidated as to how AVP favour obesity and DM. AVP mediates gluconeogenesis and glycogenolysis through vasopressin 1a receptors (V1aRs) in the liver^{1,2} and stimulates the secretion of either glucagon or insulin, depending on the actual level of glycaemia, through vasopressin 1b receptors (V1bRs) in pancreatic islets.³ Further, AVP exerts an anti-lipolytic action, possibly through haemodynamic effects.²⁷ However, the contribution of AVP to glucose and lipid metabolism seems to be rather complex. Mice with selective deletion of V1aR exhibit elevated glucose levels, predisposition for obesity and diabetes, low TG levels and enhanced lipid metabolism,^{28,29} whereas mice lacking V1bR display a phenotype of low glucose levels and better insulin sensitivity.³⁰ In humans, the rs1042615 polymorphism of the V1aR gene was among men associated with altered body mass index before and after walking training and features resembling the phenotype of the mouse with V1aR deletion, including elevated glucose levels and low TG levels, as well as increased DM prevalence in subjects with a high-fat intake or overweight.^{31,32}

Moreover, AVP binding to V1bR in the anterior hypophysis mediates adrenocorticotrophic hormone release and elevate glucocorticoid levels in plasma. Indeed, mice lacking the V1bR show lower levels of corticosterone in plasma both under stress and during basal conditions.³³ The AVP-induced adrenocorticotrophic hormone release has been reported to be resistant to glucocorticoid feedback in contrast to the corticotropin-releasing hormone-induced adrenocorticotrophic hormone release.³⁴

The proportion of subjects who developed new-onset DM was substantially higher in this study than in our previous study.⁶ This difference in DM incidence was expected as the DM end point in our previous study was register-based, requiring subjects to have been in contact with the health-care system in order to be captured, whereas in the current study, we screened all the subjects for DM with OGTT and fasting plasma glucose measurements. In addition, the follow-up time was longer in the current than in our previous study. In any case, whichever method used to retrieve incident cases of DM, copeptin was an independent predictor of DM.

Copeptin and microalbuminuria

Apart from constituting the core of the diagnosis of diabetic nephropathy in DM, microalbuminuria is considered as an early and validated sign of organ damage of the cardiovascular system not only in patients with hypertension and DM but also in the general population.^{8–10,35} In the current study, the proportion of subjects with microalbuminuria at reexamination in the present study (9.3% and 8.2% among subjects who had or did not have DM at baseline, respectively) appears to be higher than that seen in other Caucasian populations.³⁶ This may be explained by the relatively high mean age at the reinvestigation (72.8 ± 5.6 years); in fact, few studies have examined the prevalence of microalbuminuria in general populations with a mean age >70 years. Further, the prevalence of microalbuminuria in hypertensive individuals ranges 7–40% (depending on age, race, and ethnicity) and the prevalence of microalbuminuria in patients with DM ranges 30–40%.⁹

Our finding that copeptin level at baseline is associated with microalbuminuria after long-term follow-up independent of baseline MetS variables and CRP supports previous cross-sectional findings in humans^{11,17} and suggests a role for the AVP system in the development of microalbuminuria. The association between copeptin and microalbuminuria could be speculated to be explained by AVP-mediated changes of glucose metabolism or BP during follow-up. However, the association was present whether or not subjects with DM at baseline were included, and it remained significant after adjustment for all MetS factors, renal function (as assessed with cystatin C) and CRP at baseline, as well as for both incident DM and hypertension. This suggests that microalbuminuria may be at least partly directly dependent upon AVP and not mediated by other cardiometabolic risk factors related to copeptin such as DM and abdominal obesity.

Regarding the pathway whereby AVP may increase albuminuria, there are some data suggesting that AVP might contribute to rise in albumin excretion as a consequence of its antidiuretic effect mediated by vasopressin 2 receptors (V2Rs).^{11,13,14} Further, V2R may have a role in renal function decline. AVP suppression lowered proteinuria and improved renal function in rats with subtotal nephrectomy.^{12,37} Conversely, chronic infusion of dDAVP, a V2R agonist, exaggerated proteinuria in a rat model of renal failure.¹⁵ Thus, in contrast to the relationship between AVP, DM and abdominal obesity, which is most likely dependent upon V1aR and/or V1bR, experimental evidence point at the V2R as a link between AVP and microalbuminuria.

Strengths and weaknesses of the study

Our study is large, prospective and has a long follow-up time. All our analyses included adjustment for a broad range of potential confounding factors, most importantly baseline levels of all factors of the MetS, cystatin C (to account for differences in glomerular filtration rate) and, in extended analyses, even incident DM, abdominal obesity and hypertension. The independent prospective relationship between copeptin at baseline and DM, abdominal obesity and microalbuminuria at the reinvestigation suggests a primary role of the AVP system as a cardiometabolic risk factor and warrants further studies testing whether interventions targeted at the AVP system may in fact prevent or reverse glucose intolerance, abdominal obesity and microalbuminuria. However, we do acknowledge limitations. First, microalbuminuria was not measured at baseline, preventing any firm statement about progression from normo- to microalbuminuria. Second, it was not possible to prospectively analyse copeptin in relation to CRP and insulin, variables that were previously found to be cross-sectionally associated with elevated copeptin,^{6,7} as these variables were not measured at reinvestigation. Third, subjects who participated in the MDC cardiovascular cohort baseline exam but died during follow-up or did not participate in the reinvestigation for

other reasons are missing. This could lead to either over- or underestimation of the strength of the prospective relationship between copeptin at baseline and the studied end points. However, comparison of copeptin levels between participants and non-participants of the reinvestigation did not reveal any significant difference ($P=0.46$), suggesting that such potential bias is of limited magnitude.

CONCLUSIONS

In this large prospective population-based cohort, copeptin predicts abdominal obesity, DM and microalbuminuria, suggesting a primary role for the AVP system in development of these conditions. These findings may have implications for risk assessment and warrant further studies testing whether interventions targeted at the AVP system may prevent the development of DM and reduce cardiometabolic risk.

CONFLICT OF INTEREST

Drs Struck and Morgenthaler are employees of B.R.A.H.M.S GmbH, which holds patent rights on the copeptin assay. The remaining authors declare no conflict of interest.

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REFERENCES

- Whitton PD, Rodrigues LM, Hems DA. Stimulation by vasopressin, angiotensin and oxytocin of gluconeogenesis in hepatocyte suspensions. *Biochem J* 1978; **176**: 893–898.
- Keppens S, de Wulf H. The nature of the hepatic receptors involved in vasopressin-induced glycogenolysis. *Biochim Biophys Acta* 1979; **588**: 63–69.
- Abu-Basha EA, Yibchok-Anun S, Hsu WH. Glucose dependency of arginine vasopressin-induced insulin and glucagon release from the perfused rat pancreas. *Metabolism* 2002; **51**: 1184–1190.
- Holmes CL, Landry DW, Granton JT. Science review: Vasopressin and the cardiovascular system part 1—receptor physiology. *Crit care* 2003; **7**: 427–434.
- Morgenthaler NG, Struck J, Alonso C, Bergmann A. Assay for the measurement of copeptin, a stable peptide derived from the precursor of vasopressin. *Clin chem* 2006; **52**: 112–119.
- Enhörning S, Wang TJ, Nilsson PM, Almgren P, Hedblad B, Berglund G et al. Plasma copeptin and the risk of diabetes mellitus. *Circulation* 2010; **121**: 2102–2108.
- Enhörning 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**: E1065–E1072.
- Mogensen CE. Microalbuminuria, blood pressure and diabetic renal disease: origin and development of ideas. *Diabetologia* 1999; **42**: 263–285.
- Keane WF, Eknoyan G. Proteinuria, albuminuria, risk, assessment, detection, elimination (PARADE). *Am J Kidney Dis* 1999; **33**: 1004–1010.
- Creager MA, Luscher TF, Cosentino F, Beckman JA. Diabetes and vascular disease: pathophysiology, clinical consequences, and medical therapy: Part I. *Circulation* 2003; **108**: 1527–1532.
- 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**: 497–506.
- Bouby N, Bachmann S, Bichet D, Bankir L. Effect of water intake on the progression of chronic renal failure in the 5/6 nephrectomized rat. *Am J Physiol* 1990; **258**: F973–F979.
- 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**: 1755–1763.

- 14 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 USA* 1999; **96**: 10397–10402.
- 15 Bouby N, Hassler C, Bankir L. Contribution of vasopressin to progression of chronic renal failure: study in Brattleboro rats. *Life Sci* 1999; **65**: 991–1004.
- 16 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**: 561–567.
- 17 Meijer E, Bakker SJ, Halbesma N, de Jong PE, Struck J, Copeptin Gansevoort RT. A surrogate marker of vasopressin is associated with microalbuminuria in a large population cohort. *Kidney Int* 2010; **77**: 29–36.
- 18 Fenske W, Wanner C, Allolio B, Drechsler C, Blouin K, Lilienthal J *et al*. Copeptin levels associate with cardiovascular events in patients with ESRD and type 2 diabetes mellitus. *J Am Soc Nephrol* 2011; **22**: 782–790.
- 19 Berglund G, Elmstahl S, Janzon L, Larsson SA. The Malmo Diet and Cancer Study. Design and feasibility. *J Intern Med* 1993; **233**: 45–51.
- 20 Persson M, Hedblad B, Nelson JJ, Berglund G. Elevated Lp-PLA2 levels add prognostic information to the metabolic syndrome on incidence of cardiovascular events among middle-aged nondiabetic subjects. *Arterioscler Thromb Vasc Biol* 2007; **27**: 1411–1416.
- 21 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**: 123–129.
- 22 Morgenthaler NG, Struck J, Thomas B, Bergmann A. Immunoluminometric assay for the midregion of pro-atrial natriuretic peptide in human plasma. *Clin chem* 2004; **50**: 234–236.
- 23 National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III) final report. *Circulation* 2002; **106**: 3143–3421.
- 24 Tencer J, Thysell H, Grubb A. Analysis of proteinuria: reference limits for urine excretion of albumin, protein HC, immunoglobulin G, kappa- and lambda-immunoreactivity, orosomucoid and alpha 1-antitrypsin. *Scand J Clin Lab Invest* 1996; **56**: 691–700.
- 25 Kshirsagar AV, Bomback AS, Bang H, Gerber LM, Vupputuri S, Shoham DA *et al*. Association of C-reactive protein and microalbuminuria (from the National Health and Nutrition Examination Surveys, 1999 to 2004). *Am J Cardiol* 2008; **101**: 401–406.
- 26 Magnusson M, Jujic A, Hedblad B, Engstrom G, Persson M, Struck J *et al*. Low plasma level of atrial natriuretic peptide predicts development of diabetes: the prospective Malmo Diet and Cancer study. *J Clin Endocrinol Metab* 2012; **97**: 638–645.
- 27 Rofe AM, Williamson DH. Mechanism for the 'anti-lipolytic' action of vasopressin in the starved rat. *Biochem J* 1983; **212**: 899–902.
- 28 Aoyagi T, Birumachi J, Hiroyama M, Fujiwara Y, Sanbe A, Yamauchi J *et al*. Alteration of glucose homeostasis in V1a vasopressin receptor-deficient mice. *Endocrinology* 2007; **148**: 2075–2084.
- 29 Hiroyama M, Aoyagi T, Fujiwara Y, Birumachi J, Shigematsu Y, Kiwaki K *et al*. Hypermetabolism of fat in V1a vasopressin receptor knockout mice. *Mol Endocrinol* 2007; **21**: 247–258.
- 30 Fujiwara Y, Hiroyama M, Sanbe A, Yamauchi J, Tsujimoto G, Tanoue A. Mutual regulation of vasopressin- and oxytocin-induced glucagon secretion in V1b vasopressin receptor knockout mice. *J Endocrinol* 2007; **192**: 361–369.
- 31 Masuki S, Mori M, Tabara Y, Miki T, Sakurai A, Morikawa M *et al*. Vasopressin V1a receptor polymorphism and interval walking training effects in middle-aged and older people. *Hypertension* 2010; **55**: 747–754.
- 32 Enhörning S, Leosdottir M, Wallstrom P, Gullberg B, Berglund G, Wirfalt E *et al*. Relation between human vasopressin 1a gene variance, fat intake, and diabetes. *Am J Clin Nutr* 2009; **89**: 400–406.
- 33 Tanoue A, Ito S, Honda K, Oshikawa S, Kitagawa Y, Koshimizu TA *et al*. The vasopressin V1b receptor critically regulates hypothalamic-pituitary-adrenal axis activity under both stress and resting conditions. *J Clin Invest* 2004; **113**: 302–309.
- 34 Rabadan-Diehl C, Aguilera G. Glucocorticoids increase vasopressin V1b receptor coupling to phospholipase C. *Endocrinology* 1998; **139**: 3220–3226.
- 35 Mansia G, De Backer G, Dominiczak A, Cifkova R, Fagard R, Germano G *et al*. 2007 ESH-ESC guidelines for the management of arterial hypertension: the task force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *Blood Press* 2007; **16**: 135–232.
- 36 Jacobs Jr DR, Murtaugh MA, Steffes M, Yu X, Roseman J, Goetz FC. Gender- and race-specific determination of albumin excretion rate using albumin-to-creatinine ratio in single, untimed urine specimens: the Coronary Artery Risk Development in Young Adults Study. *Am J Epidemiol* 2002; **155**: 1114–1119.
- 37 Perico N, Zoja C, Corna D, Rottoli D, Gaspari F, Haskell L *et al*. V1/V2 Vasopressin receptor antagonism potentiates the renoprotection of renin-angiotensin system inhibition in rats with renal mass reduction. *Kidney Int* 2009; **76**: 960–967.

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