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Cardiovascular hormones in the development of type 2 diabetes: An epidemiological perspective

vorgelegt von:

Chaterina Sujana

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Supervisors/Examiners:	Prof. Dr. Barbara Thorand, MPH
	Prof. Dr. Ulrich Mansmann
Co-Supervisor:	Prof. Dr. Christian Herder
Co-Examiners:	Priv. Doz. Dr. Ursula Storch
	Priv. Doz. Dr. Stephanie Fichtner
Dean:	Prof. Dr. med. Thomas Gudermann
Date of the defense:	May 11th, 2022

Affidavit

Affidavit

Sujana, Chaterina
Surname, first name

Ingolstädter Landstraße 1
Street

85764, Neuherberg, Germany
Zip code, town, country

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Confirmation of congruency between printed and electronic version of the doctoral thesis

Sujana, Chaterina
Surname, first name

Ingolstädter Landstraße 1
Street

85764, Neuherberg, Germany
Zip code, town, country

I hereby declare, that the submitted thesis entitled:

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is congruent with the printed version both in content and format.

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Place, date

Chaterina Sujana
Signature doctoral candidate

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Summary

Type 2 diabetes and cardiovascular disease (CVD) commonly coexist. Both conditions share many overlapping risk factors, which suggests a shared pathological ground. It is widely known that several hormones regulating cardiovascular function, such as B-type natriuretic peptide (BNP), atrial natriuretic peptide (ANP), endothelin-1 (ET-1), adrenomedullin (ADM), and arginine vasopressin (AVP) are implicated in the development of CVD. Less recognised is the fact that these hormones also have metabolic actions. For example, BNP, ANP and ADM were shown to ameliorate insulin resistance, while ET-1 and AVP could promote insulin resistance and glucose intolerance.

Using data from several population-based studies, this doctoral thesis examined the hypothesis that cardiovascular hormones could be implicated in the development of type 2 diabetes. The specific research questions were addressed in three papers constituting the basis of this doctoral thesis.

The first paper assessed whether the cardiovascular hormones ANP, ET-1, ADM, and AVP, were associated with impaired glucose metabolism. We found that elevated circulating concentrations of ANP were associated with a lower incidence of type 2 diabetes, while elevated circulating concentrations of AVP were associated with a higher incident prediabetes and type 2 diabetes. We also observed that the elevated circulating concentrations of ET-1 and ADM were associated with an increase in insulin-related traits.

In the second paper, we investigated whether the natriuretic peptides BNP and ANP could lower the risk of type 2 diabetes and specifically assessed whether the link between both natriuretic peptides and risk of type 2 diabetes could be modified by the presence of CVD. Our results demonstrate that higher concentrations of BNP and ANP were associated with a lower incidence of type 2 diabetes. Here, using a large sample size, we were able to expand the current knowledge by showing that the inverse association with incident type 2 diabetes for BNP was

modified by the presence of CVD, while similar differences were not seen for ANP. Analyses using genetic data further suggest that associations of higher concentrations of both BNP and ANP with a lower incidence of type 2 diabetes could probably be causal.

Expanding the results from the first paper, in the third paper we further examined whether ET-1 and ADM could increase the risk of type 2 diabetes. We observed that elevated concentrations of ET-1 and ADM were associated with a higher incidence of type 2 diabetes, but analyses using genetic data suggest a probable causal link for ET-1 only. Furthermore, the findings of this paper provided new evidence that the positive association between ADM and incident type 2 diabetes was more apparent in obese than in non-obese individuals.

In summary, this thesis highlights the importance of cardiovascular hormones beyond CVD. The findings suggest that several cardiovascular hormones play important roles in the development of type 2 diabetes and raise the possibility that targeting these cardiovascular hormones might be effective in preventing and managing not only CVD but also type 2 diabetes.

Papers included in this thesis

The following articles form the basis of this cumulative thesis.

Paper I

Sujana C, Seissler J, Jordan J, Rathmann W, Koenig W, Roden M, Mansmann U, Herder C, Peters A, Thorand B and Then C. Associations of cardiac stress biomarkers with incident type 2 diabetes and changes in glucose metabolism: KORA F4/FF4 study. Cardiovascular Diabetology. 2020; 19(1):178.

Paper II

Sujana C, Salomaa V, Kee F, Costanzo S, Söderberg S, Jordan J, Jousilahti P, Neville C, Iacoviello L, Oskarsson V, Westermann D, Koenig W, Kuulasmaa K, Reinikainen J, Blankenberg S, Zeller T, Herder C, Mansmann U, Peters A and Thorand B. Natriuretic Peptides and Risk of Type 2 Diabetes: Results From the Biomarkers for Cardiovascular Risk Assessment in Europe (BiomarCaRE) Consortium. Diabetes Care. 2021; 44(11):2527-35.

Paper III (Appendix)

Sujana C, Salomaa V, Kee F, Jousilahti P, Neville C, Koenig W, Kuulasmaa K, Reinikainen J, Blankenberg S, Zeller T, Herder C, Mansmann U, Peters A and Thorand B. Associations of the Vasoactive Peptides CT-proET-1 and MR-proADM with Incident Type 2 Diabetes: Results from the BiomarCaRE Consortium. Cardiovascular Diabetology (in press).

Contributions to the included papers

In all three papers included in this thesis, I am the first author and take responsibility for the integrity of the data and the accuracy of the data analysis.

Under the supervision of my doctoral supervisor, Prof. Barbara Thorand, I developed the research questions and conceptualised the study design. I performed literature reviews that provided me with an overview of cardiovascular hormones and their metabolic actions and helped me to keep up with state-of-the-art research in order to define the research questions.

I was significantly involved in the data application for this thesis. I wrote a study protocol and a statistical analysis plan for each paper. In particular for the application of Biomarkers for Cardiovascular Risk Assessment in Europe (BiomarCaRE) Consortium data used in Paper II and Paper III, I presented the study protocol and analysis plan of the project during the BiomarCaRE annual meetings. I also communicated with all principal investigators of the contributing BiomarCaRE cohorts in this thesis and the BiomarCaRE data centre regarding data availability and approval.

I conducted all statistical analyses using SAS and R statistical languages and interpreted the results together with the members of my Thesis Advisory Committee. I also collaborated with cardiologists, endocrinologists and statisticians in the data interpretation. I wrote a manuscript draft for each paper, incorporated co-authors' comments, and critically reviewed the manuscripts. Finally, I submitted the manuscripts for publication, finalised the manuscripts based on reviewers' comments, and coordinated the communication between co-authors and journal editors.

List of abbreviations

2h-glucose	2 hours after 75-gram glucose solution intake
ADM	adrenomedullin
ANP	atrial natriuretic peptide
ANCOVA	analysis of covariance
AVP	arginine vasopressin
BiomarCaRE	Biomarkers for Cardiovascular Risk Assessment in Europe
BMI	Body mass index
BNP	B-type natriuretic peptide
CT-proAVP	C-terminal-pro-arginine vasopressin
CT-proET-1	C-terminal-pro-endothelin-1
CI	confidence interval
CVD	cardiovascular disease
ERA	endothelin receptor antagonist
ET	endothelin
FDR	false discovery rate
GWA	genome-wide association
HbA1c	haemoglobin A1c
HDL	high-density lipoprotein
HOMA-B	homeostasis model assessment of beta-cell function
HOMA-IR	homeostasis model assessment of insulin resistance
HR	hazard ratio
KORA	Cooperative Health Research in the Region of Augsburg
LOD	limit of detection
MONICA	Monitoring of Trends and Determinants in Cardiovascular Diseases
MORGAM	MONICA Risk Genetics Archiving and Monograph
MR-proADM	mid-regional-pro-adrenomedullin
MR-proANP	mid-regional-pro-atrial natriuretic peptide
NT-proBNP	N-terminal-pro-B-type natriuretic peptide
OGTT	oral glucose tolerance test
OR	odds ratio
PRIME	Prospective Epidemiological Study of Myocardial Infarction
SD	standard deviation
SNP	single nucleotide polymorphism
WHO	World Health Organization

1. Introduction

Diabetes mellitus is a general term for metabolic disorders characterised by persistent hyperglycaemia. Among all diabetes cases, type 2 diabetes accounts for 90–95% of all cases (1). Type 2 diabetes has become a major public health problem, as it is among the leading causes of death worldwide (2). In 2019, the World Health Organization (WHO) estimated that 3.7 million deaths worldwide were attributed to diabetes and its complications (3).

Type 2 diabetes is a chronic and progressive disease (4). It begins with the inability of the body cells to respond properly to insulin (insulin resistance) and a failure of the pancreatic beta cells to adequately compensate for insulin resistance and is manifested clinically in elevated concentrations of fasting and postprandial plasma glucose and haemoglobin A1c (HbA1c) (4, 5). It is firmly established that type 2 diabetes is a major risk factor for cardiovascular disease (CVD) (6), but vice versa CVD, such as heart failure, myocardial infarction and stroke, can precede the development of type 2 diabetes and can give rise to abnormalities in glucose metabolism that predispose to insulin resistance and type 2 diabetes (7-9).

1.1 The link between type 2 diabetes and CVD

Type 2 diabetes shares many risk factors with CVD. For instance, low-grade inflammation has been proposed as central to the development of type 2 diabetes and CVD, although there are differences in the inflammatory profiles between both conditions (10, 11). Low-grade inflammation induces insulin resistance and also promotes atherosclerosis, the hallmarks of type 2 diabetes and CVD, respectively (12, 13). In addition, oxidative stress, hypertension, obesity, dyslipidaemia, and insulin resistance are also known to feature both type 2 diabetes and CVD. The overlapping risk factors for type 2 diabetes and CVD have led to the “common soil” hypothesis, postulating that both conditions may have common antecedents (11,

14). A recent study using genetic and functional data further suggests that type 2 diabetes shares genetic regulatory networks with CVD (15).

The close relationship between type 2 diabetes and CVD also suggests that there is a crosstalk among the organs comprising the metabolic and cardiovascular systems (16). For example, insulin signalling not only regulates glucose metabolism in skeletal muscle, adipose tissue, and liver, but also stimulates the production of nitric oxide in vascular endothelium and thereby promotes vascular relaxation (17). Conversely, vascular endothelium, which regulates the degree of vascular relaxation and constriction, plays a role in metabolic homeostasis. Vascular endothelium affects insulin signalling through endothelial cell-secreted hormones that could act as paracrine or endocrine regulators (18). Recent pre-clinical and clinical studies also have demonstrated that several cardiac hormones regulate glucose and lipid metabolism and may provide a link between cardiovascular and metabolic diseases. (19, 20). However, while it is widely known that hormones secreted by the cardiovascular system are involved in the pathological development of CVD (21, 22), less is known regarding the impact of these hormones on the pathological development of type 2 diabetes.

1.2 Cardiovascular hormones

Heart and vascular endothelium are now recognised as endocrine organs (23, 24). Hormones secreted from the heart and endothelium have been shown to modulate not only the cardiovascular function, but also the function of other distant organ systems, including renal and metabolic systems (20, 25). This thesis focusses on several cardiovascular hormones, which have been reported to have metabolic actions.

1.2.1 B-type and atrial natriuretic peptides

B-type (or brain) natriuretic peptide (BNP) and atrial natriuretic peptide (ANP) belong to the natriuretic peptide family of peptide hormones. They have similar structural homology but are coded by unique genes, the *NPPB* and *NPPA* genes,

respectively (26). BNP and ANP are synthesised in cardiomyocytes and are mainly released in response to plasma volume overload (27).

BNP and ANP predominantly bind to the same receptor in target cells. Thus, both natriuretic peptides share many physiological actions at target cells, including diuresis, natriuresis, vasodilation, and inhibition of aldosterone synthesis and renin secretion. They also act directly on the heart to counteract cardiac hypertrophy and fibrosis (26, 27). Natriuretic peptide release increases with increasing blood pressure or in case of CVD, reflecting a compensatory mechanism to restrain cardiovascular function (19). In addition, BNP and ANP exert metabolic actions in skeletal muscle and adipose tissue, such as increasing mitochondrial fat oxidative capacity, promoting glucose uptake, lipolysis, and suppression of inflammation (19, 20). These actions ameliorate blood glucose control.

1.2.2 Endothelin-1

Endothelin (ET)-1 is an endogenous substance predominantly released by vascular endothelial cells and is one of the most potent vasoconstrictors (25). ET-1 belongs to the ET family and is the main isoform in the vasculature (29).

ET-1 maintains the normal vascular tone, tissue development and repair, and angiogenesis (29). ET-1 is also pro-inflammatory and promotes vascular smooth muscle cell proliferation. These effects implicate ET-1 in the pathogenesis of hypertension and other vascular diseases such as atherosclerosis (29, 30). In addition to its known vascular effects, ET-1 could also play important roles in glucose metabolism. In skeletal muscle, ET-1 limits insulin actions and reduces glucose uptake (31). It also disrupts insulin-regulated glucose transporter 4 translocation into the plasma membrane (32). In adipose tissue, ET-1 blocks free fatty acid uptake and induces lipolysis, resulting in increased concentrations of free fatty acids (33, 34). Altogether, these metabolic effects suggest that elevated concentrations of ET-1 promote insulin resistance and impaired glucose tolerance.

1.2.3 Adrenomedullin

The peptide hormone adrenomedullin (ADM), which is a member of the calcitonin gene–related peptide family, is a vasodilator (35). ADM is expressed in a variety of different cells, including vascular endothelial cells, smooth muscle cells, adventitial fibroblasts, as well as adipocytes (35, 36).

ADM plays a pivotal role in the regulation of many physiological processes, particularly those of the cardiovascular system. ADM dilates blood vessels and can act against vascular damage (35). Moreover, evidence from *in vivo* and *in vitro* studies suggest that ADM possess metabolic actions, including counteracting oxidative stress–induced insulin resistance and the inhibition of insulin secretion from the pancreas (37-39). The latter notion implicates ADM in maintaining insulin homeostasis (39). In obesity, ADM expression is upregulated in adipocytes and circulating concentrations of ADM are increased (38). In an experimental study using a euglycaemic-hyperinsulinemic clamp technique, acute hyperinsulinemia was demonstrated to induce ADM release in obese, but not in lean individuals (40).

1.2.4 Arginine vasopressin

In addition to the aforementioned hormones that are mainly secreted by the cardiovascular system, this thesis also includes arginine vasopressin (AVP), an antidiuretic hormone. AVP, which is synthesised in hypothalamus, is one of the key hormones in the cardiovascular system (41).

AVP is released in response to increases in plasma osmolarity, volume depletion and hypotension. Thus, it is important in maintaining body's osmotic balance, blood pressure and sodium homeostasis (41, 42). Excess release of AVP into the bloodstream is associated with systemic vasoconstriction (43). In addition to its effects on the circulation, AVP is also involved in the stimulation of glycogenolysis from the liver and insulin and glucagon secretion from the pancreas. At elevated concentrations, AVP may contribute to the development of glucose intolerance (44-46). It is further suggested that AVP indirectly affects metabolic regulation via

hemodynamic effects on adipose tissue and via modulation of circadian rhythms (45).

1.3 Epidemiological evidence linking cardiovascular hormones with the risk of type 2 diabetes

To date, there is a growing number of epidemiological studies investigating the association of the natriuretic peptides BNP and ANP with risk of type 2 diabetes. Using a prospective study design, earlier studies have demonstrated that both natriuretic peptides were inversely associated with risk of type 2 diabetes (47-53). Previous analyses using Mendelian randomisation approaches (54, 55) also suggest a possible causal link between high BNP concentrations and a lower type 2 diabetes risk. However, it remains uncertain whether the associations between high natriuretic peptide concentrations and a lower risk of type 2 diabetes are also present in individuals with a history of CVD. Previous studies only examined the associations in individuals without prevalent CVD, or did not examine the associations separately for individuals with and without prevalent CVD.

In comparison with the natriuretic peptides BNP and ANP, ET-1, ADM and AVP have not been widely investigated for their associations with the risk of type 2 diabetes. Previous studies using a cross-sectional design have demonstrated that ET-1, ADM and AVP were associated with the metabolic syndrome, insulin resistance and prevalent type 2 diabetes (56-59). However, most of the few existing prospective studies failed to provide evidence linking ET-1 and ADM with risk of type 2 diabetes (47, 60, 61) and evidence on the association between AVP and type 2 diabetes risk remains inconsistent (60, 62-64). So far, only two studies reported a positive association between ET-1 and type 2 diabetes risk (65, 66).

2. Aim of the thesis

The current thesis aimed to examine whether cardiovascular hormones could be implicated in the development of type 2 diabetes. The specific research questions addressed in three papers constituting the basis of this doctoral thesis are highlighted in Figure 1.

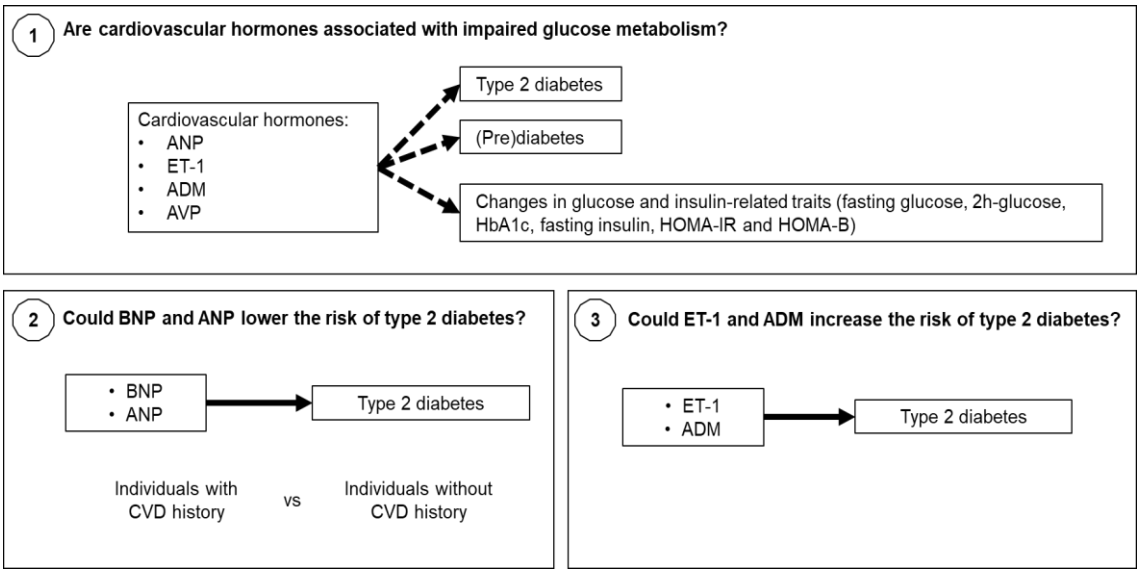

Figure 1. Thematic structure of the current thesis: cardiovascular hormones and the development of type 2 diabetes*

In Paper I, we investigated whether cardiovascular hormones that are known to have metabolic actions were associated with impaired glucose metabolism. Specifically, we examined the associations of ANP, ET-1, ADM, and AVP with incident type 2 diabetes and with changes in glucose and insulin-related traits (fasting glucose, 2 hours after glucose solution intake (2h-glucose), HbA1c, fasting insulin, homeostasis model assessment of insulin resistance (HOMA-IR) and beta-

* Each number corresponds to one of the three included papers and the respective research questions. A dashed arrow implies that an association was examined. A solid arrow implies that a possible causal link was also examined using Mendelian randomisation approaches in addition to an association. The direction of the arrow corresponds to the assumed direction of the association. (Pre)diabetes corresponds to the combined endpoint of prediabetes and type 2 diabetes. Abbreviations: ADM, adrenomedullin; ANP, atrial natriuretic peptide; AVP, arginine vasopressin; BNP, B-type natriuretic peptide; CVD, cardiovascular disease; ET-1, endothelin-1; HbA1c, haemoglobin A1c; HOMA-B, homeostasis model assessment of beta-cell function; HOMA-IR, homeostasis model assessment of insulin resistance.

cell function (HOMA-B)) in initially nondiabetic individuals. Additionally, we also examined associations of the hormones with the combined incidence of prediabetes and type 2 diabetes, referred to as incident (pre)diabetes in initially normoglycaemic individuals.

In Paper II, our focus lied on the cardiac hormones, BNP and ANP. Based on the findings of previous studies, we hypothesised that BNP and ANP could lower the risk of type 2 diabetes. We specifically aimed to assess whether the link between both natriuretic peptides and risk of type 2 diabetes differed by the presence of CVD.

In Paper III, we turned our attention to ET-1 and ADM, two vasoactive hormones that have not yet been extensively studied regarding their roles in the development of type 2 diabetes. Following the results from Paper I, we sought to examine whether both hormones could possibly increase the risk of type 2 diabetes.

3. A brief overview of methods

3.1 Study design and population

In Paper I, we applied a prospective cohort study design to investigate associations of ANP, ET-1, ADM, and AVP with impaired glucose metabolism using data from the population-based Cooperative Health Research in the Region of Augsburg (KORA) F4 and FF4 studies. In Paper II and Paper III, also a prospective cohort study design was used to investigate associations of BNP, ANP, ET-1 and ADM with incident type 2 diabetes. Here, the sample size was considerably increased by using data from several population-based studies participating in the multinational Biomarkers for Cardiovascular Risk Assessment in Europe (BiomarCaRE) Consortium (67). Additionally, in Paper II and Paper III, we performed univariate two-sample Mendelian randomisation analyses using published data on genetic variants to examine possible causal links between the hormones and type 2 diabetes.

3.1.1 KORA F4 and FF4 Study

KORA F4 (2006–2008) and FF4 (2013–2014) are follow-up examinations of the fourth KORA survey (KORA S4) conducted in 1999–2001 in Southern Germany. To examine the associations of ANP, ET-1, ADM, and AVP with impaired glucose metabolism in Paper I, we included all persons taking part in both KORA F4 (used as baseline in the analysis) and KORA FF4 studies (follow-up). Baseline measurements of ANP were available in all participants, while the baseline measurements of ET-1, ADM and AVP were only available in the first 1,596 participants. We excluded participants with prevalent diabetes and CVD at baseline and participants with missing data on type 2 diabetes status, baseline measurements of the cardiovascular hormones and other covariates. For analysing the associations with incident type 2 diabetes, we included 1,773 participants (52% women; median baseline age: 52 years (minimum: 32; maximum: 81)) with ANP measurements and 960 participants (53% women;

median baseline age: 53 years (min: 32; max: 81)) with ET-1, ADM and AVP measurements. When analysing the associations with changes in glucose and insulin-related traits and with incident (pre)diabetes, we further excluded participants who were taking glucose-lowering medication at follow-up and those with prevalent prediabetes at baseline, respectively. Details regarding the sample size for each outcome are given in Paper I.

3.1.2 BiomarCaRE Consortium

BiomarCaRE is a European Union-funded consortium that aims to investigate established and emerging biomarkers in improving risk stratification of CVD (67). BiomarCaRE relies on the Monitoring of Trends and Determinants in Cardiovascular Diseases (MONICA) Risk Genetics Archiving and Monograph (MORGAM) Project (68), which has harmonised data from numerous population-based cohorts.

In Paper II, the association between BNP and incident type 2 diabetes was analysed in five BiomarCaRE population-based cohorts. We included initially nondiabetic participants who had complete data on type 2 diabetes follow-up and baseline measurements of BNP and other covariates. The analyses comprised 45,477 participants; among these, 1,995 had a history of CVD. The median baseline age of the study participants was 51 years (min: 24; max: 98) and around 49% of the participants were women. The individual cohorts included in the analyses of the current thesis were the KORA Study (Germany), the FINRISK Study (Finland), the Prospective Epidemiological Study of Myocardial Infarction (PRIME) Belfast (United Kingdom), the Moli-sani Study (Italy), and the Northern Sweden MONICA Study (Sweden). The baseline measurements of ANP were only available in KORA, FINRISK, and PRIME Belfast. Therefore, the association between ANP and incident type 2 diabetes was examined in these three BiomarCaRE cohorts, involving 11,537 initially nondiabetic participants (783 had a history of CVD) who had complete data on type 2 diabetes follow-up and baseline measurements of ANP and other covariates. The median baseline age of the study

participants was 51 years (min: 24; max: 82) and around 42% of the participants were women.

Furthermore, to analyse the associations of ET-1 and ADM with incidence of type 2 diabetes in Paper III we included three BiomarCaRE population-based cohorts with available data on the hormones, namely KORA, FINRISK, and PRIME Belfast. The sample size comprises 12,006 participants without prevalent diabetes and CVD at baseline and who had complete follow-up data on type 2 diabetes. The median of baseline age was 51 years (min: 24; max: 74) and around 41% of the participants were women.

Figure 2 depicts the BiomaCaRE cohorts included in the analyses of the current thesis. A detailed overview of each cohort is provided in the supplementary materials of Paper II and Paper III.

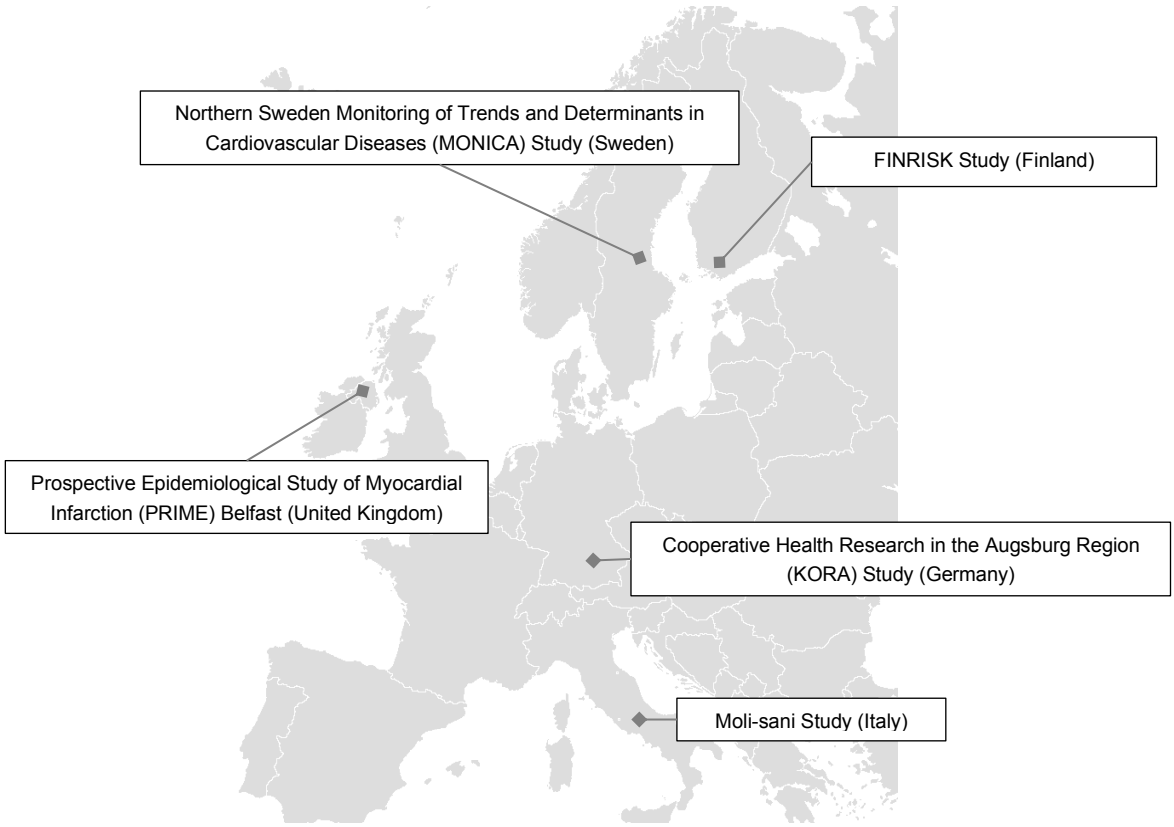

Figure 2. BiomarCaRE Consortium: cohorts included in the analyses of the current thesis

3.2 Assessment of type 2 diabetes and glucose and insulin-related traits

In KORA F4 and FF4, known type 2 diabetes was defined as self-reported diabetes that was validated through contacting the responsible physician, medical records or self-reported use of glucose-lowering medication in a personal interview. Participants without known diabetes were assigned to receive a standard 75-gram oral glucose tolerance test (OGTT). Data on fasting glucose, 2h-glucose, fasting insulin, as well as HbA1c were available for the analyses in Paper I. HOMA-IR and HOMA-B were calculated using the following formulas: HOMA-IR = (fasting insulin in µU/ml x fasting glucose mmol/l) / 22.5; and HOMA-B = (fasting insulin in µU/ml × 20) / (fasting glucose in mmol/l − 3.5).

Based on the OGTT data, normoglycaemia was defined as having fasting glucose < 6.1 mmol/l and 2h-glucose < 7.8 mmol/l; prediabetes as having fasting glucose ≥ 6.1 mmol/l but < 7.0 mmol/l and 2h-glucose < 7.8 mmol/l (impaired fasting glucose) or fasting glucose < 6.1 mmol/l and 2h-glucose ≥ 7.8 mmol/l but < 11.1 mmol/l (impaired glucose tolerance) or both impaired fasting glucose and impaired glucose tolerance; and newly diagnosed type 2 diabetes as having fasting glucose ≥ 7.0 mmol/l or 2h-glucose ≥ 11.1 mmol/l. The definitions are according to the 1999/2006 WHO criteria (69, 70). In Paper I, incident type 2 diabetes was defined as having known or newly diagnosed type 2 diabetes at follow-up in those without diabetes at baseline. Incident (pre)diabetes was defined as having prediabetes or known or newly diagnosed type 2 diabetes at follow-up, in those being normoglycaemic at baseline.

In the BiomarCaRE database, OGTT data are not available. In Paper II and Paper III, incidence of type 2 diabetes was defined as having newly diagnosed type 2 diabetes during follow-up, which was assessed either through medical records or self-report in participants without history of diabetes at baseline.

3.3 Measurement of cardiovascular hormones

Several cardiovascular hormones have a very short half-life that makes it difficult to reliably quantify the hormone release into the circulation. Thus, during the past years, assays have been developed to measure the inactive pro-hormones fragments, which are biologically more stable but are released in equimolar concentrations as the mature hormones, as surrogates (71-74).

In all three papers included in this thesis, cardiovascular hormones were measured and analysed in their stable inactive pro-hormone fragments. The natriuretic peptides, BNP and ANP, were measured as N-terminal-pro-BNP (NT-proBNP) and mid-regional-pro-ANP (MR-proANP), respectively. ET-1, ADM and AVP, were assessed as C-terminal-pro-ET-1 (CT-proET-1), mid-regional-pro-ADM (MR-proADM), and C-terminal-pro-AVP (CT-proAVP) or copeptin, respectively. Assay methods are described in the respective papers.

3.4 Statistical analysis

The exact date of prediabetes and type 2 diabetes manifestation during follow-up was not available in KORA F4 and FF4. Thus, in Paper I, associations of MR-proANP, CT-proET-1, MR-proADM, and CT-proAVP with incident type 2 diabetes and incident (pre)diabetes were calculated using logistic regression analysis rather than time-to-event analysis. To estimate associations of the hormones with changes in glucose and insulin-related traits, we performed analysis of covariance (ANCOVA). All association analyses were adjusted for age, sex, waist circumference, height, actual hypertension, ratio of total and high-density lipoprotein (HDL) cholesterol, triglycerides levels, smoking status, physical activity and parental history of diabetes. Additionally, to quantify the added value of each hormone on predicting type 2 diabetes and (pre)diabetes beyond the conventional risk factors, we calculated the area under the receiver operating characteristic curve (AUC) and the category-free net reclassification improvement (cfNRI).

In Paper II, we conducted time-to-event analysis using Cox proportional hazard models to estimate associations of NT-proBNP and MR-proANP with incident type 2 diabetes. The models were adjusted for diabetes risk factors similarly to Paper I and were stratified by study cohort. As harmonised data on physical activity and parental history of diabetes were not available in BiomarCaRE, these variables were not included in the models. Possible differences in the association of both natriuretic peptides with incident type 2 diabetes by the presence of CVD were examined by conducting separate analyses for participants with and without a history of CVD and calculating the interactions between the natriuretic peptides and CVD history on multiplicative and additive scales. History of CVD was defined as the composite history of heart failure, myocardial infarction, and stroke. The associations were also investigated for nonlinearity with restricted cubic spline regressions. We further examined the potential interactions of the natriuretic peptides with body mass index (BMI) and sex in participants with and without CVD history, separately. To correct for multiple testing, we computed false discovery rate (FDR) with the Benjamini-Hochberg method. Interaction terms were considered relevant at FDR < 0.05. As a sensitivity analysis, we performed competing-risk analyses using Fine and Gray models to account for death without experiencing diabetes as a competing event.

In Paper III, missing values of CT-proET-1, MR-proADM, and other covariates were handled with multiple imputation by chained equations using R package mice (75). The associations of CT-proET-1 and MR-proADM with incident type 2 diabetes were examined in Cox proportional hazard models as described above. We tested for interactions of both hormones with BMI, waist circumference, sex, and actual hypertension. We also examined the associations for each hormone across subgroups of BMI (obese: BMI ≥ 30 kg/m^2 vs non-obese: < 30 kg/m^2), waist circumference (obese: in men ≥ 102 cm, in women ≥ 88 cm vs non-obese: in men < 102, in women < 88 cm), sex (men vs women) and actual hypertension (yes vs no). We corrected for multiple testing by computing FDR as described previously for Paper II. Interaction terms were considered relevant at FDR < 0.05.

In Paper II and Paper III, we performed two-sample univariate Mendelian randomisation analyses to examine possible causal links between cardiovascular hormones and type 2 diabetes by including summary statistics from published genome-wide association (GWA) studies on the cardiovascular hormones (exposure GWA studies) and on type 2 diabetes (outcome GWA studies). We extracted genetic association estimates of single nucleotide polymorphisms (SNPs) that are specific to each cardiovascular hormone at a P-value of < 5E-8 from the exposure GWA studies. Only independent SNPs (i.e. SNPs that are not in linkage disequilibrium with each other) were selected as the instrumental variables. The association estimates between the selected SNPs and type 2 diabetes were extracted from the outcome GWA studies. To compute Mendelian randomisation estimates, we took a ratio of the outcome and exposure estimates using the Wald method. When more than one SNP was included as the instrumental variables, we used the inverse variance weighted meta-analysis method to combine the Wald ratio estimates. The Mendelian randomisation estimates were computed on the odds ratio (OR) scale. Details on the procedure for performing Mendelian randomisation analyses are discussed in Paper II and Paper III.

In all three papers, NT-proBNP, MR-proANP, MR-proADM and CT-proAVP were log-transformed to approximate normality. All cardiovascular hormones were z-standardised to estimate the associations per 1-standard deviation (SD) increase. Statistical analyses in the current thesis were conducted with SAS software version 9.4 (SAS Institute Inc., Cary, NC, USA) (in Paper I) and R software version 4.0.3 (76) (in Paper II and Paper III).

4. Key findings

Key finding 1: Dysregulation of ANP, ET-1, ADM, and AVP were associated with impaired glucose metabolism (Paper I)

Using data from 1,773 KORA F4 and FF4 participants (119 developed type 2 diabetes during a median follow-up time of 6.4 years), we observed that MR-proANP was significantly and inversely associated with incident type 2 diabetes. The multivariable adjusted OR (95% confidence interval, CI) was 0.75 (0.58; 0.96) per 1-SD increase of log MR-proANP. No significant association with incident type 2 diabetes was detected for other hormones in our analysis comprising 960 participants with CT-proET-1, MR-proADM and CT-proAVP measurements (72 developed type 2 diabetes during follow-up). Furthermore, we observed that CT-proAVP was significantly and positively associated with incident (pre)diabetes. The multivariable adjusted OR (95% CI) was 1.29 (1.02; 1.63) per 1-SD increase of log CT-proAVP. The analysis was conducted in 802 normoglycaemic participants with CT-proET-1, MR-proADM and CT-proAVP measurements (145 developed either prediabetes or type 2 diabetes during follow-up). No significant associations with incident (pre)diabetes were observed for other hormones. When examining whether cardiovascular hormones could improve the prediction of incident type 2 diabetes and incident (pre)diabetes, we also did not observe any substantial improvements when each hormone was added to the model containing conventional diabetes risk factors.

Moreover, our results from ANCOVA using data from 936 participants show that CT-proET-1 and MR-proADM were positively associated with 6.4-year changes in insulin-related traits. In a model adjusted for age, sex, waist circumference and height, higher CT-proET-1 concentrations were significantly associated with increased concentrations of fasting insulin and HOMA-B. The beta estimates (95% CIs) per 1-SD increase of CT-proET-1 were 0.05 (0.002; 0.10) and 0.07 (0.01; 0.12), respectively. The association with HOMA-B remained significant in a model further adjusted for hypertension, ratio of total cholesterol and HDL, triglycerides,

smoking status, physical activity and parental history of diabetes (Beta estimate (95% CI) = 0.06 (0.003; 0.11)). Higher MR-proADM concentrations were significantly associated with increased concentrations of fasting insulin, HOMA-IR and HOMA-B. The beta estimates (95% CIs) in a model adjusted for all aforementioned risk factors per 1-SD increase of log MR-proADM were 0.08 (0.02; 0.14) for fasting insulin, 0.07 (0.01; 0.13) for HOMA-IR, and 0.09 (0.02; 0.15) for HOMA-B. We did not observe significant associations with any of the glucose and insulin-related traits for other hormones.

Key finding 2: Higher concentrations of BNP and ANP were associated with a lower incidence of type 2 diabetes. Analyses using genetic data suggest a probable causal link. The inverse association between BNP and incident type 2 diabetes was modified by the presence of CVD (Paper II)

We further analysed whether NT-proBNP and MR-proANP were associated with incident type 2 diabetes in a larger sample size and sought to provide novel evidence on whether the presence of CVD could modify these associations.

Using data from the BiomarCaRE consortium, we included 45,477 participants with NT-proBNP measurements (1,707 developed type 2 diabetes during a median follow-up time of 6.5 years; among these, 209 had CVD at baseline) and 11,537 participants with MR-proANP measurements (857 developed type 2 diabetes during a median follow-up time of 13.8 years; among these, 106 had CVD at baseline) in our time-to-event analyses using Cox proportional hazard models. We observed inverse associations of both NT-proBNP and MR-proANP with incident type 2 diabetes. The multivariable adjusted hazard ratios (HRs) with 95% CIs were 0.84 (0.79; 0.89) per 1-SD increase of log NT-proBNP and 0.77 (0.71; 0.83) per 1-SD increase of log MR-proANP. In line with these findings, our Mendelian randomisation analyses show that genetically predicted NT-proBNP and MR-proANP were also inversely associated with risk of type 2 diabetes. The ORs (95% CIs) were 0.93 (0.87; 0.98) for NT-proBNP and 0.91 (0.86; 0.97) for MR-proANP.

When examining whether these inverse associations differed by the presence of CVD, for NT-proBNP, we only observed a significant association in participants without CVD history but not in those with CVD history (P-values for interaction were < 0.001 on multiplicative scale and 0.015 on additive scale). The HRs (95% CIs) per 1-SD increase of log NT-proBNP in participants without and with CVD history were 0.81 (0.76; 0.86) and 1.04 (0.90; 1.19), respectively. Conversely, for MR-proANP, there was no significant difference in the association with incident type 2 diabetes between participants without and with CVD history (P-values for interaction were 0.236 on the multiplicative scale and 0.441 on the additive scale). The HRs (95% CIs) per 1-SD increase of log MR-proANP in participants without and with CVD history were 0.75 (0.69; 0.82) and 0.81 (0.66; 0.99), respectively.

Key finding 3: Higher concentrations of ET-1 and ADM were associated with a higher incidence of type 2 diabetes, but analyses using genetic data suggest a probable causal link for ET-1 only. The association for ADM was more apparent in obese than in non-obese individuals (Paper III)

Given our previous findings from Paper I on the associations of elevated concentrations of CT-proET-1 and MR-proADM with increased insulin-related traits, in Paper III we aimed to investigate whether CT-proET-1 and MR-proADM were associated with the incidence of type 2 diabetes in a larger sample size.

We included 12,006 participants from the BiomarCaRE Consortium initially without diabetes and CVD in our time-to-event analysis. 862 participants developed type 2 diabetes over 13.8 years of median follow-up. Our results using Cox proportional hazard models show that both CT-proET-1 and MR-proADM were positively associated with incident type 2 diabetes. The multivariable adjusted HRs (95% CIs) were 1.10 (1.03; 1.18) per 1-SD increase of CT-proET-1 and 1.11 (1.02; 1.21) per 1-SD increase of log MR-proADM.

We observed a significant interaction of MR-proADM with BMI and waist circumference. The interaction terms with BMI and waist circumference remained relevant after controlling for multiple testing (FDR < 0.05). When examining the

association across BMI subgroups, the positive association between MR-proADM and incident type 2 diabetes was only significant in obese participants. The HRs [95%CIs] per 1-SD increase of log MR-proADM were 1.19 [1.05; 1.34] in obese and 1.02 [0.90; 1.15] in non-obese participants. The results were similar when we stratified by waist circumference. For CT-proET-1, no relevant interactions with BMI, waist circumference, sex and hypertension could be detected at an FDR < 0.05.

Our Mendelian randomisation analyses yielded a significant association of genetically predicted CT-proET-1 with type 2 diabetes risk. No significant association was observed between genetically predicted MR-proADM and type 2 diabetes risk. The ORs (95%CIs) were 1.12 (1.03; 1.22) and 0.97 (0.74; 1.27) for CT-proET-1 and MR-proADM, respectively.

5. Discussion

The findings of this thesis suggest that elevated circulating concentrations of BNP and ANP are associated with a lower risk of type 2 diabetes, while elevated circulating concentrations of ET-1, ADM and AVP are associated with an increased risk of type 2 diabetes. Our analyses using genetic data added further evidence that genetically predicted BNP, ANP and ET-1 were associated with type 2 diabetes risk, proposing a probable causal relationship between these hormones and type 2 diabetes development.

This thesis is the first to report a positive association of ADM with risk of developing type 2 diabetes and to demonstrate that this association appears to be confined to obese individuals. We also observed that ADM was positively associated with increased insulin resistance. These findings raise the possibility that increased ADM release might be a compensatory action attempting to restrain insulin homeostasis in obesity rather than a causal risk factor of type 2 diabetes. We were also able to expand the current knowledge by showing that the inverse association of BNP and type 2 diabetes risk was modified by the presence of CVD, while similar differences were not seen for ANP.

The results of the specific analyses have been discussed in detail in Paper I – Paper III. In the following chapter, some methodological considerations and the implications of this work on clinical practice, as well as the direction for future research are discussed.

5.1 Methodological considerations

5.1.1 Study design

All three papers included in this thesis have a prospective cohort study design that allows to clarify the temporal sequence between exposure and outcome. However, due to the nature of observational studies, causality cannot be ascertained (77). Observational studies are prone to various types of bias arising from unmeasured

confounding and reverse causation (77, 78). For example, if people who have higher circulating concentrations of cardiovascular hormones are genetically more likely to develop type 2 diabetes, then we are not able to ascertain whether higher concentrations of the hormones actually cause type 2 diabetes.

In Paper II and Paper III, we conducted a two-sample Mendelian randomisation study in tandem with a prospective cohort study to allow a more robust analysis. Using genetic variants, Mendelian randomisation reduces biases that commonly occur in observational studies and tries to make causal inferences about the exposure effect on an outcome (79). However, Burgess, et al. (80) recently have demonstrated that Mendelian randomisation is not completely immune from reverse causation. For example, if the hormones influence type 2 diabetes risk and type 2 diabetes influences the hormones at a later time-point, then the Mendelian randomisation estimates would be invalid. Therefore, it is recommended to primarily consider Mendelian randomisation as a causal null hypothesis testing rather than a causal effect estimation (81). Furthermore, most of the assumptions for a valid genetic instrumental variable in Mendelian randomisation analyses cannot be directly tested. For example, if the SNP has an effect on the outcome that is not through the exposure (horizontal pleiotropic effect), then the causal estimates can be misleading (82). In this thesis, we tried to minimise this possibility by including only SNPs that are specific for each hormone as instrumental variables and by focusing our analysis on homogeneous ancestry groups. To date, several approaches have been developed to allow for SNPs with horizontal pleiotropic effects in a Mendelian randomisation analysis and even to decompose the direct and indirect causal effects of an exposure on an outcome, such as multivariable Mendelian randomisation with mediation analysis (83). However, due to a limited number of SNPs that have been identified for each hormone included in this thesis, we were unable to perform these analyses for our Mendelian randomisation approaches. More GWA studies are needed to identify more common genetic variants associated with the cardiovascular hormones to be able to perform more robust Mendelian randomisation analyses.

5.1.2 Study population

Based on the data from the KORA F4 and FF4 studies and from the BiomarCaRE consortium, we were able to examine associations of cardiovascular hormones with risk of type 2 diabetes in population-based samples. The use of harmonised data from the BiomarCaRE consortium also allows us to include a large sample size with a long-term follow-up of type 2 diabetes. The data included in this thesis represent the so far largest population-based cohort study examining the association of BNP, ANP, ET-1 and ADM with risk of developing type 2 diabetes. However, the examined study population were predominantly of European descent, which limits the generalisability of the results. Further studies are needed to confirm our findings in other ethnic groups.

5.1.3 Data assessment

The standardised epidemiological and laboratory procedures provide us with the best possible data allowing thorough adjustments for different diabetes risk factors and subgroup analyses. The availability of OGTT data in the KORA F4 and FF4 studies also enabled us to prospectively examine the associations of cardiovascular hormones with glucose and insulin-related traits and with the progression from normoglycaemia to prediabetes or type 2 diabetes. However, due to a relatively low number of incident type 2 diabetes cases in Paper I, our study does not have enough statistical power to examine the associations with incident type 2 diabetes separately for individuals with normoglycaemia and prediabetes at baseline. Furthermore, we had only a single measurement of the cardiovascular hormones at baseline and therefore, we could not take into account intra-individual variation in our analyses. This could have led to regression dilution bias and misclassification of study participants. Measurements of several known diabetes risk factors, such as diet and family history of diabetes, were also lacking, which could have led to some extend of residual confounding.

5.2 Implications on clinical practice and future directions

Overall, this thesis provides further insights into the link between cardiovascular and metabolic regulations and addresses the potential of cardiovascular hormones in the prevention of type 2 diabetes.

5.2.1 Bridging the knowledge gaps between cardiovascular and metabolic functions

Our understanding of the physiological effects of cardiovascular hormones has significantly improved. The findings of this thesis support the notion that cardiovascular hormones also regulate glucose metabolism, and that the dysregulation of these hormones is not only associated with the risk of developing CVD, but also type 2 diabetes. However, many other questions still need to be answered. In particular, more research is required to clarify the underlying mechanisms whereby cardiovascular hormones may influence type 2 diabetes risk. The interrelationship between different cardiovascular hormones and how this interrelationship affects the risk of type 2 diabetes also need to be elucidated. A better understanding of these mechanisms may lead to more effective prevention and may open avenues for new intervention strategies.

5.2.2 Risk stratification for diabetes prevention

Cardiovascular hormones investigated in this thesis have been widely used in clinical practice as biomarkers for diagnosis and management of CVD, especially heart failure (21). However, the role of these hormones in risk stratification of type 2 diabetes remains unclear. Although in this thesis the examined cardiovascular hormones did not substantially improve the prediction of type 2 diabetes, more studies examining the use of cardiovascular hormones as predictors of type 2 diabetes are warranted to fully elucidate the potential of these biomarkers for risk stratification and to better characterise individuals at a higher risk for personalised health care decisions.

5.2.3 Potential targets for lowering the risk of type 2 diabetes and improve glucose metabolism

The findings of this thesis support the hypothesis that there is a possible causal link of low BNP and ANP concentrations and high ET-1 concentrations with type 2 diabetes. Thus, targeting these hormones may be effective in lowering the risk of type 2 diabetes.

Recently, several clinical trials and their *post hoc* analyses have shown that the antihypertensive medication class of angiotensin receptor blockers in combination with neprilysin inhibitors (ARNi) increased the release of BNP and ANP and also improved glucose metabolism in patients with heart failure (84-87). The idea that glucose metabolism could be improved by augmenting the signalling of both natriuretic peptides in patients with heart failure is nevertheless paradoxical, because heart failure is associated with an increased release of BNP and ANP. It seems that the increased natriuretic peptide release in such patients is not enough to rescue glucose metabolism (19). Future studies need to examine the potential of targeting BNP and ANP in addressing both cardiovascular and metabolic diseases in more detail, and they should also examine whether targeting a specific natriuretic peptide would exert specific effects on cardiovascular and metabolic functions.

Regarding the use of ET-1 as a target of therapy, several endothelin receptor antagonists (ERAs) have been produced. For example, bosentan, ambrisentan, and macitentan that are approved for use in patients with pulmonary hypertension (88). However, the use of these drugs in other pathophysiological conditions, including metabolic diseases, has not been widely investigated. To date, only a few clinical trials have been conducted regarding the use of ERAs in diabetic patients with renal disease, in which the primary end points were related to endothelial and kidney function (89-91). However, the effects of ERAs on glucose metabolism were not specifically examined. More research is needed to shed light on the potential use of ERAs in type 2 diabetes.

6. Concluding remarks

CVD and type 2 diabetes commonly coexist. It is widely known that type 2 diabetes is a risk factor of CVD, but the relationship between the two diseases is actually bidirectional. An impairment in one system can directly affect pathophysiological developments in the other system, which suggests a shared pathological ground.

This thesis highlights the importance of cardiovascular hormones beyond CVD. Using epidemiological methodologies, we demonstrated that BNP, ANP, ET-1, ADM, and AVP are associated with the risk of type 2 diabetes and our findings suggest that dysregulation of these hormones, especially BNP, ANP and ET-1, could be implicated in the development of type 2 diabetes. Furthermore, these findings raise the possibility that targeting these cardiovascular hormones might be effective in preventing and managing not only CVD but also type 2 diabetes.

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8. Publications

Paper I. Associations of cardiac stress biomarkers with incident type 2 diabetes and changes in glucose metabolism

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Cardiovascular Diabetology

ORIGINAL INVESTIGATION

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Associations of cardiac stress biomarkers with incident type 2 diabetes and changes in glucose metabolism: KORA F4/FF4 study

Chaterina Sujana[1,2,3], Jochen Seissler[4], Jens Jordan[5], Wolfgang Rathmann[6,7], Wolfgang Koenig[8,9,10], Michael Roden[7,11,12], Ulrich Mansmann[2,3], Christian Herder[7,11,12], Annette Peters[1,7], Barbara Thorand[1,7*†] and Cornelia Then[4†]

Abstract

Background: High N-terminal pro-brain-type natriuretic peptide levels have been associated with a lower risk of type 2 diabetes mellitus (T2D). However, less is known about other cardiac stress biomarkers in this context. Here we evaluated the association of mid-regional pro-atrial natriuretic peptide (MR-proANP), C-terminal pro-arginine vasopressin (copeptin), C-terminal pro-endothelin-1 (CT-proET-1) and mid-regional pro-adrenomedullin (MR-proADM) with incident T2D and changes in glucose metabolism.

Methods: We performed a prospective cohort study using data from the population-based KORA F4/FF4 study. 1773 participants (52.3% women) with MR-proANP measurements and 960 (52.7% women) with copeptin, CT-proET-1 and MR-proADM measurements were included. We examined associations of circulating plasma levels of MR-proANP, copeptin, CT-proET-1 and MR-proADM with incident T2D, the combined endpoint of incident prediabetes/T2D and with fasting and 2 h-glucose, fasting insulin, HOMA-IR, HOMA-B and HbA1c at follow-up. Logistic and linear regression models adjusted for age, sex, waist circumference, height, hypertension, total/HDL cholesterol ratio, triglycerides, smoking, physical activity and parental history of diabetes were used to compute effect estimates.

Results: During a median follow-up time of 6.4 years (25th and 75th percentiles: 6.0 and 6.6, respectively), 119 out of the 1773 participants and 72 out of the 960 participants developed T2D. MR-proANP was inversely associated with incident T2D (odds ratio [95% confidence interval]: 0.75 [0.58; 0.96] per 1-SD increase of log MR-proANP). Copeptin was positively associated with incident prediabetes/T2D (1.29 [1.02; 1.63] per 1-SD increase of log copeptin). Elevated levels of CT-proET-1 were associated with increased HOMA-B at follow-up, while elevated MR-proADM levels were associated with increased fasting insulin, HOMA-IR and HOMA-B at follow-up. These associations were independent of previously described diabetes risk factors.

Conclusions: High plasma concentrations of MR-proANP contributed to a lower risk of incident T2D, whereas high plasma concentrations of copeptin were associated with an increased risk of incident prediabetes/T2D. Furthermore, high plasma concentrations of CT-proET-1 and MR-proADM were associated with increased insulin resistance. Our

*Correspondence: thorand@helmholtz-muenchen.de
†Barbara Thorand and Cornelia Then shared last authors
[1] Institute of Epidemiology, Helmholtz Zentrum München-German Research Centre for Environmental Health, Ingolstädter Landstrasse 1, 85764 Neuherberg, Germany
Full list of author information is available at the end of the article

study provides evidence that biomarkers implicated in cardiac stress are associated with incident T2D and changes in glucose metabolism.

Keywords: MR-proANP, Copeptin, CT-proET-1, MR-proADM, Cardiac stress biomarkers, Type 2 diabetes, Prediabetes, Insulin resistance, Incidence, Cohort study

Background

Type 2 diabetes mellitus (T2D) is a known major risk factor for heart failure [1], but the identification of biological pathways linking both diseases remains challenging. In recent years, several circulating biomarkers implicated in cardiac stress conditions have been shown to be associated with diabetes risk factors. For example, N-terminal pro-B-type natriuretic peptide (NT-proBNP) and mid-regional pro-atrial natriuretic peptide (MR-proANP) were inversely associated with metabolic syndrome and insulin resistance [2–4]. Several novel cardiac stress biomarkers like C-terminal pro-arginine-vasopressin (copeptin), C-terminal pro-endothelin-1 (CT-proET-1) and mid-regional pro-adrenomedullin (MR-proADM) were positively associated with metabolic syndrome and insulin resistance in cross-sectional settings [5, 6]. Copeptin was also associated with a family history of T2D in a recent study [7]. Collectively, these findings implicate cardiac stress biomarkers in the pathogenesis of T2D.

To date, a growing number of studies suggest that low NT-proBNP levels are associated with incident T2D [8–10]. Similarly, low MR-proANP levels were associated with incident T2D [8, 11]. Conversely, copeptin, that is commonly known as a biomarker for diabetes insipidus [12, 13], was positively associated with incident T2D in some studies [14–16], but others observed this positive association only in women [17]. In comparison to NT-proBNP, MR-proANP and copeptin have been less widely investigated regarding their roles in the development of T2D.

Furthermore, cross-sectional studies have shown that levels of CT-proET-1 and MR-proADM were elevated in patients with T2D [5, 18], but the few existing prospective studies failed to provide evidence of their associations with incident T2D [8, 16]. However, several animal studies have demonstrated that high endothelin-1 but low adrenomedullin were involved in the development of insulin resistance [19, 20], suggesting that both vasoactive peptides may be associated with changes in glucose metabolism.

The present study aimed to evaluate plasma levels of MR-proANP, copeptin, CT-proET-1 and MR-proADM for their putative associations with incident T2D, the combined endpoint of incident prediabetes/T2D and traits of glycaemia and insulin resistance (fasting and 2 h-glucose, fasting insulin, homeostasis model assessment of insulin resistance (HOMA-IR) and β-cell function (HOMA-B) and haemoglobin A1c (HbA1c)) at follow-up.

Methods

Study design and participants

We performed a prospective cohort study using data from Cooperative Health Research in the Augsburg Region (KORA) F4 (2006–2008) and FF4 (2013–2014) studies. KORA F4 and FF4 are follow-up examinations of the fourth survey of the population-based KORA study (KORA S4; 1999–2001) conducted in the South of Germany. The study design has been described previously in detail [21]. The three examinations were carried out in accordance with Declaration of Helsinki, including obtaining written informed consent from all participants. The study was approved by the ethics board of the Bavarian Chamber of Physicians (Munich, Germany).

This study included all persons aged 32–81 years participating in both KORA F4 (as baseline in the present analysis) and KORA FF4 studies (follow-up). Baseline characteristics of KORA F4 participants who did not participate in KORA FF4 and thus were excluded from the current analysis are summarised in Additional file 1: Table S1. Reasons for non-participation were: individuals had died, moved out of the study area, refused, were too ill/not interested/too busy to participate, or could not be contacted. We further excluded participants with diabetes at baseline, unclear diabetes status at baseline and follow-up, self-reported history of myocardial infarction and stroke, non-fasting participants prior to oral glucose tolerance test (OGTT) and participants with missing values on cardiac stress biomarkers or covariables.

For analysing the association with incident T2D, 1773 nondiabetic participants at baseline (278 had prediabetes) with MR-proANP measurements and 960 (158 had prediabetes) with copeptin, CT-proET-1 and MR-proADM measurements were included. For analysing the association with incident prediabetes/T2D, 1495 normoglycaemic participants at baseline with MR-proANP measurements and 802 with copeptin, CT-proET-1 and MR-proADM measurements were included. For analysing the associations with traits of glycaemia and insulin resistance we excluded participants who were taking

glucose-lowering medication at baseline and at follow-up. Exclusions are described in Fig. 1 in detail.

Assessment of T2D and prediabetes

Known T2D was defined as self-reported diabetes that was validated through contacting the responsible physician or medical chart review or current self-reported use of glucose-lowering medication. Participants without known diabetes were assigned to receive a standard 75 g OGTT. Normoglycaemia was defined as having glucose after overnight fasting (fasting glucose) < 6.1 mmol/l and 2 hours after glucose solution intake (2 h-glucose) < 7.8 mmol/l; prediabetes as having fasting glucose ≥ 6.1 mmol/l but < 7.0 mmol/l and 2 h-glucose < 7.8 mmol/l (isolated impaired fasting glucose [IFG]) or fasting glucose < 6.1 mmol/l and 2 h-glucose ≥ 7.8 mmol/l but < 11.1 mmol/l (isolated impaired glucose tolerance [IGT]) or both IFG and IGT; newly diagnosed diabetes as having fasting glucose ≥ 7.0 mmol/l or 2 h-glucose ≥ 11.1 mmol/l [21, 22].

Incident T2D was defined as having normoglycaemia or prediabetes at baseline and known or newly diagnosed T2D at follow-up. Incident prediabetes/T2D was defined as having normoglycaemia at baseline and prediabetes or known or newly diagnosed T2D at follow-up.

Laboratory measurements

During the baseline examinations in 2006–2008, blood samples from all participants were collected without stasis and kept at 4 °C until centrifugation. All included cardiac stress biomarkers were measured in plasma samples that were stored at − 80 °C until assayed. MR-proANP was measured in all KORA F4 study participants, while copeptin, CT-proET-1 and MR-proADM were measured in the first 1596 participants of the KORA F4 study. These biomarkers were assayed with novel sandwich fluoro-immunoassay (BRAHMS, Hennigsdorf, Berlin, Germany) using the automated system BRAHMS KRYPTOR. Copeptin, MR-proADM and CT-proET-1 were assayed simultaneously in 2010, while MR-proANP was assayed in 2016–2017. Intra- and inter-assay coefficients of variation were 3.5 and 3.4% for MR-proANP; 5.9 and 8.9% for copeptin; 4.8 and 6.9% for CT-proET-1; 4.5 and 7.8% for MR-proADM, respectively.

Glucose levels were measured in serum using a hexokinase method (GLUFlex, Dade Behring, Deerfield,

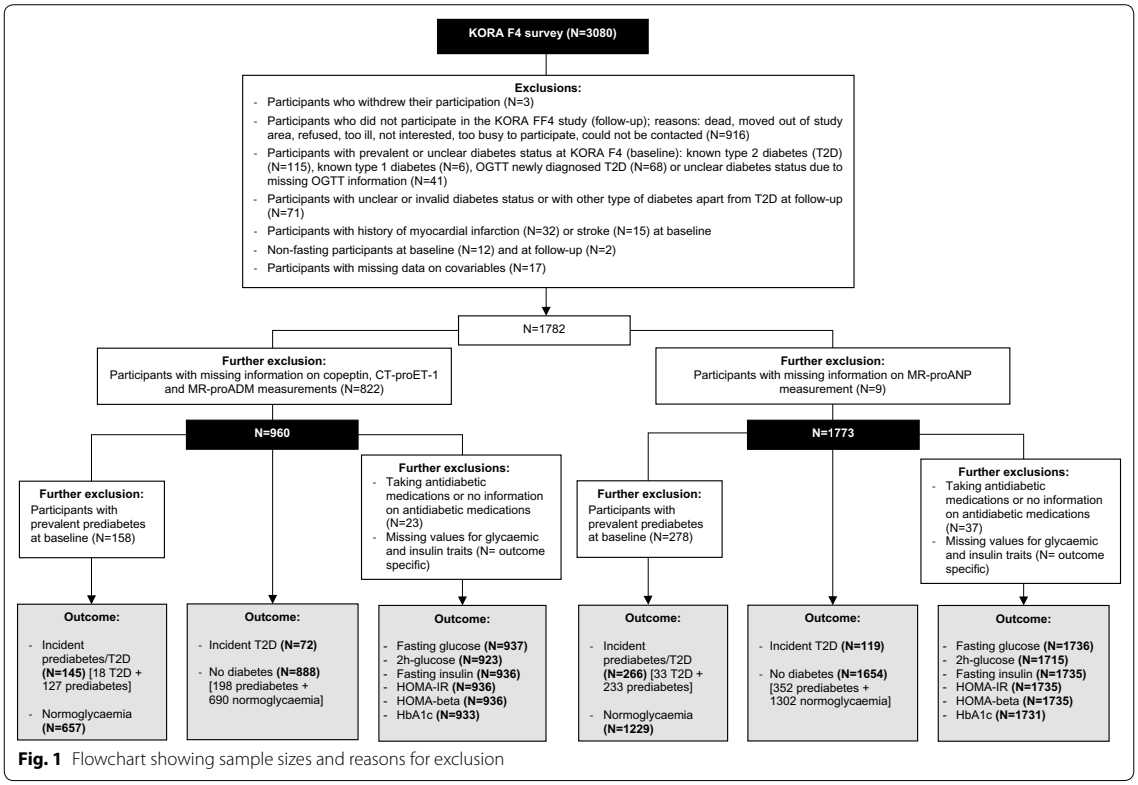

Fig. 1 Flowchart showing sample sizes and reasons for exclusion

USA) at baseline and an enzymatic colorimetric method on a Dimension Vista 1500 instrument (Siemens Healthcare Diagnostics Inc., Newark, NJ, USA) or the GLUC3 assay on a Cobas c702 instrument (Roche Diagnostics GmbH, Mannheim, Germany) at follow-up. Fasting insulin levels were measured in serum using an electrochemiluminescence immunoassay on a Cobas e602 instrument (Roche) at baseline and a solid-phase enzyme-labelled chemiluminescent immunometric assay on an Immulite 2000 systems analyser (Siemens) or an electrochemiluminescence immunoassay on a Cobas e602 instrument (Roche) at follow-up. HOMA-IR was calculated as (fasting insulin [μU/ml] x fasting glucose [mmol/l])/22.5 and HOMA-B was calculated as (fasting insulin [μU/ml] × 20)/(fasting glucose [mmol/l] − 3.5). HbA1c at baseline was assayed in haemolysed whole blood using high performance liquid chromatography on an Adams HA 8160 Haemoglobin Analysis System (Arkray Inc., distributed by A. Menarini Diagnostics, Florence, Italy) and at follow-up on Variant™ II Turbo HbA1c Kit–2.0 (Bio-Rad Laboratories Inc., Hercules, CA, USA). Calibration for different methods was performed as previously described [23].

Serum total cholesterol (TC) and high-density lipoprotein (HDL) were measured with enzymatic methods (CHOL Flex and AHDL Flex, Dade Behring, Marburg, Germany) and serum triglycerides with the GPO-PAP method (Dade Behring). All blood parameters, except for 2 h-glucose, were based on fasting blood samples.

Assessment of other covariables

Trained medical interviewers collected information on medical history, lifestyle and parental history of diabetes of all participants as described elsewhere [24]. Smoking status was categorised into never, former or current smoking. Physical activity was assessed according to duration of leisure time sport activities with four possible answers: [1] > 2 h/week, [2] 1–2 h/week, [3] < 1 h/week, [4] none, separately in winter and in summer. A total score for physical activity was obtained by summing the possible answers in winter and in summer. Participants who had a total score ≥ 5 were classified as 'physically inactive', otherwise 'physically active'. Parental history of diabetes was categorised into at least one parent with diabetes, no parent with diabetes or unknown (all remaining participants).

Waist circumference and height were measured by trained personnel as described previously [24]. Actual hypertension was defined as blood pressure ≥ 140/90 mmHg or use of antihypertensive medication given that the participants were aware of being hypertensive.

Statistical analysis

Participants characteristics are reported as mean (standard deviation (SD)) or geometric mean (antilog of SD) for continuous variables and percentages for categorical variables stratified by cases vs non-cases of incident T2D and of incident prediabetes/T2D. Characteristics between cases and non-cases were compared using analysis of variance and Chi squared test for continuous and categorical variables, respectively. Characteristics were calculated for all included participants, i.e. 1773 participants for the analysis of incident T2D and 1495 for the analysis of incident prediabetes/T2D.

The associations of cardiac stress biomarkers with incident T2D and incident prediabetes/T2D were analysed by calculating odds ratio (OR) with 95% confidence interval (CI) in logistic regression models. The exact date of prediabetes and T2D manifestation during follow-up was unknown, thus we conducted logistic regression analysis rather than survival (time-to-event) analysis. Cardiac stress biomarkers were investigated as continuous measures per 1-SD increase. MR-proANP, copeptin and MR-proADM were log-transformed to approximate normality. The distribution of CT-proET-1 was approximately normal and it was thus not log-transformed. All included cardiac stress biomarkers were z-standardized.

We performed analysis of covariance to estimate the associations of cardiac stress biomarkers with traits of glycaemia and insulin resistance at follow-up. The effect estimates (beta) with 95% CI were computed by assigning follow-up values of the continuous traits as outcome variables and including the baseline values as covariables in the linear regression models. To approximate normality, the continuous trait variables were log-transformed and z-standardized.

All association analyses were adjusted for sex (male/female), age, waist circumference, and height (all continuous) (model 1), plus actual hypertension (yes/no), ratio of total/HDL cholesterol (TC/HDL) (continuous), triglyceride concentration (continuous), smoking (current/former/never), physical activity (inactive/active) and parental history of diabetes (at least one parent/unknown/no) (model 2). We also performed sex-stratified analyses and tested for interaction by sex.

Additionally, we calculated area under the receiver operating characteristic curve (AUC) and category-free net reclassification improvement (cfNRI) to quantify the added predictive value of the cardiac stress biomarkers beyond the conventional diabetes risk factors. Differences in AUC with 95% CI were computed using the method described by DeLong et al. [25]. Overall cfNRI represents the sum of net proportions of persons correctly assigned a higher predicted risk for cases ($cfNRI_{cases}$) and a lower predicted risk for non-cases ($cfNRI_{non\text{-}cases}$) [26]. The

95% CIs for cfNRIs were computed using the percentile bootstrap method with 1000 iterations.

All statistical analyses were performed in participants with complete data of baseline and follow-up measurements and were conducted with SAS version 9.4 (SAS Institute Inc., Cary, NC, USA), except for cfNRI calculation that was conducted with R version 3.6.3 [27]. The *P*-values presented were two-tailed, and $P < 0.05$ was considered statistically significant.

Results

During a median follow-up time of 6.4 years (minimum: 5.1; 25th percentile: 6.0; 75th percentile: 6.6; maximum: 7.7), out of 1773 nondiabetic participants with MR-proANP measurements, 119 developed T2D, and out of 960 nondiabetic participants with copeptin, CT-proET-1 and MR-proADM measurements, 72 developed T2D. Furthermore, out of 1495 normoglycaemic participants with MR-proANP measurement, 266 developed prediabetes/T2D, and out of 802 participants with copeptin, CT-proET-1 and MR-proADM measurements, 145 developed prediabetes/T2D during follow-up.

The characteristics of study participants are presented in Table 1. The cases (both incident T2D and incident prediabetes/T2D) comprised more men than women. At baseline, the cases were on average older, had a higher waist circumference and TC/HDL ratio, higher triglyceride concentrations, were more frequently hypertensive, physically less active and more likely to have parents with diabetes. The cases had higher levels of MR-proANP, copeptin, CT-proET-1 and MR-proADM than the non-cases.

Table 1 Characteristics of study participants

	Incident T2D			Incident prediabetes/T2D		
	Cases[a] N = 119	Non-cases[b] N = 1654	*P*	Cases[c] N = 266	Non-cases[d] N = 1229	*P*
Male	56.3%	47.1%	0.052	54.5%	44.3%	*0.003*
Age, years	61.5 (10.8)	52.1 (11.8)	*< 0.001*	57.1 (11.3)	50.1 (11.4)	*< 0.001*
Waist circumference, cm	102.5 (13.0)	90.8 (13.3)	*< 0.001*	97.3 (11.7)	88.4 (12.2)	*< 0.001*
Height, cm	168.2 (9.4)	169.7 (9.5)	0.067	168.8 (9.5)	170.2 (9.6)	*0.032*
Actual hypertension	53.8%	26.5%	*< 0.001*	42.1%	20.3%	*< 0.001*
Parental diabetes history			*0.010*			*< 0.001*
At least 1 parent	30.3%	23.3%		29.3%	21.4%	
Unknown	21.0%	14.2%		21.8%	12.8%	
No diabetic parents	48.7%	62.5%		48.9%	65.8%	
Physically inactive	49.6%	39.3%	*0.027*	43.2%	37.2%	*0.034*
Smoking status			*0.011*			0.865
Current	10.1%	17.5%		18.1%	18.4%	
Former	34.5%	40.2%		36.8%	39.6%	
Never	55.5%	42.3%		45.1%	42.0%	
Ratio of total cholesterol/HDL	4.70 (1.21)	3.97 (1.14)	*< 0.001*	4.35 (1.20)	3.83 (1.09)	*< 0.001*
Triglycerides, mmol/l[e]	1.57 (1.68)	1.10 (1.71)	*< 0.001*	1.30 (1.71)	1.02 (1.67)	*< 0.001*
MR-proANP, pmol/l[e]	63.6 (1.7)	57.8 (1.6)	*0.026*	62.7 (1.6)	56.3 (1.6)	*< 0.001*
Copeptin, pmol/l[e,f]	6.29 (3.45)	5.19 (3.65)	0.225	7.22 (3.25)	4.93 (3.65)	*< 0.001*
CT-proET-1, pmol/l[f]	48.9 (9.6)	44.4 (11.6)	*< 0.001*	48.2 (13.6)	43.1 (10.9)	*< 0.001*
MR-proADM, nmol/l[e,f]	0.55 (1.24)	0.47 (1.26)	*< 0.001*	0.52 (1.27)	0.46 (1.25)	*< 0.001*

Characteristics were calculated in the cohort comprising of 1773 participants for the analysis of incident T2D and 1495 for the analysis of incident prediabetes/T2D. Data are presented as mean (SD) or geometric mean (antilog SD) for continuous variables and as percentages for categorical variables

Italic values indicate significant *P* values ($P < 0.05$)

CT-proET-1, C-terminal pro-endothelin-1; MR-proADM, mid-regional pro-adrenomedullin; MR-proANP, mid-regional pro-atrial natriuretic peptide; SD, standard deviation; T2D, type 2 diabetes

[a] No diabetes (normoglycaemia or prediabetes) at baseline and T2D at follow-up

[b] No diabetes (normoglycaemia or prediabetes) at baseline and follow-up

[c] Normoglycaemia at baseline and prediabetes or T2D at follow-up

[d] Normoglycaemia at baseline and follow-up

[e] Data with skewed distributions are presented as geometric mean (antilog SD)

[f] Data were calculated in 960 nondiabetic participants at baseline (72 developed T2D and 888 remained nondiabetic at follow-up) and 802 normoglycaemic participants (145 developed prediabetes/T2D and 657 remained normoglycaemic at follow-up)

Differences were also observed for traits of glycaemia and insulin resistance between cases and non-cases. The cases displayed higher levels of fasting and 2 h-glucose, fasting insulin, HOMA-IR, HOMA-B and HbA1c than the non-cases at baseline and at follow-up (Table 2).

Associations between cardiac stress biomarkers and incident T2D

MR-proANP was inversely associated with incident T2D in model 1. The OR [95% CI] was 0.70 [0.55; 0.89], $P = 0.004$ per 1-SD increase of log MR-proANP. The association was attenuated, but remained significant after additional adjustment according to model 2 (OR [95% CI] 0.75 [0.58; 0.96], $P = 0.025$; Table 3). When we excluded participants with prediabetes at baseline the associations remained significant (OR [95% CI] 0.60 [0.37; 0.96], $P = 0.033$; Additional file 1: Table S2).

Copeptin, CT-proET-1 and MR-proADM were not significantly associated with incident T2D (Table 3).

Table 3 Associations between cardiac stress biomarkers and incident type 2 diabetes

Biomarkers	$N_{cases/non\text{-}cases}$	Model	OR [95% CI]	*P*
MR-proANP	119/1645	Model 1	*0.70 [0.55; 0.89]*	*0.004*
		Model 2	*0.75 [0.58; 0.96]*	*0.025*
Copeptin	72/888	Model 1	1.03 [0.78; 1.36]	0.824
		Model 2	1.05 [0.79; 1.40]	0.730
CT-proET-1	72/888	Model 1	0.93 [0.69; 1.25]	0.626
		Model 2	0.82 [0.59; 1.14]	0.234
MR-proADM	72/888	Model 1	0.87 [0.62; 1.20]	0.389
		Model 2	0.85 [0.59; 1.21]	0.358

ORs [95% CI] were calculated per 1-SD increment of cardiac stress biomarkers

Model 1: adjusted for age, sex, waist circumference and height

Model 2: Model 1 + actual hypertension, ratio of total cholesterol and HDL, triglycerides, smoking status, physical activity and parental history of diabetes

Italic values indicate significant *P* values ($P < 0.05$)

CI, confidence interval; CT-proET-1, C-terminal pro-endothelin-1; MR-proADM, mid-regional pro-adrenomedullin; MR-proANP, mid-regional pro-atrial natriuretic peptide; OR, odds ratio; SD, standard deviation

Table 2 Traits of glycaemia and insulin resistance at baseline and follow-up

	Incident T2D			Incident prediabetes/T2D		
	Cases[a]	Non-cases[b]	*P*	Cases[c]	Non-cases[d]	*P*
Measurements at baseline						
Fasting serum glucose, mmol/l[e,f]	5.78 (1.09)	5.12 (1.10)	*< 0.001*	5.38 (1.07)	5.01 (1.08)	*< 0.001*
2 h serum glucose, mmol/l[e,g]	7.89 (1.23)	5.51 (1.29)	*< 0.001*	6.04 (1.20)	5.12 (1.25)	*< 0.001*
Fasting serum insulin, µU/ml[e,h]	13.46 (1.66)	8.14 (1.63)	*< 0.001*	9.98 (1.64)	7.46 (1.57)	*< 0.001*
HOMA-IR[e,h]	3.46 (1.71)	1.85 (1.70)	*< 0.001*	2.39 (1.68)	1.66 (1.62)	*< 0.001*
HOMA-B[e,h]	119.9 (1.7)	103.8 (1.6)	*0.004*	107.6 (1.6)	101.9 (1.5)	0.073
HbA1c, mmol/mol[i]	39.1 (3.5)	35.1 (3.5)	*< 0.001*	36.9 (3.2)	34.4 (3.3)	*< 0.001*
Measurements at follow-up						
Fasting serum glucose, mmol/l[e,f]	6.82 (1.22)	5.35 (1.10)	*< 0.001*	5.93 (1.12)	5.20 (1.08)	*< 0.001*
2h serum glucose, mmol/l[e,g]	11.75 (1.31)	5.73 (1.31)	*< 0.001*	8.02 (1.25)	5.19 (1.24)	*< 0.001*
Fasting serum insulin, µU/ml[e,h]	15.18 (1.69)	8.65 (1.73)	*< 0.001*	12.62 (1.72)	7.71 (1.66)	*< 0.001*
HOMA-IR[e,h]	4.60 (1.87)	2.06 (1.82)	*< 0.001*	3.33 (1.79)	1.78 (1.71)	*< 0.001*
HOMA-B[e,h]	95.0 (1.7)	96.0 (1.7)	0.864	106.2 (1.7)	92.5 (1.6)	*< 0.001*
HbA1c, mmol/mol[i]	43.2 (9.3)	35.2 (3.8)	*< 0.001*	37.8 (4.8)	34.6 (3.5)	*< 0.001*

Characteristics were calculated in the sample for analysing the association of cardiac stress biomarkers with glycaemic and insulin traits at the follow-up after excluding participants taking glucose-lowering medication. Data are presented as mean (SD) or geometric mean (antilog SD)

Italic values indicate significant *P* values ($P < 0.05$)

HbA1c, haemoglobin A1c; HOMA-B, homeostasis model assessment of beta-cell function; HOMA-IR, homeostasis model assessment of insulin resistance; SD, standard deviation; T2D, type 2 diabetes

[a] No diabetes (normoglycaemia or prediabetes) at baseline and T2D at follow-up

[b] No diabetes (normoglycaemia or prediabetes) at baseline and follow-up

[c] Normoglycaemia at baseline and prediabetes or T2D at follow-up

[d] Normoglycaemia at baseline and follow-up

[e] Data with skewed distributions are presented as geometric mean (antilog SD)

[f] Data were calculated in 1736 nondiabetic participants at baseline (84 developed T2D and 1652 remained nondiabetic) and 1484 normoglycaemic participants (257 developed prediabetes/T2D and 1227 remained normoglycaemic at follow-up)

[g] Data were calculated in 1715 nondiabetic participants at baseline (63 developed T2D and 1652 remained nondiabetic) and 1478 normoglycaemic participants (251 developed prediabetes/T2D and 1227 remained normoglycaemic at follow-up)

[h] Data were calculated in 1735 nondiabetic participants at baseline (84 developed T2D and 1651 remained nondiabetic) and 1484 normoglycaemic participants (257 developed prediabetes/T2D and 1227 remained normoglycaemic at follow-up)

[i] Data were calculated in 1731 nondiabetic participants at baseline (84 developed T2D and 1647 remained nondiabetic) and 1479 normoglycaemic participants (256 developed prediabetes/T2D and 1223 remained normoglycaemic at follow-up)

Sex-specific associations between the cardiac stress biomarkers and incident T2D are presented in Additional file 1: Table S3.

The AUC [95% CI] of conventional T2D risk factors (model 2) without any cardiac stress biomarkers predicting incident T2D in the full study population was 0.833 [0.799; 0.867] and 0.853 [0.814; 0.891] in the subpopulation with copeptin, CT-proET-1 and MR-proADM measurements. None of the cardiac stress biomarkers individually improved the AUC significantly on top of model 2 (Additional file 1: Table S4). The overall cfNRI was significantly improved when MR-proANP was added to model 2 ($cfNRI_{overall}$ [95% CI] 0.211 [0.015; 0.466]), but cfNRI for cases and non-cases were not significantly improved ($cfNRI_{cases}$ [95% CI] 0.109 [−0.018; 0.273] and $cfNRI_{non\text{-}cases}$ [95% CI] 0.102 [−0.009; 0.231]). None of the other cardiac stress biomarkers significantly improved the cfNRI when added to model 2 (Additional file 1: Table S4).

Associations between cardiac stress biomarkers and incident prediabetes/T2D

Copeptin was positively associated with incident prediabetes/T2D. The OR [95% CI] was 1.30 [1.03; 1.63], $P=0.027$ per 1-SD increase of log copeptin in model 1. The association remained similar after further adjustment according to model 2 (OR [95% CI] 1.29 [1.02; 1.63], $P=0.033$; Table 4). In a sensitivity analysis excluding participants who progressed from normoglycaemia at baseline to T2D at follow-up, copeptin was also positively associated with incident prediabetes alone (OR [95% CI] 1.43 [1.10; 1.86], $P=0.008$; Additional file 1: Table S2).

We observed no significant associations of MR-proANP, CT-proET-1 and MR-proADM with incident prediabetes/T2D (Table 4). Sex-specific associations between the cardiac stress biomarkers and incident prediabetes/T2D are presented in Additional file 1: Table S5.

The AUC [95% CI] of the conventional T2D risk factors (model 2) predicting incident prediabetes/T2D without any cardiac stress biomarkers was 0.779 [0.750; 0.807] in the total study population and 0.796 [0.760; 0.832] in the subpopulation with available copeptin, CT-proET-1 and MR-proADM measurements. The addition of the cardiac stress biomarkers individually to model 2 did not significantly improve the AUC and overall cfNRI (Additional file 1: Table S6).

Table 4 Associations between cardiac stress biomarkers and the combined incident prediabetes/type 2 diabetes endpoint

Biomarkers	$N_{cases/non\text{-}cases}$	Model	OR [95% CI]	*P*
MR-proANP	266/1229	Model 1	0.91 [0.76; 1.09]	0.297
		Model 2	0.94 [0.78; 1.14]	0.539
Copeptin	145/657	Model 1	*1.30 [1.03; 1.64]*	*0.027*
		Model 2	*1.29 [1.02; 1.63]*	*0.033*
CT-proET-1	145/657	Model 1	1.15 [0.95; 1.40]	0.154
		Model 2	1.15 [0.94; 1.41]	0.169
MR-proADM	145/657	Model 1	1.09 [0.85; 1.38]	0.497
		Model 2	1.08 [0.84; 1.39]	0.543

ORs [95% CI] were calculated per 1-SD increment of cardiac stress biomarkers

Model 1: adjusted for age, sex, waist circumference and height

Model 2: Model 1 + actual hypertension, ratio of total cholesterol and HDL, triglycerides, smoking status, physical activity and parental history of diabetes

Italic values indicate significant *P* values ($P < 0.05$)

CI, confidence interval; CT-proET-1, C-terminal pro-endothelin-1; MR-proADM, mid-regional pro-adrenomedullin; MR-proANP, mid-regional pro-atrial natriuretic peptide; OR, odds ratio; SD, standard deviation

Associations of cardiac stress biomarkers with traits of glycaemia and insulin resistance at follow-up

Elevated MR-proANP levels were only significantly associated with reduced HbA1c in model 1, but the association was attenuated to non-significance after further adjustment in model 2 (Fig. 2). Copeptin was not significantly associated with any of the assessed continuous traits at follow-up.

Elevated levels of CT-proET-1 and MR-proADM at baseline were significantly associated with higher fasting insulin and HOMA-B at follow-up in model 1. Elevated MR-proADM levels were also significantly associated with higher 2 h-glucose and HOMA-IR in model 1 (Fig. 2, model 1). Further adjustment (model 2) attenuated the positive association between CT-proET-1 and fasting insulin and also the positive association between MR-proADM and 2 h-glucose to non-significance (Fig. 2, model 2). Additionally, we observed significant inverse associations of CT-proET-1 and MR-proADM with HbA1c, even after further adjustment in model 2.

Discussion

In the current study elevated MR-proANP levels were associated with a lower risk of incident T2D, elevated copeptin levels were associated with a higher risk of incident prediabetes/T2D, and elevated CT-proET-1 and MR-proADM levels were associated with increases in several traits of insulin resistance during the follow-up period. Overall, the examined cardiac stress biomarkers did not substantially improve the prediction of incident T2D and incident prediabetes/T2D when added to the models containing conventional diabetes risk factors. As previous research demonstrated, the predictive ability of established diabetes risk scores is rarely considerably improved by the addition of single novel biomarkers,

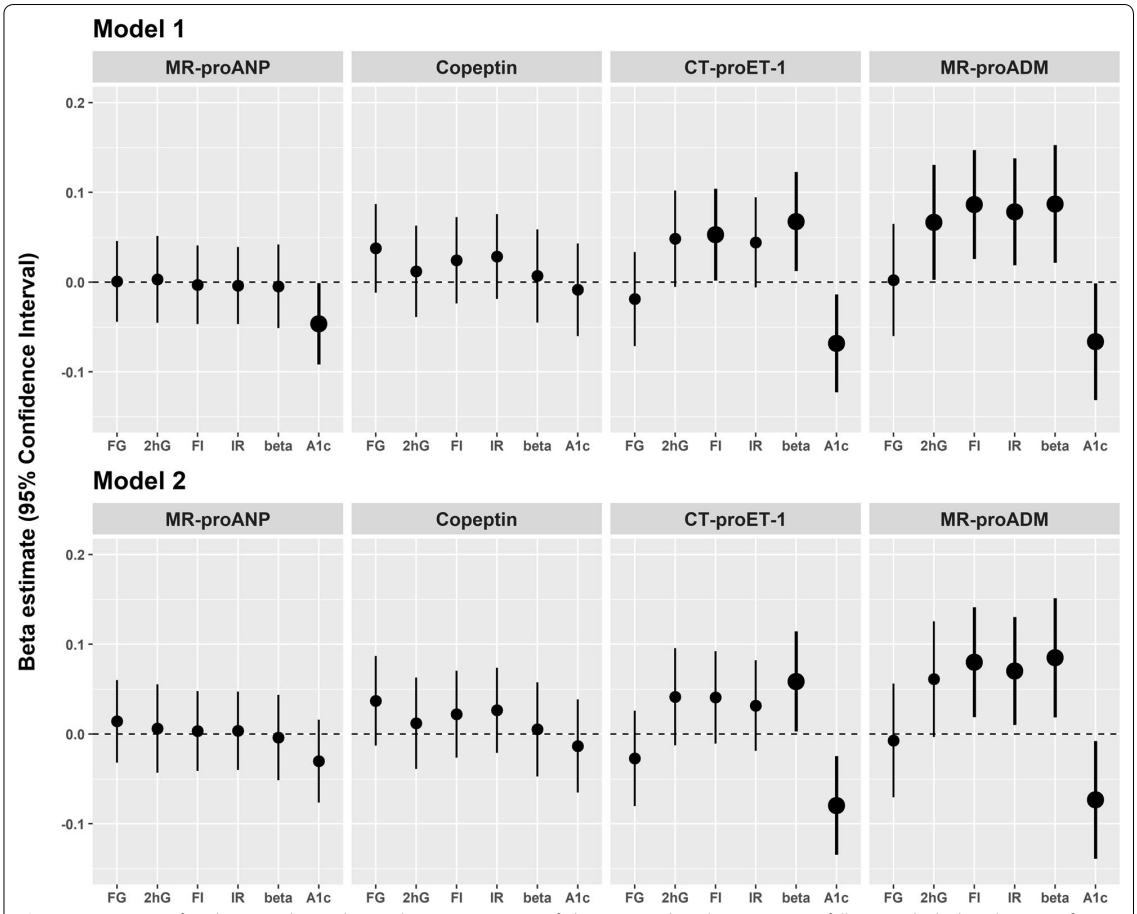

Fig. 2 Associations of cardiac stress biomarkers with continuous traits of glycaemia and insulin resistance at follow-up. Thick plots show significant associations. The analyses were adjusted for baseline value of glycaemic or insulin traits, age, sex, waist circumference and height (model 1) and were further adjusted for actual hypertension, ratio of total cholesterol and HDL, triglycerides, smoking status, physical activity and parental history of diabetes (model 2). Abbreviations: 2 hG, 2 h-glucose; A1c, haemoglobin A1c; beta, homeostasis model assessment of beta-cell function; CT-proET-1, C-terminal pro-endothelin-1; FG, fasting glucose; FI, fasting insulin; IR, homeostasis model assessment of insulin resistance; MR-proADM, mid-regional pro-adrenomedullin; MR-proANP, mid-regional pro-atrial natriuretic peptide

however, this does not preclude relevant associations between the biomarkers and T2D [28].

MR-proANP and incident T2D

Our results describing the inverse association of MR-proANP with incident T2D are consistent with results from previous studies [8, 11]. Additionally, we observed a significant improvement of overall cfNRI but not AUC in predicting incident T2D after the addition of MR-proANP to model 2. This may be explained by the fact that the cfNRI is more sensitive to small changes in predicted risk after the addition of a new marker than the AUC [29]. We are not aware of any other studies addressing improvement in T2D prediction by the addition of MR-proANP.

Furthermore, our study adds evidence that higher MR-proANP levels were associated with decreased HbA1c at follow-up in a model adjusted for age, sex, waist circumference and height. However, further adjustment attenuated this association to non-significance.

Low atrial natriuretic peptide (ANP) levels were associated with the activation of the renin-angiotensin system [30], which in turn could promote the development of insulin resistance and thus T2D [31]. However, in the present study we observed no significant associations of

baseline MR-proANP with any of the assessed insulin traits at follow-up. By contrast, a recent investigation from the MDC study [32] among 2243 nondiabetic participants who were followed up for 16.5 years reported a significant inverse association of MR-proANP with fasting insulin and HOMA-IR at follow-up. However, the authors reported no significant associations of MR-proANP with fasting and 2 h-glucose at follow-up as seen in our results.

Findings from experimental studies suggest that natriuretic peptides directly affect metabolism in skeletal muscle and adipose tissue. Natriuretic peptides were shown to induce fat oxidative capacity, reduce lipotoxicity and enhance insulin signalling in skeletal muscle and promote lipolysis, browning and glucose uptake in adipose tissue [33]. ANP also inhibited the secretion of adipokines and cytokines involved in inflammation and insulin resistance [34]. These biological effects of ANP improved insulin sensitivity and blood glucose control [33] and may thereby explain the inverse association of MR-proANP and incident T2D.

Copeptin and incident prediabetes/T2D

Our findings on the positive association between copeptin and incident prediabetes/T2D among normoglycaemic participants at baseline are in accordance with findings from the DESIR study [15] showing a positive association between copeptin and incident IFG/T2D among participants with normal fasting glucose at baseline. However, in the present study, copeptin was not significantly associated with incident T2D among nondiabetic participants at baseline. Similar to our finding, the FINRISK study [8] also observed no significant associations between copeptin and incident T2D among nondiabetic participants at baseline. In contrast, investigations from the MDC study [14] and the British Regional Heart Study [35] reported positive associations between copeptin and incident T2D among nondiabetic participants at baseline. These associations were stronger in participants without IFG than in all nondiabetic participants at baseline. Recently, higher copeptin levels were reported in participants with prediabetes than in participants with T2D [7]. This finding corroborates our results on a more pronounced association of copeptin with incident prediabetes alone than with the combined incident prediabetes/T2D. Unfortunately, our study is underpowered to examine the association between copeptin and incident T2D alone among participants with normoglycaemia at baseline.

Previously, elevated copeptin was reported to be associated with increased insulin resistance [14, 15] which may partly explain our findings. Of note, we also observed a trend towards a positive association of copeptin with fasting insulin and HOMA-IR. The active peptide arginine vasopressin directly stimulates glucagon and insulin release from pancreas and induces hepatic glycogenolysis [36]. Interestingly, higher rather than lower copeptin levels were reported in participants treated with empagliflozin, which is known to reduce hyperglycaemia, than in participants treated with placebo [37]. Although this finding might be due to a mild volume depletion caused by sodium-glucose cotransporter-2 inhibition of empagliflozin [37], further studies are needed to understand the mechanism of copeptin-induced hyperglycaemia.

The utility of copeptin in the prediction of prediabetes/T2D is not widely understood. In the present analysis, we did not observe a significantly improved prediction of incident prediabetes/T2D after the addition of copeptin to models containing conventional diabetes risk factors. However, Abbasi et al. [17] shows that copeptin significantly improved the prediction of T2D in addition to conventional diabetes risk factors in women, but not in men. More studies are needed to understand the clinical significance of copeptin in the prediction of prediabetes/T2D beyond the known role of predicting clinical outcomes of heart failure [38] and other major cardiovascular events in T2D patients [39].

CT-proET-1, MR-proADM and insulin resistance

In the present study we also observed positive associations of CT-proET-1 and MR-proADM with increased traits of insulin resistance during follow-up in nondiabetic participants. Our findings extend findings from previous cross-sectional analyses demonstrating that CT-proET-1 and MR-proADM were positively associated with metabolic risk factors and insulin resistance [5, 40, 41]. To the best of our knowledge, this is the first study to examine the prospective associations of CT-proET-1 and MR-proADM with fasting insulin, HOMA-IR and HOMA-B in a nondiabetic population.

Several in vitro and experimental studies have shown that endothelin-1 stimulated insulin secretion directly from pancreas [19, 42]. Endothelin-1 also limited insulin action [43] and decreased adiponectin mRNA levels in adipocytes [44]. These biological effects may lead to the development of insulin resistance and thus, support our findings. The positive association between CT-proET-1 with higher HOMA-B at follow-up in our study was most likely a consequence of its positive association with insulin resistance. Furthermore, there is evidence that endothelin-1 inhibits glucose uptake in human skeletal muscle [45] that may further explain the trend of a positive association between CT-proET-1 and 2 h-glucose at follow-up in our study as 2 h-glucose mainly reflects muscle glucose uptake [46]. Interestingly, we also observed an inverse association between CT-proET-1

and HbA1c. The reason for this finding is still unknown and further confirmation is needed.

Regarding the association between MR-proADM and insulin resistance, we found that higher MR-proADM levels were positively rather than inversely associated with fasting insulin, HOMA-IR and HOMA-B at follow-up. Our findings are contradictory to the results of a previous animal study [20]. The mechanistic evidence linking MR-proADM and insulin resistance is not well understood and controversial. In the previous animal study [20] adrenomedullin deficiency increased oxidative stress and induced insulin resistance. Adrenomedullin also inhibited insulin secretion on the pancreatic islets and reduced insulin levels accompanied by an increase in postprandial glucose [47]. In contrast, high adrenomedullin levels stimulated interleukin-6 and remarkably potentiated stimulatory effects of tumor necrosis factor-α, interleukin-1β and lipopolysaccharide that contribute to the development of insulin resistance [48]. A recent epidemiological study further demonstrated that high MR-proADM was associated with increased BMI and waist circumference at follow-up [49]. The study also reported that high MR-proADM was associated with lower fasting glucose levels that contradicts evidence from the animal models [47]. More studies are needed to confirm our findings on the associations between high MR-proADM and increased insulin resistance at follow-up.

Strengths and limitations

Strengths of the current study include the prospective study design, the population-based sample and the availability of OGTT data at baseline and follow-up. This enabled us to examine prospective associations between the included cardiac stress biomarkers with progression from normoglycaemia to prediabetes/T2D and with the continuous traits of glycaemia and insulin resistance at follow-up.

Limitations of this study are: Our study has a relatively low number of incident T2D cases and is therefore not sufficiently powered to examine associations with incident T2D among participants with normoglycaemia and prediabetes at baseline separately. Furthermore, participants who participated in KORA FF4 tended to be healthier than those who did not participate, which could have introduced some selection bias. Due to the lack of data on history of heart failure at baseline, we were unable to exclude participants with heart failure. Due to the lack of follow-up data on the included cardiac stress biomarkers, we were also unable to examine changes of the biomarkers in the progression towards diabetes. Our study participants were predominantly European descent, which means that further studies need to confirm our findings in other ethnic groups.

Conclusions

High plasma concentrations of MR-proANP were associated with a lower risk of incident T2D, whereas high plasma concentrations of copeptin were associated with an increased risk of incident prediabetes/T2D. Furthermore, high plasma concentrations of CT-proET-1 and MR-proADM were associated with increased insulin resistance during follow-up time. Our study provides evidence that biomarkers implicated in cardiac stress are associated with incident T2D and changes in glucose metabolism.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s12933-020-01117-1.

Additional file 1: Table S1. Characteristics of KORA F4 study participants stratified by participation in the follow-up study KORA FF4. **Table S2.** Associations of cardiac stress biomarkers with incident type 2 diabetes and incident prediabetes among different subgroups. **Table S3.** Sex-specific associations between cardiac stress biomarkers and incident type 2 diabetes. **Table S4.** Predictive performance of cardiac stress biomarkers in predicting incident type 2 diabetes. **Table S5.** Sex-specific association between cardiac stress biomarkers and incident prediabetes/type 2 diabetes. **Table S6.** Predictive performance of cardiac stress biomarkers in predicting incident prediabetes/type 2 diabetes.

Abbreviations

ANP: Atrial natriuretic peptide; AUC: Area under the receiver operating characteristic curve; $cfNRI_{cases}$: Category-free net reclassification improvement for cases; $cfNRI_{non\text{-}cases}$: Category-free net reclassification improvement for non-cases; $cfNRI_{overall}$: Overall category-free net reclassification improvement; CI: Confidence interval; CT-proET-1: C-terminal pro-endothelin-1; HbA1c: Haemoglobin A1c; HDL: High density lipoprotein; HOMA-B: Homeostasis model assessment of insulin resistance β-cell function; HOMA-IR: Homeostasis model assessment of insulin resistance; IFG: Isolated impaired fasting glucose; IGT: Isolated impaired glucose tolerance; KORA: Cooperative Health Research in the Region of Augsburg; MR-proADM: Mid-regional pro-adrenomedullin; MR-proANP: Mid-regional pro-atrial natriuretic peptide; NT-proBNP: N-terminal pro-B-type natriuretic peptide; OGTT: Oral glucose tolerance test; OR: Odds ratio; SD: Standard deviation; TC: Total cholesterol; T2D: Type 2 diabetes mellitus.

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Authors' contributions

CS and BT conceptualized the current study design. CS wrote the manuscript and conducted the statistical analyses. JS, JJ, WR, WK, MR, CH, AP, BT and CT collected and researched data. CS, BT, CT, UM, and CH contributed to data interpretation. All authors contributed to and critically reviewed the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The data are subject to national data protection laws and restrictions were imposed by the Ethics Committee of the Bavarian Chamber of Physicians to ensure data privacy of the study participants. Therefore, data cannot be made freely available in a public repository. However, data can be requested through an individual project agreement with KORA via the online portal KORA.passt (https://epi.helmholtz-muenchen.de/).

Ethics approval and consent to participate

The KORA F4/FF4 study was approved by the local authorities and carried out in accordance with the Declaration of Helsinki. The ethics approval number is EC No. 06068. All participants provided written informed consent.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

[1] Institute of Epidemiology, Helmholtz Zentrum München-German Research Centre for Environmental Health, Ingolstädter Landstrasse 1, 85764 Neuherberg, Germany. [2] Institute for Medical Information Processing, Biometry, and Epidemiology (IBE), Ludwig-Maximilians-Universität, Munich, Germany. [3] Pettenkofer School of Public Health, Munich, Germany. [4] Medizinische Klinik und Poliklinik IV, Klinikum der Universität München, Ludwig-Maximilians-Universität, Munich, Germany. [5] Institute of Aerospace Medicine, German Aerospace Center (DLR) and University of Cologne, Cologne, Germany. [6] Institute for Biometrics and Epidemiology, German Diabetes Center, Leibniz Center for Diabetes Research at Heinrich Heine University Düsseldorf, Düsseldorf, Germany. [7] German Center for Diabetes Research (DZD), Munich-Neuherberg, Germany. [8] Deutsches Herzzentrum München, Technische Universität München, Munich, Germany. [9] German Centre for Cardiovascular Research (DZHK), Partner Site Munich Heart Alliance, Munich, Germany. [10] Institute of Epidemiology and Medical Biometry, University of Ulm, Ulm, Germany. [11] Institute for Clinical Diabetology, German Diabetes Center, Leibniz Center for Diabetes Research at Heinrich Heine University Düsseldorf, Düsseldorf, Germany. [12] Division of Endocrinology and Diabetology, Medical Faculty, Heinrich Heine University Düsseldorf, Düsseldorf, Germany.

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Paper II. Natriuretic peptides and risk of type 2 diabetes

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Natriuretic Peptides and Risk of Type 2 Diabetes: Results From the Biomarkers for Cardiovascular Risk Assessment in Europe (BiomarCaRE) Consortium

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Chaterina Sujana,[1,2,3] Veikko Salomaa,[4] Frank Kee,[5] Simona Costanzo,[6] Stefan Söderberg,[7] Jens Jordan,[8] Pekka Jousilahti,[4] Charlotte Neville,[5] Licia Iacoviello,[6,9] Viktor Oskarsson,[7] Dirk Westermann,[10] Wolfgang Koenig,[11,12,13] Kari Kuulasmaa,[4] Jaakko Reinikainen,[4] Stefan Blankenberg,[10,14] Tanja Zeller,[10,14] Christian Herder,[15,16,17] Ulrich Mansmann,[2] Annette Peters,[1,3,13] and Barbara Thorand,[1,3] for the BiomarCaRE Consortium

OBJECTIVE

Natriuretic peptide (NP) concentrations are increased in cardiovascular diseases (CVDs) but are associated with a lower diabetes risk. We investigated associations of N-terminal pro-B-type NP (NT-proBNP) and midregional proatrial NP (MR-proANP) with incident type 2 diabetes stratified by the presence of CVD.

RESEARCH DESIGN AND METHODS

Based on the Biomarkers for Cardiovascular Risk Assessment in Europe (BiomarCaRE) Consortium, we included 45,477 participants with NT-proBNP measurements (1,707 developed type 2 diabetes over 6.5 years of median follow-up; among these, 209 had CVD at baseline) and 11,537 participants with MR-proANP measurements (857 developed type 2 diabetes over 13.8 years of median follow-up; among these, 106 had CVD at baseline). The associations were estimated using multivariable Cox regression models.

RESULTS

Both NPs were inversely associated with incident type 2 diabetes (hazard ratios [95% CI] per 1-SD increase of log NP: 0.84 [0.79; 0.89] for NT-proBNP and 0.77 [0.71; 0.83] for MR-proANP). The inverse association between NT-proBNP and type 2 diabetes was significant in individuals without CVD but not in individuals with CVD (0.81 [0.76; 0.86] vs. 1.04 [0.90; 1.19]; *P* multiplicative interaction = 0.001). There was no significant difference in the association of MR-proANP with type 2 diabetes between individuals without and with CVD (0.75 [0.69; 0.82] vs. 0.81 [0.66; 0.99]; *P* multiplicative interaction = 0.236).

CONCLUSIONS

NT-proBNP and MR-proANP are inversely associated with incident type 2 diabetes. However, the inverse association of NT-proBNP seems to be modified by the presence of CVD. Further investigations are warranted to confirm our findings and to investigate the underlying mechanisms.

B-type natriuretic peptide (BNP) and atrial natriuretic peptide (ANP) are cardiac hormones that exert physiological actions not only on cardiovascular homeostasis but also on glucose and lipid metabolism. Both natriuretic peptides (NPs) increase mitochondrial fat oxidative capacity in skeletal muscle and promote lipolysis,

[1]Institute of Epidemiology, Helmholtz Zentrum München–German Research Center for Environmental Health, Neuherberg, Germany
[2]Institute for Medical Information Processing, Biometry, and Epidemiology (IBE), Pettenkofer School of Public Health, Ludwig-Maximilians-Universität, Munich, Germany
[3]German Center for Diabetes Research (DZD), Partner Munich-Neuherberg, Munich-Neuherberg, Germany
[4]Department of Public Health and Welfare, Finnish Institute for Health and Welfare, Helsinki, Finland
[5]Centre for Public Health, Queens University of Belfast, Belfast, Northern Ireland, U.K.
[6]Department of Epidemiology and Prevention, IRCCS Neuromed, Pozzilli, Isernia, Italy
[7]Department of Public Health and Clinical Medicine, Umeå University, Umeå, Sweden
[8]Institute of Aerospace Medicine, German Aerospace Center (DLR) and University of Cologne, Cologne, Germany
[9]Research Center in Epidemiology and Preventive Medicine (EPIMED), Department of Medicine and Surgery, University of Insubria, Varese, Italy
[10]Department for General and Interventional Cardiology, University Heart Center Hamburg, Hamburg, Germany
[11]Institute of Epidemiology and Medical Biometry, University of Ulm, Ulm, Germany
[12]Deutsches Herzzentrum München, Technische Universität München, Munich, Germany
[13]German Centre for Cardiovascular Research (DZHK e.V.), partner site Munich Heart Alliance, Munich, Germany
[14]German Centre for Cardiovascular Research (DZHK e.V.), partner site Hamburg, Lübeck, Kiel, Hamburg, Germany
[15]Institute for Clinical Diabetology, German Diabetes Center, Leibniz Center for Diabetes Research at Heinrich-Heine-University Düsseldorf, Düsseldorf, Germany

browning of white adipocytes, oxygen consumption, glucose uptake in adipose tissue and also modulate cytokine and adipokine responses (1,2). Altogether, these biological effects ameliorate insulin resistance and blood glucose control (3).

In previous epidemiological studies, higher concentrations of N-terminal pro-BNP (NT-proBNP) (4–9) and midregional pro-ANP (MR-proANP) (4,10–12), the inactive fragments of BNP and ANP, respectively, were associated with a lower risk of type 2 diabetes. However, circulating concentrations of both NPs are elevated in cardiovascular diseases (CVDs) to compensate for cardiac pressure overload (2) and predict CVD prognosis (13). CVDs, such as heart failure (HF), myocardial infarction (MI), and stroke, can give rise to abnormalities in glucose metabolism (14–16). For instance, HF invokes a compensatory neurohumoral response, which increases free fatty acids, thereby inhibiting muscular glucose uptake and insulin signaling (14). These effects, in turn, predispose to insulin resistance and type 2 diabetes. To date, it remains uncertain whether high NP concentrations are also associated with a lower risk of diabetes in individuals with CVD. Existing studies investigating the association of NPs with diabetes have only included individuals without diabetes and without prevalent CVD or did not report the association separately for individuals with and without prevalent CVD.

The current study aimed to investigate the prospective associations of NT-proBNP and MR-proANP with incident type 2 diabetes in several population-based studies from the multinational Biomarkers for Cardiovascular Risk Assessment in Europe (BiomarCaRE) Consortium (17). We specifically aimed to assess whether these associations differed by the presence of CVD. Additionally, to allow a more robust analysis, we applied two-sample Mendelian randomization (MR) approaches by using published data on genetic variants that are specific for NT-proBNP or MR-proANP.

RESEARCH DESIGN AND METHODS

Study Design and Population

BiomarCaRE is based on the Monitoring of Trends and Determinants in Cardiovascular Diseases (MONICA) Risk Genetics Archiving and Monograph (MORGAM) Project (18), which has harmonized data from a large number of population-based cohorts. Our study complied with the Declaration of Helsinki. All participating cohorts were approved by local ethical review boards, and written informed consent was obtained from all study participants.

To investigate the association between NT-proBNP and incident type 2 diabetes, we included five BiomarCaRE population-based cohorts comprising 45,477 participants initially without diabetes and who had baseline measurements of NT-proBNP. The participating cohorts were the Cooperative Health Research in the Region of Augsburg Study Survey 3 and 4 (KORA S3-S4), the 1997 survey of the FINRISK Study, the Prospective Epidemiological Study of Myocardial Infarction (PRIME) Belfast, the Moli-sani Study, and the Northern Sweden MONICA Study. To investigate the association between MR-proANP and incident type 2 diabetes, we included three BiomarCaRE population-based cohorts involving 11,537 participants who initially did not have diabetes and who had baseline measurements of MR-proANP. The participating cohorts were the reexamination study of KORA S4 in 2006–2008 (KORA F4), FINRISK, and PRIME Belfast. An overview of each participating cohort is provided in Supplementary Table 1. The exclusion criteria for analyzing NT-proBNP and MR-proANP are described in Supplementary Figs. 1 and 2, respectively.

For each cohort, the following harmonized variables were available at baseline: age, sex, BMI, systolic blood pressure, antihypertensive medication, smoking status, total and HDL cholesterol, diabetes status, and history of CVD. History of CVD was defined as having documented or self-reported history of HF, MI, or stroke.

Assessment of Type 2 Diabetes

Prevalent diabetes was defined as a documented diagnosis of diabetes at baseline, either identified by record linkage or through self-report of the participants. In some cohorts, self-reported data were verified by medical record review or through information obtained from the treating physician. Incident type 2 diabetes was defined as a new diagnosis of type 2 diabetes during follow-up, either identified by record linkage or through self-report of the participants without prevalent diabetes at baseline. Details on the assessment of type 2 diabetes and the general follow-up procedures in each cohort are provided in Supplementary Table 1.

Laboratory Measurements

Baseline concentrations of NT-proBNP were measured using electrochemiluminescence immunoassay (ECLIA; Roche Diagnostics GmbH, Mannheim, Germany) on the Elecsys 2010 or the cobas e411 system. Baseline concentrations of MR-proANP were measured using an immunoluminometric assay (B·R·A·H·M·S/Thermo Fisher Scientific, Hennigsdorf, Berlin, Germany) on the B·R·A·H·M·S KRYPTOR automated system. The study-specific intra- and interassay coefficients of variation for each NP are described in Supplementary Table 2. Description of laboratory procedures in detail are provided in Supplementary Text 1.

Statistical Analysis

Participant characteristics stratified by history of CVD were calculated separately for the study sample with baseline measurements of NT-proBNP and MR-proANP.

In our data, we distinguished between missing values, which were below the limit of detection (LOD); that is, below the range to which the assay has been calibrated and missing values due to

[16]*Division of Endocrinology and Diabetology, Medical Faculty and University Hospital Düsseldorf, Heinrich-Heine-University Düsseldorf, Düsseldorf, Germany*
[17]*German Center for Diabetes Research (DZD), Partner Düsseldorf, Munich-Neuherberg, Germany*

Corresponding author: Barbara Thorand, thorand@helmholtz-muenchen.de

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other reasons (e.g., samples were not available, sample volumes were inadequate, sample mix-up or a technical problem). Only missing values due to NP values below the LOD ($n = 2{,}725$ for NT-proBNP and $n = 5$ from the FINRISK study for MR-proANP) were imputed to the lower LOD (i.e., 5 pg/mL for NT-proBNP and 4.6 pmol/L for MR-proANP).

The associations of NT-proBNP and MR-proANP with incident type 2 diabetes were estimated by calculating hazard ratios (HRs) with 95% CIs in Cox proportional hazard models. Age (continuous, in years) was used as the time scale. The models were stratified by study cohort and were adjusted for sex (men/women) in model 1 and were further adjusted for BMI (continuous, in kg/m^2), current smoking (yes/no), systolic blood pressure (continuous, in mmHg), use of antihypertensive medication (yes/no), total and HDL cholesterol (continuous, in mmol/L), and history of CVD (yes/no) in model 2. Additionally, we included history of CVD as a time-varying covariate in a sensitivity analysis of model 2. The distributions of NT-proBNP and MR-proANP were right-skewed (Supplementary Fig. 3). Thus, both NPs were log-transformed and (0,1)-standardized in the overall study population to approximate normality and to evaluate the HRs per 1-SD increase. These associations were further investigated for nonlinearity with restricted cubic spline regressions, with three knots at the 10th, 50th, and 90th percentiles applied to model 2. The proportional hazard assumption was tested by plotting scaled Schoenfeld residuals against follow-up time for each covariate. No indication of nonproportionality was observed.

To examine possible differences in the association of NPs with incident type 2 diabetes by CVD status at baseline, we conducted separate analyses for individuals with and without CVD history. Because some individuals may experience more than one CVD at baseline, which was seen in our data, history of CVD was defined as the composite history of HF, MI, and stroke. We also tested for multiplicative and additive interactions between NPs and CVD history in model 2. The multiplicative interaction was examined by incorporating both factors and their cross-product term in the same model. We calculated the additive interaction by estimating the relative excess risk due to interaction (RERI). RERI was calculated by comparing the joint and separate regression coefficients of NPs and CVD history from the same model using the following formula:

$$RERI = e^{(\beta_{NPs} + \beta_{CVD\ \text{history}} + \beta_{\text{NPs*}CVD\ \text{history}})} - e^{\beta_{NPs}} - e^{\beta_{CVD\ \text{history}}} + 1.$$

To test the null hypothesis that RERI $= 0$, we computed the 95% CIs and P values using the delta method (19). To further evaluate whether other diabetes-related biomarkers might account for the observed associations, we additionally included the baseline measurement of the estimated glomerular filtration rate (eGFR), hs-CRP, leptin, and adiponectin individually in model 2. We log-transformed hs-CRP, leptin, and adiponectin to approximate normality. The distribution of eGFR was approximately normal and was therefore not log-transformed. We further examined the potential interactions of NPs with BMI and sex in individuals with and without CVD history separately. False discovery rate with the Benjamini-Hochberg method was used to correct for multiple testing. An interaction was considered statistically significant at a false discovery rate of <0.05.

As a sensitivity analysis, we examined the associations of NT-proBNP and MR-proANP with incident type 2 diabetes in the same individuals by including only participants with data on both NPs. We also calculated the HRs for each participating cohort and used the Cochran Q to evaluate heterogeneity between cohorts. To account for death without experiencing diabetes as a competing event, we conducted competing-risk analyses using Fine and Gray models.

Finally, we performed two-sample univariate MR analyses using results from published genome-wide association (GWA) studies to examine the associations between genetically predicted NPs and type 2 diabetes risk. We identified single nucleotide polymorphisms (SNPs) with effects specific to either NT-proBNP or MR-proANP at a P value of $<5E{-}8$ as the genetic instrumental variables (IVs) from a published GWA study of European ancestry from Salo et al. (20). The association estimates of the IVs with type 2 diabetes were extracted from a meta-analysis of GWA studies by Xue et al. (21) because of the data availability for populations of European ancestry and the large sample size (62,892 case subjects and 596,424 control subjects). The procedure for the MR analysis is provided in detail in Supplementary Text 2.

All statistical analyses were performed using R 4.0.3 software (22). P values <0.05 were considered statistically significant.

RESULTS

Participant Characteristics

Characteristics of the study participants stratified by CVD history for the study samples with data on NT-proBNP and MR-proANP, respectively, are presented in Table 1. At baseline, compared with participants without CVD, participants with CVD had higher concentrations of NT-proBNP and MR-proANP, were on average older and more frequently male, had a higher BMI and systolic blood pressure, were more likely to take antihypertensive medication, had lower eGFR, had lower concentrations of HDL cholesterol and adiponectin, and had higher concentrations of leptin and hs-CRP. Throughout the follow-up period, participants with CVD were more likely to develop type 2 diabetes than participants without CVD. Characteristics of the study participants in the quarters of baseline NT-proBNP and MR-proANP concentrations are shown in Supplementary Tables 3 and 4, respectively. Characteristics for each participating cohort are provided in Supplementary Table 5.

Associations of NT-proBNP and MR-proANP With Incident Type 2 Diabetes

During a median follow-up of 6.5 years (interquartile range [IQR] 9.9), among the 45,477 participants with NT-proBNP data, 1,707 developed type 2 diabetes. Of these, 209 had a history of CVD at baseline. Among the 11,537 participants with MR-proANP data, 857 developed type 2 diabetes during a median follow-up of 13.8 years (IQR 5.0). Of these, 106 had a history of CVD at baseline.

Both NT-proBNP and MR-proANP were inversely associated with incident type 2 diabetes in model 1 in the overall study population. The HRs (95% CIs) were 0.89 (0.84; 0.94) per 1-SD increase of log NT-proBNP and 0.79 (0.73; 0.86) per 1-SD increase of log MR-proANP. The associations remained significant after additional adjustment according to model 2 (HRs

Table 1—Participant characteristics in the total study population and stratified by history of CVD

	Study population with NT-proBNP measurement			Study population with MR-proANP measurement		
	Overall *n* = 45,477	With CVD* *n* = 1,995	Without CVD *n* = 43,482	Overall *n* = 11,537	With CVD† *n* = 783	Without CVD *n* = 10,754
Incident type 2 diabetes	1,707 (3.8)	209 (10.5)	1,498 (3.4)	857 (7.4)	106 (13.5)	751 (7.0)
NT-proBNP, pg/mL (antilog SD)	41.3 (3.0)	122.7 (3.6)	39.3 (2.9)	39.3 (2.9)‡	107.8 (3.4) ‡	36.2 (2.8)‡
MR-proANP, pmol/L (antilog SD)	47.9 (1.6)‡	76.7 (1.8)‡	46.1 (1.6)‡	49.9 (1.6)	82.3 (1.8)	47.9 (1.6)
Study cohort						
KORA S3-S4	5,130 (11.3)	328 (16.4)	4,802 (11.0)	NA	NA	NA
KORA F4	NA	NA	NA	2,265 (19.6)	128 (16.3)	2,137 (19.9)
FINRISK	7,240 (15.9)	518 (26.0)	6,722 (15.5)	7,301 (63.3)	532 (67.9)	6,769 (62.9)
PRIME Belfast	2,332 (5.1)	147 (7.4)	2,185 (5.0)	1,971 (17.1)	123 (15.7)	1,848 (17.2)
Moli-sani	21,357 (47.0)	589 (29.5)	20,768 (47.8)	NA	NA	NA
Northern Sweden	9,418 (20.7)	413 (20.7)	9,005 (20.7)	NA	NA	NA
Age, years (SD)	51.5 (12.7)	62.7 (10.1)	51.0 (12.6)	49.9 (12.0)	60.9 (9.7)	49.1 (11.8)
Male	23,045 (50.7)	1,386 (69.5)	21,659 (49.8)	6,677 (57.9)	552 (70.5)	6,125 (57.0)
BMI, kg/m^2 (SD)	27.2 (4.6)	28.7 (4.6)	27.2 (4.6)	26.6 (4.3)	28.4 (4.6)	26.5 (4.3)
Current smoking	11,333 (24.9)	367 (18.4)	10,966 (25.2)	2,998 (26.0)	166 (21.2)	2,832 (26.3)
Systolic blood pressure, mmHg (SD)	135.5 (20.6)	142.2 (22.4)	135.2 (20.5)	132.5 (20.3)	140.3 (22.4)	132.0 (20.0)
Use of antihypertensive medication	8,424 (18.5)	964 (48.3)	7,460 (17.2)	1,533 (13.3)	331 (42.3)	1,202 (11.2)
Total cholesterol, mmol/L (SD)	5.67 (1.13)	5.59 (1.20)	5.67 (1.13)	5.58 (1.05)	5.61 (1.01)	5.58 (1.05)
HDL cholesterol, mmol/L (SD)	1.45 (0.40)	1.31 (0.38)	1.45 (0.40)	1.38 (0.37)	1.26 (0.37)	1.39 (0.37)
eGFR, mL/min/1.73 m^2 (SD)§	94.5 (17.3)	82.8 (18.7)	95.1 (17.0)	88.6 (19.3)	78.1 (18.9)	89.4 (19.1)
hs-CRP, mg/L (antilog SD)|	1.35 (3.03)	1.99 (3.00)	1.34 (3.03)	1.26 (3.03)	1.97 (3.00)	1.22 (3.00)
Leptin, ng/mL (antilog SD)¶	7.77 (2.36)	9.30 (2.36)	7.69 (2.36)	8.67 (2.56)	10.59 (2.59)	8.58 (2.53)
Adiponectin, μg/mL (antilog SD)#	5.42 (1.88)	4.90 (1.95)	5.47 (1.88)	5.64 (1.90)	5.31 (1.99)	5.64 (1.90)

Data are presented as *n* (%) for categorical variables and as mean (SD) for continuous variables. Continuous variables with skewed distributions are presented as geometric mean (antilog SD). NA, data not available. *Among 1,995 participants with CVD history and NT-proBNP measurement, 570 had HF (120 with MI, 28 with stroke, 16 with MI and stroke), 1,114 had MI (120 with HF, 65 with stroke, and 16 with HF and stroke), and 556 had stroke (28 with HF, 65 with MI and 16 with HF and MI). †Among 783 participants with CVD history and MR-proANP measurement, 315 had HF (88 with MI, 34 with stroke, 10 with MI and stroke), 411 had MI (88 with HF, 19 with stroke and 10 with HF and stroke), and 218 had stroke (34 with HF, 19 with MI and 10 with HF and MI). ‡Data were available and calculated in 8,695 (612 with and 8,083 without a history of CVD). §Data were available and calculated in 44,219 (1,922 with and 42,297 without a history of CVD) participants with NT-proBNP measurement and 11,341 (764 with and 10,577 without a history of CVD) participants with MR-proANP measurement. |Data were available and calculated in 45,032 (1,981 with and 43,051 without a history of CVD) participants with NT-proBNP measurement and 11,073 (758 with and 10,315 without a history of CVD) participants with MR-proANP measurement. ¶Data were available and calculated in 17,927 (1,038 with and 16,889 without a history of CVD) participants with NT-proBNP measurement and 10,311 (734 with and 9,577 without a history of CVD) participants with MR-proANP measurement. #Data were available and calculated in 8,669 (611 with and 8,058 without a history of CVD) participants with NT-proBNP measurement and 9,673 (738 with and 8,935 without a history of CVD) participants with MR-proANP measurement.

0.84 [95% CI 0.79; 0.89] per 1-SD increase of log NT-proBNP and 0.77 [0.71; 0.83] per 1-SD increase of log MR-proANP). The results were similar when we included history of CVD as a time-varying covariate (HR 0.84 [95% CI 0.79; 0.89] per 1-SD increase of log NT-proBNP and 0.77 [0.71; 0.83] per 1-SD increase of log MR-proANP). The associations of NT-proBNP and MR-proANP with incident type 2 diabetes were also examined in each cohort without significant heterogeneity (Supplementary Figs. 4 and 5).

We observed a significant interaction between NT-proBNP and history of CVD on both multiplicative and additive scales with respect to incident type 2 diabetes ($P = 0.001$ for interaction on multiplicative scale and $P = 0.015$ on additive scale) (Table 2). When stratified by CVD history, NT-proBNP was significantly inversely associated with incident type 2 diabetes in participants without but not in participants with CVD history. The association between NT-proBNP and incident type 2 diabetes in participants without CVD was approximately linear (Fig. 1*A*). The multi-variable HRs (95% CIs) per 1-SD increase were 0.81 (0.76; 0.86) and 1.04 (0.90; 1.19) in participants without and with CVD history, respectively. These results were consistent after further adjustment for eGFR, hs-CRP, leptin, and adiponectin (Supplementary Table 6). In our subgroup analyses, we observed a significant interaction of NT-proBNP with BMI and sex in participants without CVD history, with a stronger association in women than in men and in obese than in nonobese participants (Supplementary Fig. 6).

For MR-proANP, we did not observe a significant difference in the association with incident type 2 diabetes between individuals with and without CVD history

Table 2—Association between NPs and incident type 2 diabetes

	Cases/person-years (*n*)	HR (95% CI) per 1-SD increase	*P* value	Multiplicative interaction	*P* value	Additive interaction*	*P* value
NT-proBNP							
Overall	1,707/429,620	0.84 (0.79; 0.89)	<0.001				
History of CVD				1.25 (1.09; 1.43)	0.001†	0.20 (0.04; 0.37)	0.015†
Yes	209/18,249	1.04 (0.90; 1.19)	0.608				
No	1,498/411,372	0.81 (0.76; 0.86)	<0.001				
MR-proANP							
Overall	857/141,206	0.77 (0.71; 0.83)	<0.001				
History of CVD				1.12 (0.93; 1.36)	0.236	0.08 (−0.12; 0.27)	0.441
Yes	106/8,441	0.81 (0.66; 0.99)	0.042				
No	751 / 132,765	0.75 (0.69; 0.82)	<.001				

The Cox models used age (continuous, in years) as time scale and were stratified by study cohort and adjusted for sex (men/women), BMI (continuous, in kg/m^2), current smoking (yes/no), systolic blood pressure (continuous, in mmHg), use of antihypertensive medication (yes/no), total and HDL (continuous, in mmol/L), and history of CVD (yes/no). NT-proBNP and MR-proANP were log-transformed and (0,1)-standardized in the total study population to approximate normality and to evaluate the HRs per 1-SD increase. *Interaction on additive scale was estimated with RERI (95% CI). †False discovery rate-adjusted *P* values <0.05 using Benjamini-Hochberg method.

($P = 0.236$ for interaction on the multiplicative scale and $P = 0.441$ on the additive scale) (Table 2). The multivariable HRs (95% CIs) per 1-SD increase were 0.81 (0.66; 0.99) and 0.75 (0.69; 0.82) in participants with and without CVD history, respectively. Inspection of restricted cubic splines indicated an inverse linear relationship between MR-proANP and incident type 2 diabetes in participants with and without CVD history (Fig. 1*B*). Further individual adjustment for eGFR, hs-CRP, leptin, and adiponectin only marginally affected the association (Supplementary Table 6). No significant differences in the association between MR-proANP and incident type 2 diabetes were observed across BMI and sex categories (Supplementary Fig. 7).

In a sensitivity analysis including only participants with complete data on both NT-proBNP and MR-proANP ($n = 8,695$), the results were consistent. We only observed a significant difference by CVD history in the association between NT-proBNP and incident type 2 diabetes (Supplementary Table 7). Our competing risk analyses yielded similar results (Supplementary Table 8).

Two-Sample MR Analyses on the Associations of Genetically Predicted NT-proBNP and MR-proANP With Type 2 Diabetes Risk

We included one SNP located in the natriuretic peptide precursor B (*NPPB*) gene (rs198379) for NT-proBNP and two independent SNPs in the natriuretic peptide precursor A (*NPPA*) gene for MR-proANP (rs4845875 and rs3753584) as the genetic IVs. The genetic associations with each NP and with type 2 diabetes were extracted from the previously mentioned GWA studies (20,21) and are provided in Supplementary Table 9. In line with the results from the survival analysis, our MR analyses showed that genetically predicted NT-proBNP and MR-proANP were inversely associated with type 2 diabetes risk. The odds ratios (95% CIs) were 0.93 (0.87; 0.98) for NT-proBNP and 0.91 (0.86; 0.97) for MR-proANP. Sensitivity analyses using the likelihood-based and the weighted mode-based methods yielded similar results (Table 3). We did not observe significant heterogeneity between the two IVs for MR-proANP with respect to the association with type 2 diabetes (Table 3 and Supplementary Fig. 8).

CONCLUSIONS

Our results show that higher circulating concentrations of NT-proBNP and MR-proANP were significantly associated with a lower incidence of type 2 diabetes. We were able to show for the first time that the association between NT-proBNP and incident type 2 diabetes was modified by the presence of CVD, while there was no significant difference in the inverse association of MR-proANP with incident type 2 diabetes between individuals with and without CVD history. We only observed an inverse association of NT-proBNP with incident type 2 diabetes in individuals without but not in individuals with CVD history. Further adjustment for eGFR, hs-CRP, leptin, and adiponectin did not substantially alter the results. In addition, our MR analyses yielded significant associations of genetically predicted NT-proBNP and MR-proANP with the risk of type 2 diabetes.

Our findings support the growing evidence associating high concentrations of NT-proBNP and MR-proANP with a lower risk of type 2 diabetes in individuals without CVD at baseline (5–12) and provide further information regarding these associations in individuals with CVD. Furthermore, our MR analyses corroborate findings from previous studies (5,23) suggesting a potential causal relationship between higher concentrations of NT-proBNP and a lower risk of type 2 diabetes in individuals without prevalent diabetes and CVD and additionally provide the same evidence for MR-proANP.

The underlying mechanisms whereby higher concentrations of BNP and ANP are associated with a lower risk of type 2 diabetes are not fully understood. In adipose tissue, ANP stimulates lipolysis via cyclic guanosine monophosphate–mediated phosphorylation thereby inhibits visceral adipocyte hypertrophy (6), while BNP induces browning of white adipose tissue. ANP also inhibits leptin release, and BNP and ANP both reduce the secretion of proinflammatory cytokines from adipose tissue and enhance adiponectin secretion via the activation of NP receptor A, which suppresses low-grade inflammation of the adipose tissue (24). Thus, BNP and ANP may counteract insulin res-

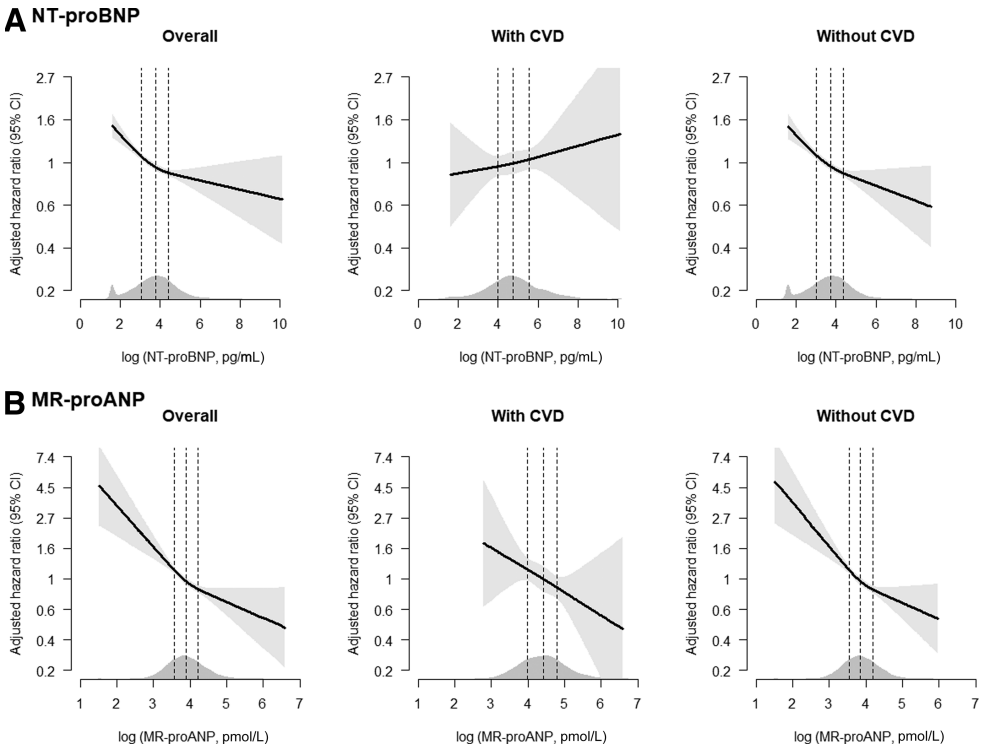

Figure 1—Shape of the association between NPs and incident type 2 diabetes in the total study population and stratified by history of CVD. The linearity was assessed with restricted cubic splines with three knots at the 10th, 50th, and 90th percentiles. Data and the smoothed splines are fitted using Cox models. The models used age as the time scale and were stratified by study cohort. The models were adjusted for sex (men/women), BMI (continuous, in kg/m^2), current smoking (yes/no), systolic blood pressure (continuous, in mmHg), use of antihypertensive medication (yes/no), total and HDL cholesterol (continuous, in mmol/L), and history of CVD (yes/no). NT-proBNP and MR-proANP were log-transformed and (0,1)-standardized in the total study population to approximate normality and to evaluate the HRs per 1-SD increase. Shaded areas around the curves depict the 95% CI. Kernel density plots are imposed along the *x* axis, with vertical dotted lines depicting (from the left) the 25th, 50th, and 75th percentiles of the population.

istance. However, in our study, the inverse association of NT-proBNP and MR-proANP concentrations with incident type 2 diabetes remained stable after further adjustment for hs-CRP, leptin, and adiponectin.

Another potential mechanism for a lower risk of type 2 diabetes is via the direct effects of BNP and ANP on the oxidative metabolism of skeletal muscles (1). NP receptor A is upregulated in the muscle of exercise-trained individuals, suggesting that some of the metabolic adaptations of skeletal muscle in response to exercise may be mediated by NPs (1,24). In healthy individuals, increased NT-proBNP concentrations are also associated with prolonged physical activity (25). In contrast, the concentrations of NPs are reduced in obesity (26), possibly due to the deleterious effects of cardiac ectopic fat and the upregulation of the NP receptor C in adipose tissue that increases NPs clearance (24). Moreover, evidence from previous epidemiological studies suggests that NPs are inversely associated with insulin resistance and obesity (26,27). Intriguingly, we observed a stronger association of NT-proBNP and incident type 2 diabetes in obese than in nonobese participants without CVD history. Of note, a recent study indicates that the inverse association between NT-proBNP and obesity could be modified by sex, with a more pronounced association in women than in men (28), which could be explained by the sex differences in body composition and fat distribution. This observation is in line with our findings and a previous study (9) reporting a stronger inverse association between NT-proBNP and incident type 2 diabetes in women than in men without CVD history, which could possibly be further explained by sex hormones, especially testosterone. Testosterone suppresses NT-proBNP production (29) and, in turn, may partially account for higher circulating NT-proBNP concentrations in women than in men (24). Interestingly, in men, testosterone was inversely associated with type 2 diabetes, while this association in women was positive in cross-sectional settings (30). This evidence is similar in prospective studies; however, the positive association in women was no longer significant after controlling for diabetes risk factors (30,31). The apparent sex-specific associations between testosterone and type 2 diabetes may be driven by the extreme spectrum of testosterone concentrations; for instance, abnormally high testosterone concentrations (hyperandrogenism) in women and abnormally low testosterone concentrations (hypogonadism) in men are associated with a higher risk of

Table 3—MR results of the association between genetically predicted NPs and the risk of type 2 diabetes

Phenotype	No. of IVs	Methods	MR estimates on odds ratio scale (95% CI)	*P*	Cochran's Q	*P* for Cochran *Q*	I^2 (%)
NT-proBNP	1 (rs198379)	Wald ratio	0.93 (0.87; 0.98)	0.012	—	—	—
		Maximum likelihood	0.93 (0.87; 0.98)	0.013	—	—	—
MR-proANP	2 (rs4845875 & rs3753584)	IVW fixed effect model	0.91 (0.86; 0.97)	0.002	0.701	0.402	0
		Maximum likelihood	0.91 (0.85; 0.97)	0.003	0.649	0.421	—
		Weighted mode	0.92 (0.86; 0.98)	0.016	—	—	—

Cochran *Q* and I^2 statistics to test for heterogeneity were calculated when more than one IV was included in the analysis. IVW, inverse-variance weighted.

type 2 diabetes (32). However, in the previous study reporting sex-specific associations between NT-proBNP and incident type 2 diabetes (9), the sex differences were still observed after adjustment for testosterone and other sex hormones, suggesting other possible explanations.

Furthermore, previous studies have shown that elevated blood pressure is associated with increased NP concentrations, which reflects a compensatory response to restrain blood pressure (2,26). Cardiovascular and metabolic regulations are tightly linked; therefore, lowering blood pressure may lower type 2 diabetes risk (2,33). However, some classes of antihypertensive medication may exhibit differential effects on type 2 diabetes risk. Thiazides and β-blockers tend to increase diabetes risk, while neprilysin blockers, angiotensin receptor blockers, ACE inhibitors, and calcium channel blockers decrease the risk (34,35). Due to lack of data on specific antihypertensive medications, we were unable to examine the differential effects of antihypertensive medication classes on the association between NPs and type 2 diabetes.

Differences between BNP and ANP in their cardiometabolic effects have not been widely studied. Within the heart, ANP is considered to be mainly secreted from the atria, while BNP is mainly from the ventricles (36). Although both NPs are known to lower blood pressure, data from animal models (37) and a recent GWA study (20) implicate ANP rather than BNP as a strong blood pressure-lowering hormone. However, in individuals with left ventricular dysfunction, BNP concentrations are markedly increased compared with ANP, suggesting BNP rather than ANP as an emergency defense against ventricular overload (36). Elevated BNP concentrations are also strongly correlated with the severity of CVD (38), which is associated with a higher risk of type 2 diabetes (14,39,40). This could have attenuated the inverse association of NT-proBNP with incident type 2 diabetes in individuals with CVD and could thus explain the difference in the association between individuals with and without CVD seen in our study. Unfortunately, due to lack of relevant data, we were unable to further examine whether the observed associations could be influenced by the severity of CVD. Furthermore, as NT-proBNP concentrations are higher in individuals with CVD compared with individuals without, one could speculate that there is a plateau effect and that the inverse association between NT-proBNP and incident type 2 diabetes is only seen in the lower concentration range. However, our results did not support this hypothesis. An inverse association between NT-proBNP and incident type 2 diabetes in individuals without CVD was also observed within the concentration range seen in individuals with CVD, although with some uncertainty due to the wide 95% CIs at the upper end of NT-proBNP concentrations (Fig. 1*A*). Of note, a nonsignificant association is not evidence for no association. Indeed, based on the 95% CIs from the present analysis, we cannot rule out a small effect of NT-proBNP on type 2 diabetes risk in individuals with CVD. Furthermore, our interaction analyses for MR-proANP may have been underpowered to detect differences between individuals with and without CVD history. More studies with larger study populations, particularly with a large number of incident type 2 diabetes cases in individuals with CVD history are needed to confirm our findings.

The strengths of the current study include the prospective, population-based design, the large sample size, and the thorough adjustment for different cardiometabolic risk factors. Since 1998, we have harmonized data from population-based cohort studies in the MORGAM Data Centre in Helsinki, providing the best possible exposure and covariate alignment as well as end point validation. Standardized epidemiological and laboratory procedures based on individual level data also allow for the best possible data analyses.

Our study has some limitations that merit consideration. Although the assessment of type 2 diabetes incidence was systematic and detailed, it was mainly based on medical reviews and for a small number of participants on self-report, which may have led to misclassification of incident cases. However, since we expect people under regular review by their physician for CVD to have more opportunities for the detection of diabetes, any bias introduced by these means of ascertainment could not explain the lower risk of diabetes seen in individuals with elevated NP concentrations. History of CVD was based on medical review or self-report; therefore, it is possible that some of the individuals classified as having no CVD could have had underlying undiagnosed CVD. We had only a single measurement of NT-proBNP and MR-proANP at baseline, and therefore, intraindividual variation could not be taken into account. This could have led to misclassification of participants and biased the estimates toward the null. Harmonized data on other known cardiometabolic risk factors, such as physical activity and diet, were lacking in the current study, which could have led to some degree of residual confounding. Furthermore, the exclusion of eligible individuals due to

missing values of the NPs or of cardiometabolic risk factors (10.1% in the subsample for NT-proBNP and 10.7% in the subsample for MR-proANP) decreased the statistical power of the analyses and could have led to biased association estimates if the data were not missing completely at random. Finally, due to a limited number of genetic IVs, we were unable to perform more robust sensitivity analyses for our MR.

In conclusion, our findings suggest that NT-proBNP and MR-proANP are inversely associated with incident type 2 diabetes. However, the inverse association of NT-proBNP seems to be modified by the presence of CVD. Future studies are needed to examine the underlying mechanisms, the differences in the metabolic actions between both NPs, and their potential as targets for therapeutic interventions of type 2 diabetes.

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Appendix

Paper III. Associations of the vasoactive peptides CT-proET-1 and MR-proADM with incident type 2 diabetes

Title: Associations of the vasoactive peptieds CT-proET-1 and MR-proADM with Incident Type 2 Diabetes: Results from the BiomarCaRE Consortium

Authors: Chaterina Sujana, Veikko Salomaa, Frank Kee, Jochen Seissler, Pekka Jousilahti, Charlotte Neville, Cornelia Then, Wolfgang Koenig, Kari Kuulasmaa, Jaakko Reinikainen, Stefan Blankenberg, Tanja Zeller, Christian Herder, Ulrich Mansmann, Annette Peters, Barbara Thorand, for the BiomarCaRE Consortium.

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Associations of the Vasoactive Peptides CT-proET-1 and MR-proADM with Incident Type 2 Diabetes: Results from the BiomarCaRE Consortium

Chaterina Sujana[1,2,3], Veikko Salomaa[4], Frank Kee[5], Jochen Seissler[6], Pekka Jousilahti[4], Charlotte Neville[5], Cornelia Then[6], Wolfgang Koenig[7,8,9], Kari Kuulasmaa[4], Jaakko Reinikainen[4], Stefan Blankenberg[10,11], Tanja Zeller[10,11], Christian Herder[12,13,14], Ulrich Mansmann[2], Annette Peters[1,3,9], Barbara Thorand[1,3], for the BiomarCaRE Consortium

[1] Institute of Epidemiology, Helmholtz Zentrum München - German Research Center for Environmental Health, Neuherberg, Germany;

[2] Institute for Medical Information Processing, Biometry, and Epidemiology (IBE), Pettenkofer School of Public Health, Ludwig-Maximilians-Universität München, Munich, Germany;

[3] German Center for Diabetes Research (DZD), Partner München-Neuherberg, Neuherberg, Germany.

[4] Department of Public Health and Welfare, Finnish Institute for Health and Welfare, Helsinki, Finland;

[5] Centre for Public Health, Queens University of Belfast, Belfast, Northern Ireland, UK;

[6] Diabetes Zentrum, Medizinische Klinik und Poliklinik IV, Klinikum der Ludwig-Maximilians-Universität München, Munich, Germany;

[7] Institute of Epidemiology and Medical Biometry - University of Ulm, Ulm, Germany;

[8] Deutsches Herzzentrum München, Technische Universität München, Munich, Germany;

[9] German Centre for Cardiovascular Research (DZHK e.V.), partner site Munich Heart Alliance, Munich, Germany;

[10] Department for General and Interventional Cardiology, University Heart Center Hamburg, Hamburg, Germany;

[11] German Centre for Cardiovascular Research (DZHK e.V.), partner site Hamburg, Lübeck, Kiel, Hamburg, Germany;

[12] Institute for Clinical Diabetology, German Diabetes Center, Leibniz Center for Diabetes Research at Heinrich-Heine-University Düsseldorf, Düsseldorf, Germany;

[13] Division of Endocrinology and Diabetology, Medical Faculty and University Hospital Düsseldorf, Heinrich-Heine-University Düsseldorf, Düsseldorf, Germany;

[14] German Center for Diabetes Research (DZD), Partner Düsseldorf, Neuherberg, Germany.

ORCid numbers: 0000-0002-0663-2557 (C. Sujana), 0000-0001-7563-5324 (V. Salomaa), 0000-0002-2064-9603 (W. Koenig), 0000-0003-2165-1411 (K. Kuulasmaa), 0000-0002-7825-5359 (J. Reinikanen), 0000-0002-2050-093X (C. Herder), 0000-0002-9955-8906 (U. Mansmann), 0000-0001-6645-0985 (A. Peters), 0000-0002-8416-6440 (B. Thorand)

Corresponding author:

Prof. Dr. Barbara Thorand, MPH

Institute of Epidemiology

Helmholtz Zentrum München - German Research Center for Environmental Health

Ingolstädter Landstrasse 1

85764 Neuherberg, Germany

e-mail: thorand@helmholtz-muenchen.de

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Abstract

Background: Endothelin-1 (ET-1) and adrenomedullin (ADM) are commonly known as vasoactive peptides that regulate vascular homeostasis. Less recognised is the fact that both peptides could affect glucose metabolism. Here, we investigated whether ET-1 and ADM, measured as C-terminal-proET-1 (CT-proET-1) and mid-regional-proADM (MR-proADM), respectively, were associated with incident type 2 diabetes.

Methods: Based on the population-based Biomarkers for Cardiovascular Risk Assessment in Europe (BiomarCaRE) Consortium data, we performed a prospective cohort study to examine associations of CT-proET-1 and MR-proADM with incident type 2 diabetes in 12,006 participants. During a median follow-up time of 13.8 years, 862 participants developed type 2 diabetes. The associations were examined in Cox proportional hazard models. Additionally, we performed two-sample Mendelian randomisation analyses using published data.

Results: CT-proET-1 and MR-proADM were positively associated with incident type 2 diabetes. The multivariable hazard ratios (HRs) [95% confidence intervals (CI)] were 1.10 [1.03; 1.18], P=0.008 per 1-SD increase of CT-proET-1 and 1.11 [1.02; 1.21], P=0.016 per 1-SD increase of log MR-proADM, respectively. We observed a stronger association of MR-proADM with incident type 2 diabetes in obese than in non-obese individuals (P-interaction with BMI < 0.001). The HRs [95%CIs] were 1.19 [1.05; 1.34], P=0.005 and 1.02 [0.90; 1.15], P=0.741 in obese and non-obese individuals, respectively. Our Mendelian randomisation analyses yielded a significant association of CT-proET-1, but not of MR-proADM with type 2 diabetes risk.

Conclusions: Higher concentrations of CT-proET-1 and MR-proADM are associated with incident type 2 diabetes, but our Mendelian randomisation analysis suggests a probable causal link for CT-proET-1 only. The association of MR-proADM seems to be modified by body composition.

Keywords: adrenomedullin, C-terminal-proendothelin-1, endothelin-1, epidemiology, incident type 2 diabetes, Mendelian randomisation, mid-regional-proadrenomedullin, cohort study

Background

Metabolic and vascular diseases commonly coexist. However, the pathophysiological mechanisms linking both diseases are not well understood. A possible link is the dysregulation of vasoactive peptides that could be implicated in both vascular and metabolic homeostasis (1), such as endothelin-1 (ET-1) and adrenomedullin (ADM).

ET-1, a 21-amino acid peptide primarily secreted by vascular endothelial cells, is a potent vasoconstrictor and pro-inflammatory peptide (2). ET-1 has been implicated in the pathogenesis of several chronic diseases, including hypertension and chronic kidney disease (3). In addition to its known effect on the vascular function, ET-1 also limits insulin actions in skeletal muscles and adipocytes leading to insulin resistance and impaired glucose tolerance (4-6). ADM, a 52-amino acid peptide that belongs to the calcitonin gene–related peptide family, is a vasodilator. ADM is secreted by a variety of different cells, including vascular endothelial cells, smooth muscle cells, adventitial fibroblasts as well as adipocytes (7, 8). In a later investigation, adipose tissue was suggested to be the major source of ADM (8). ADM has several metabolic actions, including counteracting oxidative stress–induced insulin resistance (9, 10) and inhibition of insulin secretion from the pancreatic islets (11).

Measurements of circulating concentrations of ET-1 and ADM are very difficult because of the short half-life, the existence of binding proteins, and other technical difficulties. Therefore, assays have been developed to measure the inactive fragments of ET-1 and ADM as the surrogates, C-terminal-proendothelin-1 (CT-proET-1) and mid-regional-proadrenomedullin (MR-proADM), respectively, which are biologically stable and are correlated with the active peptides in equimolar concentrations (12, 13).

In epidemiological studies using a cross-sectional design, both CT-proET-1 and MR-proADM were positively associated with the metabolic syndrome, insulin

150 resistance and prevalent type 2 diabetes (14-16). Previously, using a prospective
151 study design, we have also shown that higher circulating concentrations of CT-proET-
152 1 and MR-proADM were associated with increased insulin resistance, suggesting that
153 both vasoactive peptides could play a role in the pathogenesis of type 2 diabetes (17).
154 However, most of the existing prospective studies failed to provide evidence for an
155 association of both CT-proET-1 and MR-proADM with incident type 2 diabetes (17-
156 20). So far, only two prospective studies reported a positive association between CT-
157 proET-1 and incident type 2 diabetes (21, 22). Thus, we aimed to examine the putative
158 association of CT-proET-1 and MR-proADM with incident type 2 diabetes by
159 performing a prospective cohort study with a larger sample size using data from the
160 multinational Biomarkers for Cardiovascular Risk Assessment in Europe
161 (BiomarCaRE) Consortium (23) in tandem with a two-sample Mendelian
162 randomisation study using published data on genetic variants that are specific for CT-
163 proET-1 or MR-proADM, to allow a more robust analysis.
164

165 ## Methods

166 ### Study population

167 BiomarCaRE is an EU-funded consortium that aims to determine the value of
168 established and emerging biomarkers in improving risk estimation of cardiovascular
169 disease. BiomarCaRE relies on the Monitoring of Trends and Determinants in
170 Cardiovascular Diseases (MONICA) Risk Genetics Archiving and Monograph
171 (MORGAM) Project (24), which includes harmonized data from a large number of
172 population-based cohorts. All participating cohorts were approved by local ethical
173 review boards and written informed consent was obtained from all study participants.
174 The study was conducted according to the Declaration of Helsinki.

In the prospective cohort study, we included three BiomarCaRE population-based cohorts involving 12,006 participants initially without diabetes and cardiovascular diseases and with follow-up data on type 2 diabetes. The exclusion criteria are described in Additional File 1: Figure S1. The participating cohorts were the FINRISK Study (Finland), the Prospective Epidemiological Study of Myocardial Infarction (PRIME) Belfast (UK), and the Cooperative Health Research in the Region of Augsburg Study (KORA) F4 (Germany). An overview of each participating cohort is provided in Additional File 1: Table S1. The following harmonized variables were available for each cohort: age, sex, body mass index (BMI), waist circumference, systolic and diastolic blood pressure, antihypertensive medication, smoking status, total and high-density lipoprotein (HDL) cholesterol and diabetes status.

Ascertainment of type 2 diabetes cases

We defined prevalent diabetes as a documented diagnosis of diabetes at baseline, either identified by record linkage or through self-report of the participants that were verified by medical chart review or through information obtained from the treating physician. Incident type 2 diabetes was defined as a new diagnosis of type 2 diabetes during follow-up, either identified by record linkage or through self-report of the participants initially without diabetes at baseline that were verified by medical record review or through information obtained from the treating physician. Details of the assessment of type 2 diabetes in each participating cohort are provided in Additional File 1: Table S1.

Laboratory measurements

Baseline concentrations of CT-proET-1 and MR-proADM were measured from plasma with immunoluminometric assay (BRAHMS/Thermo Fisher Scientific, Hennigsdorf, Berlin, Germany) on the BRAHMS KRYPTOR automated system. The data were measured centrally in the MORGAM/BiomarCaRE core laboratory for FINRISK and PRIME Belfast (in 2008) and locally for KORA F4 (in 2010). The cohort-specific intra- and interassay coefficients of variation for CT-proET-1 and MR-proADM are described in Additional File 1: Table S2. Laboratory procedures for other diabetes-related biomarkers used in the analyses are provided in Additional File 1: Text S1.

Statistical analysis

Measurement values below the limit of detection (LOD) (N=121 for CT-proET-1 and N=166 for MR-proADM, all from the FINRISK study) were set to the lower LOD (i.e. 9.44 pmol/l for CT-proET-1 and 0.24 nmol/l for MR-proADM). Other missing values of the vasoactive peptides or missing values of diabetes risk factors (Additional File 1: Table S3) were handled with multiple imputation by chained equations (MICE), performed using R package mice (25), version 3.13. The imputation was done separately for each cohort. A total of 200 imputed data sets were created. Additional variation due to imputation was taken into account according to the Rubin's rules for multiple imputation (26).

Descriptive statistics are reported for the participants stratified by incident type 2 diabetes during follow-up and shown as frequency (percentage) for categorical variables and as mean (standard deviation (SD)) for continuous variables. Continuous variables with skewed distributions are presented as geometric mean (antilog SD).

The associations of both CT-proET-1 and MR-proADM with incident type 2 diabetes were estimated by calculating hazard ratios (HRs) with 95% confidence

intervals (95% CIs) in Cox proportional hazard (PH) models. The models were stratified by study cohort and were adjusted for age (continuous, in years) and sex (men/women) in model 1 and were further adjusted for current smoking (yes/no), total and HDL cholesterol (continuous, in mmol/l), actual hypertension (yes/no) and BMI (continuous, in kg/m^2) in model 2. Actual hypertension was defined as having systolic blood pressure ≥ 140 mmHg, diastolic blood pressure ≥ 90 mmHg or using antihypertensive medication. The distribution of MR-proADM was right-skewed (Additional File 1: Figure S2) and thus was log-transformed to approximate normality. Both peptides were (0,1)-standardized to estimate the HRs per 1-SD increase. To further evaluate whether other diabetes-related biomarkers might account for the observed associations, we additionally included the baseline measurement of estimated glomerular filtration rate (eGFR), insulin, high-sensitivity C-reactive protein (hsCRP), leptin, and fasting glucose individually and simultaneously in model 2. The PH assumption was tested by plotting scaled Schoenfeld residuals against follow-up time for each covariate. No indication of non-proportionality was observed.

We tested for interactions of both peptides with BMI, sex and actual hypertension by creating cross-product terms and evaluating the significance level. Additionally, we also tested for the interaction with waist circumference as an alternative to BMI. False discovery rate (FDR) with the Benjamini–Hochberg method was used to correct for multiple testing. An interaction was considered relevant at FDR < 0.05. Subgroup analyses were conducted by examining the associations across BMI (≥ 30 kg/m^2 vs < 30 kg/m^2), waist circumference (men: ≥ 102 cm, women: ≥ 88 cm vs men: < 102, women: < 88 cm), sex (men vs women) and actual hypertension (yes vs no) categories. We also calculated the associations of CT-proET-1 and MR-proADM with incident type 2 diabetes for each participating cohort. Heterogeneity in the

association across cohorts were examined by testing the interaction by study cohort and by examining Cochran's Q and I^2.

To examine the associations of genetically predicted CT-proET-1 and MR-proADM with type 2 diabetes risk, we performed two-sample univariate Mendelian randomisation analyses using results from published genome-wide association (GWA) studies. We identified single nucleotide polymorphisms (SNPs) with effects specific to either CT-proET-1 or MR-proADM at a *P*-value < 5E-8 as the genetic instrumental variables (IVs) from a published GWA study of European ancestry from Verweij, et al (27). Estimates of the genetic association with type 2 diabetes were extracted from meta-analyses of GWA studies for populations of European ancestry by Mahajan et al. (28) and Bonàs-Guarch et al. (29), depending on the data availability. The procedure for the Mendelian randomisation analysis is provided in detail in Additional File 1: Text S2.

All statistical analyses were performed using R version 4.0.3 (30). *P*-values less than 0.05 were considered statistically significant.

Results

Baseline characteristics of study participants

Baseline characteristics of participants according to incident type 2 diabetes status during follow-up are summarized in Table 1. During a median follow-up time of 13.8 years (interquartile range of 4.8), 862 out of 12,006 participants developed type 2 diabetes. Participants who developed type 2 diabetes were more frequently men. At baseline, in comparison to non-cases, the cases of incident type 2 diabetes were on average older, had higher concentrations of CT-proET-1 and MR-proADM, had a higher BMI and waist circumference, were more frequently hypertensive, had lower

eGFR, had lower concentrations of HDL cholesterol and higher concentrations of total cholesterol, hsCRP, insulin and leptin. Participant characteristics for each participating cohort are presented in Additional File 1: Table S4.

-- Table 1 should appear here --

Associations of CT-proET-1 and MR-proADM with incident type 2 diabetes

Both CT-proET-1 and MR-proADM were positively associated with incident type 2 diabetes in the overall study population. The HRs [95% CIs] in model 1 were 1.30 [1.21; 1.39] per 1-SD increase of CT-proET-1 and 1.57 [1.45; 1.69] per 1-SD increase of log MR-proADM. The associations were attenuated, but remained statistically significant after additional adjustment for diabetes risk factors according to model 2 (HR [95% CI]: 1.10 [1.03; 1.18] per 1-SD increase of CT-proET-1 and 1.11 [1.02; 1.21] per 1-SD increase of log MR-proADM). The association for CT-proET-1 remained stable when we further adjusted for eGFR, insulin, hsCRP, leptin, and fasting glucose (Table 2 and Additional File 1: Table S5). However, it was no longer significant for MR-proADM when insulin, hsCRP or leptin were added to the model (Table 2). The associations of CT-proET-1 and MR-proADM with incident type 2 diabetes were also examined in each participating cohort with a negligible level of heterogeneity (Additional File 1: Figures S3 and S4, respectively).

-- Table 2 should appear here --

We observed significant interactions of MR-proADM with BMI and waist circumference with respect to the association with incident type 2 diabetes (FDR < 0.05) (Table 3). When stratified by BMI, the positive association between MR-proADM and incident type 2 diabetes was only significant in obese participants. The HRs [95%CIs] per 1-

SD increase of log MR-proADM were 1.19 [1.05; 1.34] in obese and 1.02 [0.90; 1.15] in non-obese participants. The results were similar when we stratified by waist circumference (Table 3). In an analysis where we further adjusted for eGFR, insulin, hsCRP and leptin, the association between MR-proADM and incident type 2 diabetes was attenuated, but remained significant in obese participants (HRs [95%CIs] per 1-SD increase of log MR-proADM: 1.14 [1.01; 1.29] in obese and 1.00 [0.88; 1.13] in non-obese participants). No significant differences could be detected in the association between MR-proADM and incident type 2 diabetes across sex and hypertension categories (Table 3).

For CT-proET-1, no relevant interactions with BMI, waist circumference, sex and hypertension were observed with respect to incident type 2 diabetes under FDR < 0.05 (Table 3). The distribution of CT-proET-1 and MR-proADM by subgroup are presented in Additional File 1: Figures S5.

-- Table 3 should appear here --

Mendelian randomisation analysis

We identified one SNP that is specific for CT-proET-1 in the *EDN-1* gene (rs5370) and one SNP that is specific for MR-proADM in the *ADM* gene (rs2957692) and included them as the genetic IVs. The genetic associations with each vasoactive peptide and with type 2 diabetes were extracted from the previously mentioned GWA studies (27-29).

In line with the findings from the time-to-event analysis, our Mendelian randomisation analysis showed a significant positive association between genetically predicted CT-proET-1 and type 2 diabetes risk. The OR [95%CI] was 1.12 [1.03; 1.22]. Conversely, we did not observe a significant association between genetically predicted

MR-proADM and type 2 diabetes risk. The OR [95%CI] for MR-proADM was 0.97 [0.74; 1.27]. Sensitivity analyses using the likelihood-based method yielded similar results (OR [95%CI]: 1.12 [1.02; 1.22] for CT-proET-1 and 0.96 [0.73; 1.27] for MR-proADM) (Table 4).

-- Table 4 should appear here --

Discussion

In the current study, we observed that higher concentrations of both CT-proET-1 and MR-proADM were significantly associated with a higher incidence of type 2 diabetes. This is the first study to demonstrate a positive association between MR-proADM and incident type 2 diabetes independently of classical diabetes risk factors and that the association was more apparent in obese than in non-obese individuals. Using Mendelian randomisation approaches, we added further evidence that genetically predicted CT-proET-1 was significantly associated with a higher risk of type 2 diabetes. No significant association between genetically predicted MR-proADM and type 2 diabetes risk was documented.

Our results corroborate the few existing prospective analyses reporting a positive association of CT-proET-1 with incident type 2 diabetes (21, 22) and added evidence for a similar association for MR-proADM, particularly in obese individuals. In a previous study using data from 7,953 participants of the Prevention of Vascular and Renal End-stage Disease Cohort (18), the authors also reported a significant positive association between MR-proADM and incident type 2 diabetes in a model adjusted for age and sex. However, the association was no longer significant in a multivariable model adjusted for classical diabetes risk factors. Compared with the previous prospective studies, our study represents the largest population-based cohort study

examining the association of CT-proET-1 and MR-proADM with incident type 2 diabetes.

Factors underlying the association between CT-proET-1 and incident type 2 diabetes are not well understood. Studies conducted thus far have demonstrated that overexpression of ET-1 directly limits insulin actions. In skeletal muscles, the activation of endothelin receptor type-A by ET-1 suppresses insulin-mediated Akt phosphorylation and reduces glucose uptake (5, 31). ET-1 also disrupts insulin-regulated glucose transporter 4 translocation to the plasma membrane (32). In adipocytes, ET-1 blocks free fatty acid uptake and induces lipolysis, resulting in increased free fatty acid concentrations (33, 34). Moreover, the interplay between increased free fatty acids and impaired glucose uptake may further exacerbate the dysregulation of lipid metabolism and energy homeostasis in insulin-resistant states (33). Conversely, the inhibition of ET-1 signalling improves insulin sensitivity (35, 36). Altogether, these biological effects suggest that ET-1 promotes insulin resistance and impaired glucose tolerance and thereby increases the risk of type 2 diabetes.

Furthermore, ET-1 is a potent vasoconstrictor, which plays an important role in the pathogenesis of hypertension and chronic kidney disease (3, 37), both are known to be associated with type 2 diabetes. ET-1 signalling also has been linked to increased leptin production (38) and stimulates the secretion of pro-inflammatory cytokines known to be involved in the development of metabolic disorders (6, 39). However, in the present study, the positive association between CT-proET-1 and incident type 2 diabetes remained stable after additional adjustment for eGFR, insulin, hsCRP, leptin, and fasting glucose suggesting other possible explanations.

With regard to ADM, the underlying mechanisms linking higher concentrations with an increased risk of type 2 diabetes seem to be less straightforward. Evidence

374 from *in vivo* and *in vitro* studies suggest that ADM could counteract insulin resistance
375 through its antioxidant effects and the inhibition of insulin secretion (9, 11). The latter
376 notion also implicates ADM in maintaining insulin homeostasis (11). ADM also has
377 anti-inflammatory actions (40). In obesity, ADM expression is upregulated in
378 adipocytes and circulating ADM concentrations are increased (10). Evidence from
379 previous epidemiological studies also suggest positive associations of ADM with BMI
380 and waist circumference (41, 42). Factors that upregulate ADM production in obesity
381 are incompletely understood. In an experimental study using a euglycaemic-
382 hyperinsulinemic clamp technique, acute hyperinsulinemia was demonstrated to
383 induce circulating ADM concentrations in obese, but not in lean individuals (43). This
384 evidence could explain the more apparent association of MR-proADM with incident
385 type 2 diabetes in obese than in non-obese individuals seen in the current study.
386 Furthermore, oxidative stress, insulin resistance, low-grade inflammation and
387 dyslipidaemia, conditions that are commonly found in obesity, were also associated
388 with increased MR-proADM concentrations (16, 44). The increased ADM release in
389 adipocytes seems to be a compensatory action attempting to restrain insulin
390 homeostasis rather than a causal factor of insulin resistance thus, type 2 diabetes. Of
391 note, in our data, the association between MR-proADM and incident type 2 diabetes
392 was attenuated when we further controlled for insulin, hsCRP and leptin. We also did
393 not observe a significant association between genetically predicted MR-proADM and
394 type 2 diabetes risk in our Mendelian randomisation analysis. However, a non-
395 significant association is not evidence for no association. Further studies are needed
396 to confirm our findings.
397 Our study has some limitations that should be considered. As only single
398 measurements of CT-proET-1 and MR-proADM were available at baseline we could

not take into account the intra-individual variation. This could have led to exposure misclassification and regression dilution bias. In the current study, the harmonized data on several known diabetes risk factors, such as physical activity, diet and family history of diabetes, were lacking, which could have led to some degree of residual confounding. Our study participants were predominantly of European descent, which means that further studies need to confirm our findings in other ethnic groups. Finally, due to a very limited number of genetic IVs, we were unable to perform more robust analyses for our Mendelian randomisation.

Our study also has several strengths including the prospective, population-based design and the long-term follow-up with a median of 13.8 years. The use of harmonized data from the population-based cohorts participating in the BiomarCaRE project allows us to include a large sample size. To our knowledge, our study represents the so far largest population-based cohort study examining the association of CT-proET-1 and MR-proADM with incident type 2 diabetes. Furthermore, standardized epidemiological and laboratory procedures based on individual level data also allow for the best possible data analyses, including thorough adjustments for different diabetes risk factors and subgroup analyses.

Conclusions

In conclusion, higher concentrations of CT-proET-1 and MR-proADM were associated with incident type 2 diabetes. However, the positive association between MR-proADM and incident type 2 diabetes seemed to be modified by body composition, with a more apparent association in obese than in non-obese individuals. Our Mendelian randomisation analysis further suggests a probable causal link between CT-proET-1 and type 2 diabetes. These findings raise the possibility that ET-1 might be implicated

in the pathogenesis of type 2 diabetes. Future studies are warranted to examine the utility of both peptides in risk stratification of type 2 diabetes for a better health care decision and their potential as targets for antidiabetic therapy.

List of abbreviations

ADM: adrenomedullin; BiomarCaRE: Biomarkers for Cardiovascular Risk Assessment in Europe; BMI: body mass index; CT-proET-1: C-terminal-proendothelin-1; CI: confidence interval; eGFR estimated glomerular filtration rate; GWA: genome-wide association; ET-1: endothelin-1; FDR: false discovery rate; HDL: high density lipoprotein; HR: hazard ratio; hsCRP: high-sensitivity C-reactive protein; IV: instrumental variable; KORA: Cooperative Health Research in the Region of Augsburg; LOD: limit of detection; MICE: multiple imputation by chained equations; MONICA: Multinational Monitoring of Trends and Determinants in Cardiovascular Disease; MORGAM: MONICA Risk, Genetics, Archiving and Monograph; MR-proADM: mid-regional-proadrenomedullin; OR: odds ratio; PH: proportional hazard; PRIME: Prospective Epidemiological Study of Myocardial Infarction; SD: standard deviation; SNP: single nucleotide polymorphism.

Declarations

Ethics approval and consent to participate

The studies contributing data were conducted in accordance with the principles of the Declaration of Helsinki and were approved by local ethical committees as follows: FINRISK: the Ethics Committee of the National Public Health Institute and the

Coordinating Ethics Committee of Helsinki and Uusimaa Hospital District; PRIME Belfast: the Research Ethics Committee of the Faculty of Medicine, Queen's University Belfast; KORA F4: the Ethics Committee of the Bavarian Chamber of Physicians. All participants provided written informed consent.

Consent for publication

Not applicable.

Availability of data and materials

Data are not made available in a public repository. Access to the data is restricted by the ethical approvals and the legislation of the European Union. Approval by the Principal Investigator of each participating cohort study and the MORGAM/ BiomarCaRE Steering Group are required for release of the data. The MORGAM Manual at https://www.thl.f/publications/morgam/manual/contents.htm gives more information on access to the data.

Competing interests

VS has received honoraria for consulting from Novo Nordisk and Sanofi. He also has ongoing research collaboration with Bayer AG (All unrelated to the present study). CH has received a research grant from Sanofi unrelated to the present study. Other authors have nothing to disclose related to the content of this manuscript.

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Contribution statement CS and BT conceptualized the current study design. CS drafted the manuscript and conducted the statistical analyses. UM and JR provided statistical analysis advice. VS, FK, PJ, CN, JS, CT, WK, KK, JR, SB, TZ, CH, AP and BT collected and researched data. CS, BT, UM, CH, VS, FK and AP contributed to data interpretation. All authors contributed to and critically reviewed the manuscript and approved the final manuscript. CS is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Additional File1: Table S1. Overview of contributing BiomarCaRE cohorts. **Table S2.** Intra-assay and inter-assay coefficients of variation for CT-proET-1 and MR-proADM by participating BiomarCaRE cohort. **Table S3.** Characteristics of participants with complete data. **Table S4.** Characteristics of participant by participating cohort. **Table S5.** Association of CT-proET-1 and MR-proADM with incident type 2 diabetes additionally controlling for baseline fasting glucose in subgroup with available fasting glucose measurements. **Figure S1.** Flowchart showing sample size and reasons for exclusion. **Figure S2.** The distribution (frequency histogram) of CT-proET-1 (A) and MR-proADM (B) in the study population. **Figure S3.** Association between CT-proET-1 and incident type 2 diabetes in each participating BiomarCaRE cohort. **Figure S4.** Association between MR-proADM and incident type 2 diabetes in each participating BiomarCaRE cohort. **Figure S5.** The distribution of CT-proET-1 and MR-proADM by subgroup. **Text S1.** Laboratory measurements for other biomarkers used in the analyses. **Text S2.** Procedure for the univariate Mendelian randomisation analysis.

670 **Table 1. Participant characteristics in the total study population and stratified**

671 **by incident type 2 diabetes status**

	Overall	Incident type 2 diabetes	
		Cases	Non-cases
Number of individuals	12,006	862	11,144
Cohort (N (%))			
FINRISK	7,336 (61.1)	531 (61.6)	6,805 (61.1)
PRIME Belfast	2,496 (20.8)	240 (27.8)	2,256 (20.2)
KORA F4	2,174 (18.1)	91 (10.6)	2,083 (18.7)
CT-proET-1, in pmol/l (mean (SD))	50.7 (13.4)	55.5 (14.2)	50.3 (13.3)
MR-proADM, in nmol/l (geometric mean (antilog SD))	0.46 (1.31)	0.52 (1.30)	0.45 (1.30)
Age, in years (mean (SD))	49.4 (11.8)	54.7 (9.2)	49.0 (11.8)
Male (N (%))	7,072 (58.9)	615 (71.3)	6,457 (57.9)
Body mass index, in kg/m^2 (mean (SD))	26.5 (4.25)	30.6 (5.04)	26.1 (4.01)
Waist circumference, in cm (mean (SD))	88.9 (12.8)	101 (12.9)	87.9 (12.3)
Actual hypertension (N (%)) [a]	4,899 (40.8)	608 (70.5)	4,291 (38.5)
Systolic blood pressure, in mmHg (mean (SD))	132.1 (20.1)	144.0 (20.7)	131.2 (19.8)
Diastolic blood pressure, in mmHg (mean (SD))	81.0 (11.3)	87.2 (11.4)	80.5 (11.2)
Use of antihypertensive medication (N (%))	1,308 (10.9)	225 (26.1)	1,083 (9.7)
Current smoker (N (%))	3,234 (26.9)	234 (27.1)	3,000 (26.9)
Total cholesterol, in mmol/l (mean (SD))	5.59 (1.05)	5.89 (1.06)	5.56 (1.05)
HDL, in mmol/l (mean (SD))	1.37 (0.37)	1.19 (0.33)	1.39 (0.37)
eGFR (ml/min/1.73m^2) (mean (SD))	89.1 (19.4)	84.8 (21.3)	89.4 (19.2)
Insulin (microU/ml) (geometric mean (antilog SD))	5.79 (1.85)	9.04 (1.89)	5.60 (1.82)

hsCRP (mg/l) (geometric mean (antilog SD))	1.23 (3.03)	2.19 (2.83)	1.18 (3.01)
Leptin (ng/ml) (geometric mean (antilog SD))	7.30 (2.69)	11.07 (2.52)	7.07 (2.69)
Fasting glucose (mmol/l) (geometric mean (antilog SD)) [b]	5.01 (1.13)	5.45 (1.23)	4.98 (1.12)

672

673 Data are presented as frequency (percentage) for categorical variables and as mean
674 (SD) for continuous variables. Continuous variables with skewed distributions are
675 presented as geometric mean (antilog SD). [a] Actual hypertension was defined as
676 having systolic blood pressure ≥ 140 mmHg, diastolic blood pressure ≥ 90 mmHg or
677 using antihypertensive medication. [b] Data were available and calculated in 9,112
678 participants of FINRISK and KORA F4 who fasted at least 4 hours (593 cases and
679 8,519 non-cases of incident type 2 diabetes).
680 Abbreviations: CT-proET-1, C-terminal-proendothelin-1; eGFR, estimated glomerular
681 filtration rate; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive
682 protein; KORA, Cooperative Health Research in the Region of Augsburg Study; MR-
683 proADM, mid-regional-proadrenomedullin; PRIME, Prospective Epidemiological
684 Study of Myocardial Infarction; SD, standard deviation.

Table 2. Association of CT-proET-1 and MR-proADM with incident type 2 diabetes

	Adjustment	Hazard ratio [95%CI] N cases / person-years = 862 / 149,937
CT-proET-1	Model 1	1.30 [1.21; 1.39], P < 0.001
	Model 2	1.10 [1.03; 1.18], P = 0.008
	Model 2 + eGFR	1.10 [1.03; 1.19], P = 0.007
	Model 2 + insulin[a]	1.10 [1.02; 1.18], P = 0.012
	Model 2 + hsCRP	1.08 [1.01; 1.16], P = 0.026
	Model 2 + leptin	1.09 [1.02; 1.17], P = 0.018
	Model 2 + eGFR, insulin, hsCRP, leptin	1.09 [1.01; 1.17], P = 0.021
MR-proADM	Model 1	1.57 [1.45; 1.69], P < 0.001
	Model 2	1.11 [1.02; 1.21], P = 0.016
	Model 2 + eGFR	1.12 [1.02; 1.22], P = 0.013
	Model 2 + insulin[a]	1.09 [1.00; 1.18], P = 0.061
	Model 2 + hsCRP	1.08 [0.99; 1.18], P = 0.073
	Model 2 + leptin	1.08 [0.99; 1.18], P = 0.089
	Model 2 + eGFR, insulin, hsCRP, leptin	1.07 [0.98; 1.17], P = 0.153

The associations were computed using Cox regression models per 1-SD increment of log (MR-proADM) and CT-proET-1. The distributions of MR-proADM, insulin, hsCRP, and leptin were right-skewed and thus, were log-transformed to approximate normality. [a] 97% of study participants were fasting at least 4 hours and the exclusion of those who were not fasting or whose fasting status was unknown did not change the results.

Model 1: adjusted for age (continuous, in years), sex (man/woman) and cohort (as a stratum variable);

Model 2: Model 1 + actual hypertension (yes/no), total and high-density lipoprotein cholesterol (continuous, in mmol/l), current smoking status (yes/no) and body mass index (continuous, in kg/m^2).

Abbreviations: CI, confidence interval; CT-proET-1, C-terminal-proendothelin-1; eGFR, estimated glomerular filtration rate; hsCRP, high-sensitivity C-reactive protein; MR-proADM, mid-regional-proadrenomedullin.

Table 3. Subgroup analysis of the association of CT-proET-1 and MR-proADM with incident type 2 diabetes

	N cases / PY	CT-proET-1		MR-proADM	
		Hazard ratio [95%CI]	*P*-interaction	Hazard ratio [95%CI]	*P*-interaction
Overall	862 / 149,937	1.10 [1.03; 1.18], P = 0.008		1.11 [1.02; 1.21], P = 0.016	
BMI (kg/m^2)			0.020		< 0.001 [a]
• Obese (≥ 30)	420 / 22,897	1.09 [0.99; 1.20], P = 0.070		1.19 [1.05; 1.34], P= 0.005	
• Non-obese (< 30)	442 / 127,040	1.11 [1.00; 1.24], P = 0.058		1.02 [0.90; 1.15], P= 0.741	
Waist circumference (cm)			0.348		0.001 [a]
• Obese (Men: ≥ 102, Women: ≥ 88)	470 / 30,126	1.09 [1.00; 1.20], P = 0.055		1.15 [1.03; 1.28], P= 0.013	
• Non-obese (Men: < 102, Women: < 88)	392 / 119,811	1.10 [0.98; 1.24], P = 0.116		1.01 [0.88; 1.15], P= 0.909	
Sex			0.145		0.157
• Men	615 / 91,156	1.07 [0.97; 1.17], P = 0.164		1.06 [0.96; 1.19], P= 0.257	
• Women	247 / 58,781	1.19 [1.05; 1.35], P = 0.006		1.25 [1.08; 1.46], P= 0.004	
Actual hypertension [b]			0.161		0.374
• Yes	608 / 60,423	1.10 [1.01; 1.19], P = 0.026		1.12 [1.02; 1.24], P = 0.023	
• No	254 / 89,513	1.16 [0.99; 1.35], P = 0.069		1.10 [0.93; 1.30], P = 0.267	

The associations were computed using Cox regression models per 1-SD increment of log (MR-proADM) and CT-proET-1. The models included study cohort as a stratum variable and were adjusted for age (continuous, in years), sex (men/women), actual hypertension (yes/no), total and HDL cholesterol (continuous, in mmol/l), current smoking status (yes/no) and BMI (continuous, in kg/m^2) (waist

circumference (continuous, in cm) instead of BMI in models for waist circumference). Actual hypertension was defined as having systolic blood pressure ≥ 140 mmHg, diastolic blood pressure ≥ 90 mmHg or using antihypertensive medication.

[a] Remained significant (FDR < 0.05) after correcting for multiple testing with the Benjamini–Hochberg method.

[b] Actual hypertension was defined as systolic blood pressure ≥ 140 mmHg, diastolic blood pressure ≥ 90 mmHg or using antihypertensive medication.

Abbreviations: BMI, body mass index; CI, confidence interval; CT-proET-1, C-terminal-proendothelin-1; MR-proADM, mid-regional-proadrenomedullin; PY, person-years.

Table 4. Results for the two-sample Mendelian randomisation analysis

SNP (Gene)	Effect allele	Phenotype	Association estimates with vasoactive peptides per 1-SD difference		Association estimates with type 2 diabetes		Methods	Mendelian randomisation estimates on odds ratio scale [95%CI]
			β (SE) [a]	P-value	β (SE)	P-value		
rs5370 (*EDN1*)	T	CT-proET-1	0.213 (0.020)	1.49E-27	0.024 (0.009)	0.002	Wald ratio	1.12 [1.03; 1.22]; P = 0.011
							Maximum likelihood	1.12 [1.02; 1.22]; P = 0.013
rs2957692 (*ADM*)	G	MR-proADM	-0.115 (0.015)	1.05E-12	0.004 (0.016)	0.798	Wald ratio	0.97 [0.74: 1.27]; P = 0.798
							Maximum likelihood	0.96 [0.73; 1.27]; P = 0.798

[a] Standardized β estimates.

Abbreviations: CI, confidence interval; CT-proET-1, C-terminal-proendothelin-1; MR-proADM, mid-regional-proadrenomedullin; SE, standard error; SNP, single nucleotide polymorphism.

Additional File 1

Associations of the Vasoactive Peptides CT-proET-1 and MR-proADM with Incident Type 2 Diabetes: Results from the BiomarCaRE Consortium

Chaterina Sujana[1,2,3], Veikko Salomaa[4], Frank Kee[5], Jochen Seissler[6], Pekka Jousilahti[4], Charlotte Neville[5], Cornelia Then[6], Wolfgang Koenig[7,8,9], Kari Kuulasmaa[4], Jaakko Reinikainen[4], Stefan Blankenberg[10,11], Tanja Zeller[10,11], Christian Herder[12,13,14], Ulrich Mansmann[2], Annette Peters[1,3,9], Barbara Thorand[1,3], for the BiomarCaRE Consortium

[1] Institute of Epidemiology, Helmholtz Zentrum München - German Research Center for Environmental Health, Neuherberg, Germany;
[2] Institute for Medical Information Processing, Biometry, and Epidemiology (IBE), Pettenkofer School of Public Health, Ludwig-Maximilians-Universität München, Munich, Germany;
[3] German Center for Diabetes Research (DZD), Partner München-Neuherberg, Neuherberg, Germany.
[4] Department of Public Health and Welfare, Finnish Institute for Health and Welfare, Helsinki, Finland;
[5] Centre for Public Health, Queens University of Belfast, Belfast, Northern Ireland, UK;
[6] Diabetes Zentrum, Medizinische Klinik und Poliklinik IV, Klinikum der Ludwig-Maximilians-Universität München, Munich, Germany;
[7] Institute of Epidemiology and Medical Biometry - University of Ulm, Ulm, Germany;
[8] Deutsches Herzzentrum München, Technische Universität München, Munich, Germany;
[9] German Centre for Cardiovascular Research (DZHK e.V.), partner site Munich Heart Alliance, Munich, Germany;
[10] Department for General and Interventional Cardiology, University Heart Center Hamburg, Hamburg, Germany;
[11] German Centre for Cardiovascular Research (DZHK e.V.), partner site Hamburg, Lübeck, Kiel, Hamburg, Germany;

[12] Institute for Clinical Diabetology, German Diabetes Center, Leibniz Center for Diabetes Research at Heinrich-Heine-University Düsseldorf, Düsseldorf, Germany;
[13] Division of Endocrinology and Diabetology, Medical Faculty and University Hospital Düsseldorf, Heinrich-Heine-University Düsseldorf, Düsseldorf, Germany;
[14] German Center for Diabetes Research (DZD), Partner Düsseldorf, Neuherberg, Germany.

Table S1. Overview of contributing BiomarCaRE cohorts

Cohort	Country	Short description
Kooperative Gesundheits-forschung in der Region Augsburg (KORA) F4	Germany	The Cooperative Health Research in the Region of Augsburg F4 (KORA F4; 2006-2008) is the first follow-up examination of the fourth survey of the population-based KORA study (KORA S4; 1999–2001). The S4 study comprises randomly selected respondents aged 25-74 years from the city of Augsburg in Bavaria, Southern Germany and its two surrounding counties. List of municipalities and population registers were used as sampling frames. Out of 4,261 S4 participants, 3,080 participated in F4. The BiomarCaRE project includes 3,060 participants from F4. Follow-up examinations of KORA S4 participants were conducted in 2006-2008 (F4) and 2013/2014 (FF4). Follow-up questionnaires were sent to all KORA S4 study participants in 2008/2009 and 2016 to obtain information on the occurrence of chronic diseases and risk factors. The core BiomarCaRE database includes follow-up data until 2009. In the current analysis, we included data from the KORA F4 study who were followed for incident type 2 diabetes up to 2016. Prevalent or incident type 2 diabetes was defined as self-reported clinically diagnosed diabetes that could be validated by the responsible physician or hospital discharge letters, or by self-reported use of glucose-lowering medication. Furthermore, participants with clinical diagnoses or comorbidity ICD-code (ICD-9: 250) on the death certificate were coded as prevalent or incident diabetes. For incident diabetes cases, the self-reported date of diagnosis was assessed and generally verified by contacting the treating physician or medical chart review. When information on the type of diabetes was not available, it was considered to be type 2 diabetes if the age of the person was above 35 years at the time of diagnosis. https://www.thl.fi/publications/morgam/cohorts/full/germany/ger-auga.htm
FINRISK	Finland	The FINRISK study is a series of population-based cardiovascular risk factor surveys carried out every five years in five (or six in 2002) districts of Finland, including North Karelia, Northern Savo (former Kuopio), South-western Finland, Oulu Province, Lapland province (in 2002 only) and the region of Helsinki and Vantaa. A stratified random sample was drawn for each survey from the national population register; the age-range was 25-74 years. All individuals enrolled in the study received a physical examination, a self-administered questionnaire, and a blood sample examination.

		In 1997, altogether 11,500 individuals were invited and 8,444 (73%) participated in the clinical examination. During the follow-up time the National Hospital Discharge Register, the National Causes of Death Register and the National Drug Reimbursement Register were used to identify endpoints. Participants were followed up until December 31st, 2010. The cohorts were linked to the Hospital Discharge Register and Causes of Death Register and drug reimbursement registers. A hospitalization or death with the ICD-8/9 code 250 or with any of the ICD-10 codes E10, E11 and E14 was considered to indicate diabetes. Likewise, the appearance of "special reimbursements" for diabetes mellitus, i.e., KELA code 103, or purchases of drugs with the ATC code A10* were taken as diabetes. The type of diabetes was then determined as follows: If the age of the patient was < 35 at the time of diagnosis and the treatment was insulin only, the person was considered to have type 1 diabetes. All others were considered to have type 2 diabetes. http://www.thl.fi/publications/morgam/cohorts/full/finland/fin-fina.htm
Prospective Epidemiological Study of Myocardial Infarction (PRIME) Belfast	United Kingdom	The PRIME study examined the classic and putative cardiovascular risk factors to explain the large difference in heart disease incidence between Ireland and France. The study includes four cohorts of men aged 50-59; from Belfast, Northern Ireland (N= 2,745) and Lille (N= 2,633), Toulouse (N= 2,610) and Strasbourg (N= 2,612) in France. The current study only includes the Belfast cohort, since data on vasoactive peptides of interest was not available for the French cohorts. Baseline examinations took place in 1991-1994 and targeted cohorts that had broadly similar social class structures to the background population. Study participants were followed up until 2012 through annual follow up questionnaires with verification against national death registers, medical records, and hospital discharge diagnoses. Endpoints were validated by expert medical committee. Type 2 diabetes cases identified by contacting the practitioner of each subject who self-reported type 2 diabetes to validate diabetes type, treatment and diagnosis date. http://www.thl.fi/publications/morgam/cohorts/full/uk/unk-bela.htm

Table S2. Intra-assay and inter-assay coefficients of variation for CT-proET-1 and MR-proADM by participating BiomarCaRE cohort

Cohort	CT-proET-1		MR-proADM	
	Intra-assay (%)	Inter-assay (%)	Intra-assay (%)	Inter-assay (%)
FINRISK	2.16 - 2.61	1.74 - 8.79	2.17	2.43
PRIME Belfast	2.61	3.57	2.17	2.43
KORA F4	4.8	6.9	4.5	7.8

Abbreviations: CT-proET-1, C-terminal-proendothelin-1; KORA, Cooperative Health Research in the Region of Augsburg Study; MR-proADM, mid-regional-proadrenomedullin; PRIME, Prospective Epidemiological Study of Myocardial Infarction.

Table S3. Characteristics of participants with complete data

	values	% missing
Number of individuals	12,006	
Event (N (%))		0%
Incident type 2 diabetes	862 (7.2%)	
Censored	11,144 (92.8%)	
Cohort (N (%))		0%
FINRISK	7,336 (61.1%)	
PRIME Belfast	2,496 (20.8%)	
KORA F4	2,174 (18.1%)	
CT-proET-1, in pmol/l (mean (SD))	51.3 (13.5)	17.9%
MR-proADM, in nmol/l (geometric mean (antilog SD))	0.45 (1.31)	17.9%
Age, in years (mean (SD))	49.4 (11.8)	0%
Male (N (%))	7,072 (58.9%)	0%
Body mass index, in kg/m^2 (mean (SD))	26.5 (4.25)	0.1%
Actual hypertension [a] (N (%))	4,886 (41.0%)	0.7%
Systolic blood pressure, in mmHg (mean (SD))	132.1 (20.1)	0.1%
Diastolic blood pressure, in mmHg (mean (SD))	81.0 (11.3)	0.1%
Use of antihypertensive medication (N (%))	1,294 (11.0%)	1.6%
Current smoker (N (%))	3,234 (26.9%)	0%
Total cholesterol, in mmol/l (mean (SD))	5.59 (1.05)	0.4%
HDL, in mmol/l (mean (SD))	1.38 (0.37)	0.4%
eGFR (ml/min/1.73m^2) (mean (SD))	89.5 (19.1)	10.5%
hsCRP (mg/l) (geometric mean (antilog SD))	1.22 (3.03)	5.7%
Insulin (microU/ml) (geometric mean (antilog SD))	5.81 (1.84)	8.1%
Leptin (ng/ml) (geometric mean (antilog SD))	7.37 (2.69)	6.1%
Fasting glucose (mmol/l) (geometric mean (antilog SD)) [b]	5.03 (1.13)	27.0%

Data are presented as frequency (percentage) for categorical variables and as mean (SD) for continuous variables. Continuous variables with skewed distributions are presented as geometric mean (antilog SD). [a] Actual hypertension was defined as having systolic blood pressure ≥ 140 mmHg, diastolic blood pressure ≥ 90 mmHg or using antihypertensive medication. [b] Data were available and calculated in participants of FINRISK and KORA F4 who fasted at least 4 hours.

Abbreviations: eGFR, estimated glomerular filtration rate; CT-proET-1, C-terminal-proendothelin-1; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; KORA, Cooperative Health Research in the Region of Augsburg Study; MR-proADM, mid-regional-proadrenomedullin; PRIME, Prospective Epidemiological Study of Myocardial Infarction; SD, standard deviation.

Table S4. Characteristics of participant by participating cohort

	FINRISK	PRIME Belfast	KORA F4
N	7,336	2,496	2,174
Examination years	1997	1991-1994	2006-2008
Median of follow-up time in years (IQR)	13.8 (0.11)	18.0 (4.38)	8.1 (1.02)
Incident type 2 diabetes (N (%))	531 (7.2)	240 (9.6)	91 (4.2)
Person-years	95,093	38,380	16,464
Incidence rate of type 2 diabetes per 1000 person-years (95% CI)	5.58 (5.12; 6.08)	6.25 (5.49; 7.10)	5.53 (4.45; 6.79)
CT-proET-1, in pmol/l (mean (SD))	53.2 (14.5)	49.2 (9.7)	44.2 (10.5)
MR-proADM, in nmol/l (geometric mean (antilog SD))	0.46 (1.34)	0.44 (1.23)	0.47 (1.26)
Age, in years (mean (SD))	47.0 (13.1)	54.7 (2.90)	51.5 (10.7)
Male (N (%))	3,569 (48.7)	2,496 (100.0)	1,007 (46.3)
Body mass index, in kg/m^2 (mean (SD))	26.4 (4.42)	26.2 (3.38)	26.9 (4.55)
Waist circumference, in cm (mean (SD))	87.4 (13.3)	91.1 (9.3)	91.2 (13.6)
Actual hypertension (N (%)) [a]	3,273 (44.6)	1,044 (41.8)	582 (26.8)
Systolic blood pressure, in mmHg (mean (SD))	134.9 (19.7)	133.7 (20.3)	120.8 (17.5)
Diastolic blood pressure, in mmHg (mean (SD))	82.1 (11.3)	81.8 (11.4)	76.1 (9.9)
Use of antihypertensive medication (N (%))	748 (10.2)	217 (8.7)	343 (15.8)
Current smoker (N (%))	1,991 (27.1)	786 (31.5)	457 (21.0)
Total cholesterol, in mmol/l (mean (SD))	5.49 (1.06)	5.88 (1.02)	5.58 (1.00)
HDL, in mmol/l (mean (SD))	1.41 (0.36)	1.19 (0.32)	1.47 (0.38)
eGFR (ml/min/1.73m^2) (mean (SD))	89.7 (19.9)	83.9 (20.9)	92.8 (14.2)
hsCRP (mg/l) (geometric mean (antilog SD))	1.16 (3.04)	1.63 (2.87)	1.09 (3.06)

Insulin (microU/ml) (geometric mean (antilog SD))	5.18 (1.87)	5.77 (1.70)	8.48 (1.68)
Leptin (ng/ml) (geometric mean (antilog SD))	7.98 (2.57)	4.32 (2.10)	9.85 (3.20)
Fasting glucose (mmol/l) (geometric mean (antilog SD)) [b]	4.96 (1.14)	NA	5.17 (1.11)

Data are presented as frequency (percentage) for categorical variables and as mean (SD) for continuous variables. Continuous variables with skewed distributions are presented as geometric mean (antilog SD). [a] Actual hypertension was defined as having systolic blood pressure ≥ 140 mmHg, diastolic blood pressure ≥ 90 mmHg or using antihypertensive medication. [b] Data were available and calculated in 6,952 FINRISK participants and 2,160 KORA F4 participants who fasted at least 4 hours.
Abbreviations: eGFR, estimated glomerular filtration rate; CT-proET-1, C-terminal-proendothelin-1; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; KORA, Cooperative Health Research in the Region of Augsburg Study; MR-proADM, mid-regional-proadrenomedullin; PRIME, Prospective Epidemiological Study of Myocardial Infarction; SD, standard deviation.

Table S5. Association of CT-proET-1 and MR-proADM with incident type 2 diabetes additionally controlling for baseline fasting glucose in subgroup with available fasting glucose measurements

	Adjustment	Hazard ratio [95%CI] N cases / person-years = 593 / 106,651
CT-proET-1	Model 1	1.32 [1.23; 1.43], P < 0.001
	Model 2	1.13 [1.04; 1.22], P = 0.004
	Model 2 + eGFR	1.13 [1.04; 1.23], P = 0.003
	Model 2 + fasting insulin	1.12 [1.03; 1.22], P = 0.006
	Model 2 + hsCRP	1.11 [1.02; 1.20], P = 0.012
	Model 2 + leptin	1.12 [1.04; 1.22], P = 0.005
	Model 2 + fasting glucose	1.17 [1.08; 1.27], P < 0.001
	Model 2 + eGFR, fasting insulin, hsCRP, leptin, fasting glucose	1.16 [1.07; 1.25], P = 0.001
MR-proADM	Model 1	1.66 [1.51; 1.81], P < 0.001
	Model 2	1.15 [1.04; 1.28], P = 0.006
	Model 2 + eGFR	1.16 [1.05; 1.29], P = 0.005
	Model 2 + fasting insulin	1.12 [1.02; 1.24], P = 0.024
	Model 2 + hsCRP	1.12 [1.01; 1.24], P = 0.033
	Model 2 + leptin	1.14 [1.02; 1.26], P = 0.017
	Model 2 + fasting glucose	1.23 [1.11; 1.36], P < 0.001
	Model 2 + eGFR, fasting insulin, hsCRP, leptin, fasting glucose	1.19 [1.07; 1.32], P = 0.001

The associations were computed using Cox regression models per 1-SD increment of log (MR-proADM) and CT-proET-1. Data on fasting glucose were only available in FINRISK and KORA; thus, the analyses were performed in these cohorts (N= 9,112). The distributions of MR-proADM, insulin, hsCRP, leptin, and fasting glucose were right-skewed and thus, were log-transformed to approximate normality.

Model 1: adjusted for age (continuous, in years), sex (man/woman) and cohort (as a stratum variable);

Model 2: Model 1 + actual hypertension (yes/no), total and high-density lipoprotein cholesterol (continuous, in mmol/l), current smoking status (yes/no) and body mass index (continuous, in kg/m2).

Abbreviations: CI, confidence interval; CT-proET-1, C-terminal-proendothelin-1; eGFR, estimated glomerular filtration rate; hsCRP, high-sensitivity C-reactive protein; MR-proADM, mid-regional-proadrenomedullin.

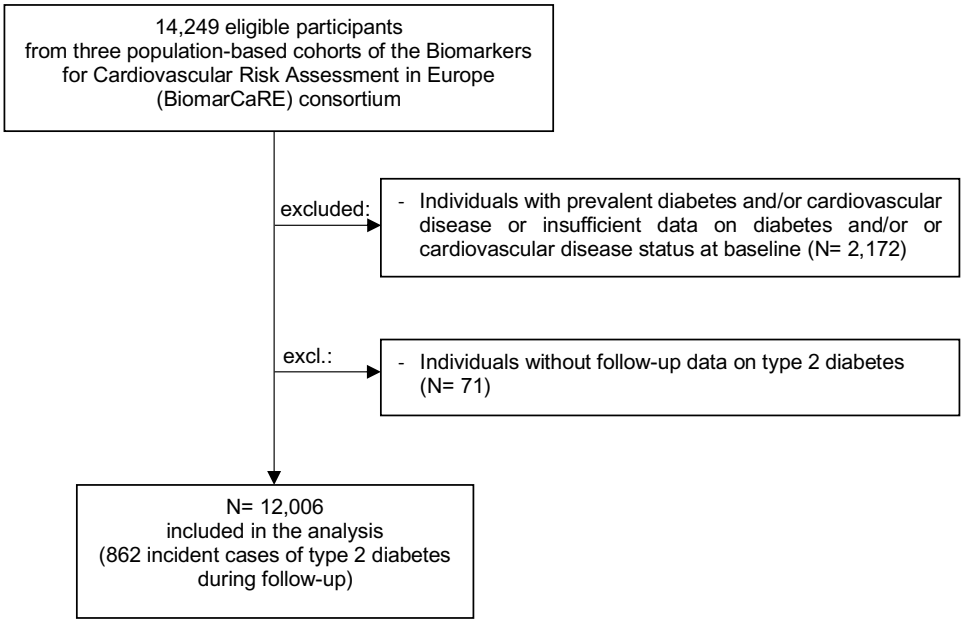


Figure S1. Flowchart showing sample size and reasons for exclusion

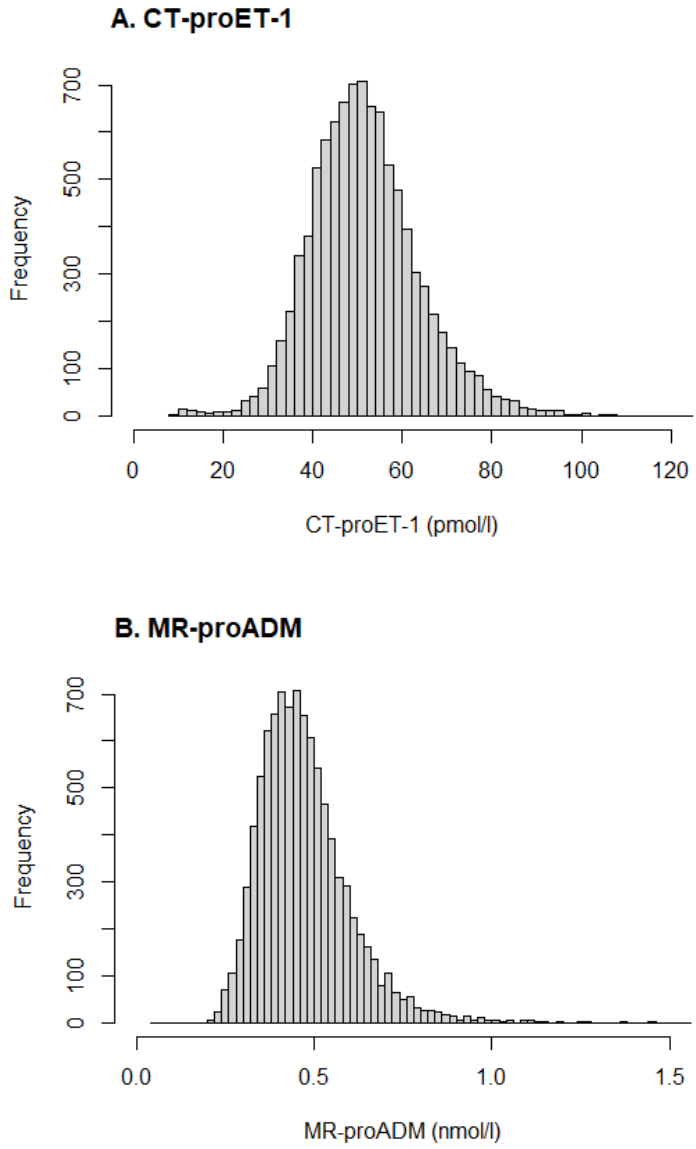


Figure S2. The distribution of CT-proET-1 (A) and MR-proADM (B) in the study population. The distributions of both vasoactive peptides were examined in all study participants with complete data for the main analysis. Abbreviations: CT-proET-1, C-terminal-proendothelin-1; MR-proADM, mid-regional-proadrenomedullin.

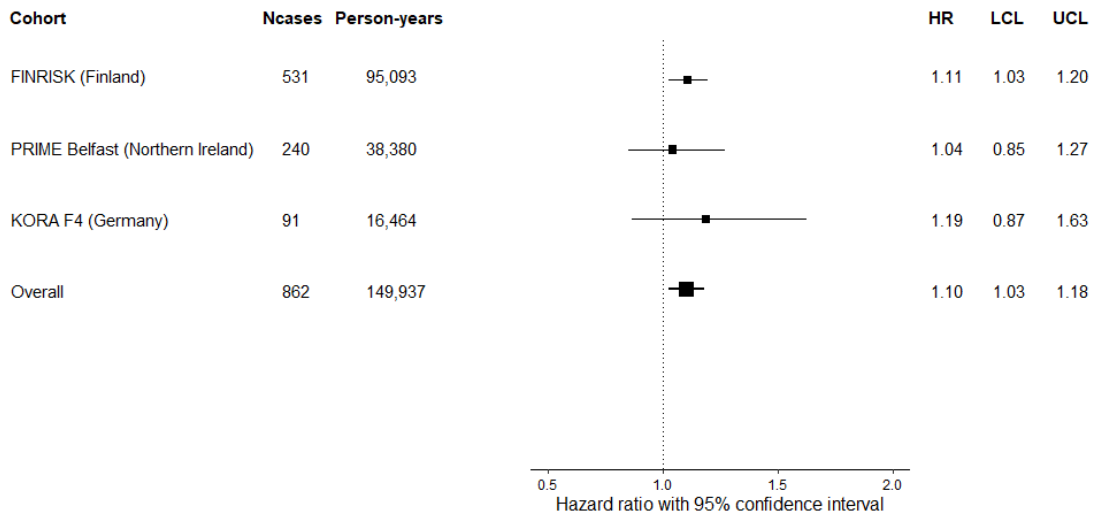


Figure S3. Association between CT-proET-1 and incident type 2 diabetes in each participating BiomarCaRE cohort. Hazard ratios for each study cohort were computed using Cox models. The models were adjusted for age (continuous, in years), sex (men/women), body mass index (continuous, in kg/m^2), current smoking (yes/no), actual hypertension (yes/no), total and high-density lipoprotein cholesterol (continuous, in mmol/l). CT-proET-1 was (0,1)-standardized in the total study population to evaluate the hazard ratios per 1-standard deviation increase. Overall estimate was calculated using DerSimonian-Laird random-effects model. Heterogeneity: *P*-value for interaction between CT-proET-1 and study cohort = 0.682; *P*-value for Cochran's Q = 0.761; I^2 = 0%. Black squares represent hazard ratios and bars represent 95% confidence intervals per 1-standard deviation increment of CT-proET-1. Abbreviations: BiomarCaRE, Biomarkers for Cardiovascular Risk Assessment in Europe; CT-proET-1, C-terminal-proendothelin-1; HR, hazard ratio; KORA, Cooperative Health Research in the Region of Augsburg Study; LCL, lower confidence limit; Ncases, number of incident type 2 diabetes cases; PRIME, Prospective Epidemiological Study of Myocardial Infarction; UCL, upper confidence limit.

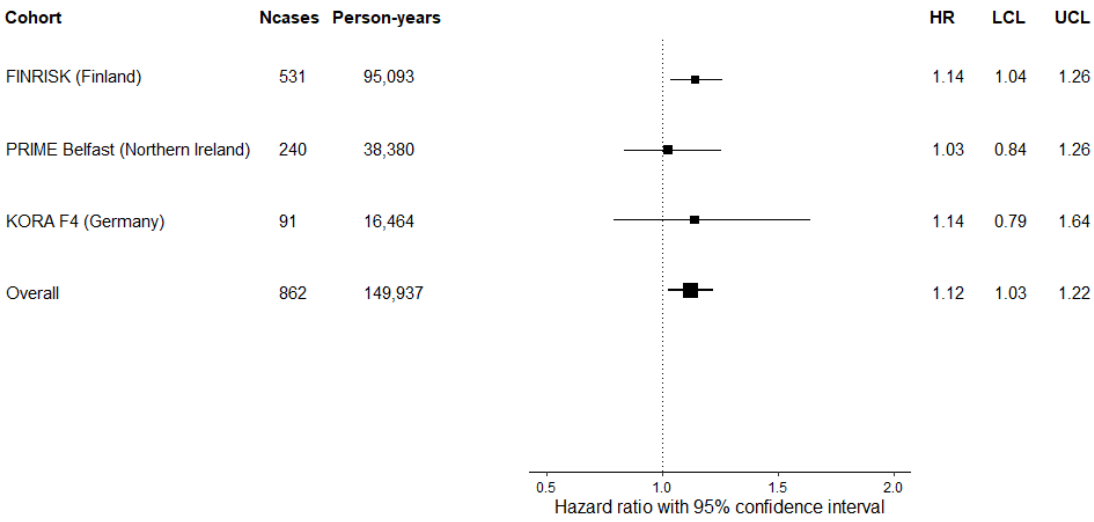
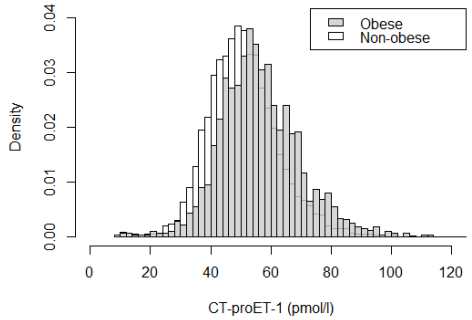
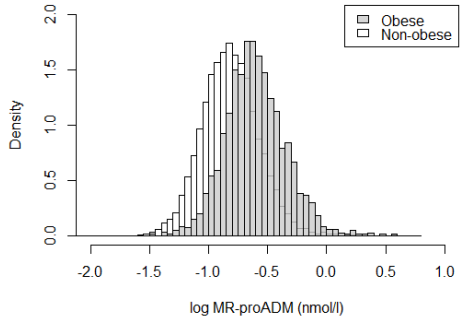
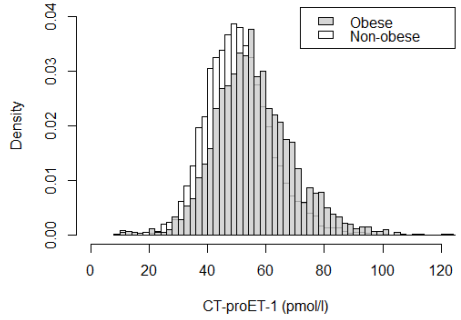
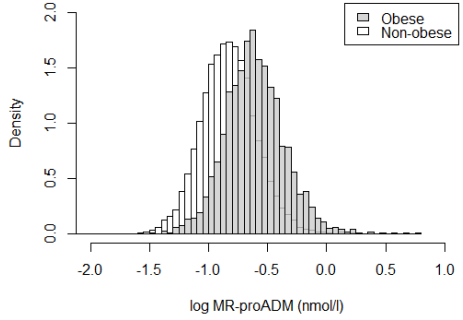


Figure S4. Association between MR-proADM and incident type 2 diabetes in each participating BiomarCaRE cohort. Hazard ratios for each study cohort were computed using Cox models. The models were adjusted for age (continuous, in years), sex (men/women), body mass index (continuous, in kg/m^2), current smoking (yes/no), actual hypertension (yes/no), total and high-density lipoprotein cholesterol (continuous, in mmol/l). MR-proADM was log-transformed and (0,1)-standardized in the total study population to approximate normality and to evaluate the hazard ratios per 1-standard deviation increase. Overall estimate was calculated using DerSimonian-Laird random-effects model. Heterogeneity: *P*-value for interaction between MR-proADM and study cohort = 0.379; *P*-value for Cochran's Q = 0.639; I^2 = 0%. Black squares represent hazard ratios and bars represent 95% confidence intervals per 1-standard deviation increment of log MR-proADM. Abbreviations: BiomarCaRE, Biomarkers for Cardiovascular Risk Assessment in Europe; HR, hazard ratio; KORA, Cooperative Health Research in the Region of Augsburg Study; LCL, lower confidence limit; MR-proADM, mid-regional-proadrenomedullin; Ncases, number of incident type 2 diabetes cases; PRIME, Prospective Epidemiological Study of Myocardial Infarction; UCL, upper confidence limit.

Subgroup	CT-proET-1	MR-proADM
BMI (kg/m^2) Obese (≥ 30) vs Non-obese (< 30)		
Waist circumference (cm) Obese (Men: ≥ 102, Women: ≥ 88) vs Non-obese (Men: < 102, Women: < 88)		
Sex		

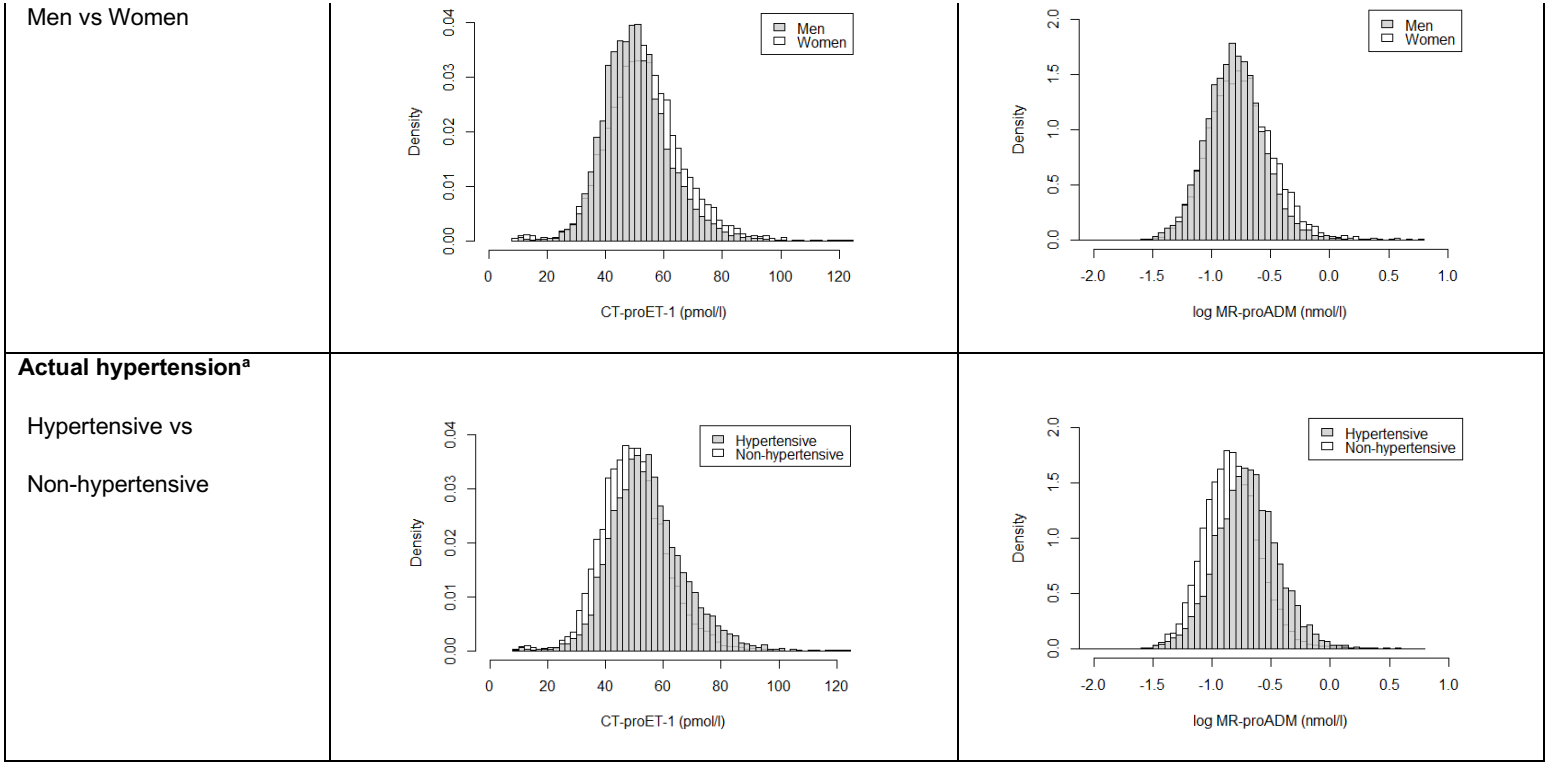


Figure S5. The distribution of CT-proET-1 and MR-proADM by subgroup. The distributions of both vasoactive peptides were examined in all study participants with complete data for the main analysis. [a]Actual hypertension was defined as systolic blood pressure ≥ 140 mmHg, diastolic blood pressure ≥ 90 mmHg or using antihypertensive medication. Abbreviations: BMI, body mass index; CT-proET-1, C-terminal-proendothelin-1; MR-proADM, mid-regional-proadrenomedullin.

Text S1. Laboratory measurements for other biomarkers used in the analyses

Baseline concentrations of high-sensitivity C-reactive protein (hsCRP) were centrally measured in MORGAM/BiomarCaRE core laboratory for the FINRISK Study and the Prospective Epidemiological Study of Myocardial Infarction (PRIME) Belfast Study. hsCRP was measured from blood serum using latex immunoassay CRP16 (Turbidimetric / Immunoturbidimetric, Architect c8000 Abbott, Wiesbaden, Germany). In the Cooperative Health Research in the Augsburg Region Study (KORA) F4 Study, hsCRP was measured from plasma using a latex-enhanced immunonephelometry (BN II, Siemens, Erlangen, Germany).

Baseline concentrations of insulin were measured from serum using chemiluminescent microparticle immunoassay CMIA (Abbott, Architect i2000) for FINRISK and PRIME Belfast. In PRIME Belfast, data were measured after at least 8 hours fasting. In FINRISK, vast majority of the participants fasted at least 4 hours. Around 5% of FINRISK study participants did not fast or for whom the fasting status was unknown. In KORA F4, the data were measured from serum after an overnight fasting of at least 8 hours using electrochemiluminescence immunoassay (ECLIA, Roche Diagnostics GmbH, Mannheim, Germany) on the Cobas e602 instrument.

Baseline concentrations of leptin were measured from serum with an enzyme immunoassay technique using the Quantikine ELISA Kit (R&D Systems, Minneapolis, MN, USA) for FINRISK and PRIME Belfast and using the Mercodia ELISA (Mercodia AB, Uppsala, Sweden) for KORA F4.

Baseline concentrations of fasting glucose were only available for FINRISK and KORA-F4. In FINRISK, fasting glucose was measured from serum or EDTA plasma using a hexokinase method (Hexokinase/G-6-PDH, Architect c8000, Abbott). The required fasting duration was 4 hours, and the majority of FINRISK participants fasted at least 4 hours but less than 8 hours. In KORA F4, fasting glucose was measured from serum after an overnight fasting of at least 8 hours on an enzymatic colorimetric method on a Dimension Vista 1500 instrument (Siemens Healthcare Diagnostics Inc., Newark, NJ, USA) or the GLUC3 assay on a Cobas c702 instrument (Roche Diagnostics GmbH, Mannheim, Germany).

Estimated glomerular filtration rate (eGFR) in all participating cohorts was estimated using the CKD-EPI formula with creatinine (1).

Text S2. Procedure for the univariate Mendelian randomisation analysis

In our study, we tried to satisfy the three main assumptions for a valid genetic instrumental variable (IV) in our Mendelian randomisation analysis. The three assumptions are: (A1) the IV must be associated with the exposure (relevance), (A2) the IV should be independent of the outcome conditional on the exposure and confounders (exclusion restriction), and (A3) the IV should not be associated with confounders of the exposure-outcome association (exchangeability) (2, 3). The A1 assumption is the only assumption that can be directly tested (2).

The A1 assumption was satisfied by including single nucleotide polymorphisms (SNPs) that are associated with C-terminal-proendothelin-1 (CT-proET-1) or mid-regional-proadrenomedullin (MR-proADM) at a *P*-value < 5E-8 as the IVs. The data were extracted from a published genome-wide association (GWA) study of European ancestry from Verweij et al. (4). The study consists of 3,444 GWA study discovery samples and 3,230 replication samples from the Prevention of Renal and Vascular End-Stage Disease (PREVEND) study. We identified 6 SNPs for each peptide at a *P*-value < 5E-8.

Violations of the A2 and A3 assumption can occur in case of horizontal pleiotropy that is a scenario where a SNP is associated with other variables on different causal pathways to the outcome (2, 3). We tried to meet the A2 and A3 assumptions by including only SNPs that are not in linkage disequilibrium with each other and are specific for either CT-proET-1 or MR-proADM. For each peptide, we identified 3 out of 6 SNPs that are not in linkage disequilibrium with each other, using the r^2 cut-off of 0.1 to obtain independent SNPs. Out of these 3 independent SNPs for each peptide, only 1 SNP that is specific for CT-proET-1 (rs5370; gene: *EDN-1*) and 1 SNP that is specific for MR-proADM (rs2957692; gene: *ADM*). Thus, we only included one SNP as the IV for each peptide in our Mendelian randomisation analysis. Furthermore, violations of the A3 assumption can also occur in case of population stratification when the study sample includes subgroups with different genetic ancestries and thus, different allele frequencies (2). We tried to overcome this issue by focusing our Mendelian randomisation analysis on homogeneous ancestry groups, in this case we focused our analysis on individuals with European ancestry.

The estimates of the genetic associations between IVs and the risk of type 2 diabetes were extracted from meta-analyses of GWA studies of European ancestry on

type 2 diabetes by Mahajan et al. (5) comprising 48,286 type 2 diabetes cases and 250,671 controls and by Bonàs-Guarch et al. (6) comprising 12,931 type 2 diabetes cases and 57,196 controls, depending on the data availability. We prioritized the meta-analysis by Mahajan et al. due to the large sample size. Before performing the Mendelian randomisation analyses, we made sure that the IV association estimates with each vasoactive peptide and with type 2 diabetes correspond to the same effect alleles.

To compute the Mendelian randomisation estimates we used the Wald ratio (7) compared the results with the likelihood-based method (8). For an easier interpretation, we reported the estimates with 95% confidence intervals on the odds ratio (OR) scale. *P*-values < 0.05 were considered statistically significant. Due to a very few numbers of IVs, we were not able to perform other robust methods, including MR-Egger regression model (9). Our Mendelian randomisation analyses were performed in the statistical software R version 4.0.3 (10) using "MendelianRandomization" R-package version 0.5.0 (11).

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The BiomarCaRE Consortium website: http://www.biomarcare.eu/

Sites and key personnel of contributing BiomarCaRE centers:

BiomarCaRE center	Investigators
FINRISK	Veikko Salomaa (Principal Investigator), Anne Juolevi, Erkki Vartiainen, Pekka Jousilahti, Tiina Laatikainen, Kennet Harald.
Prospective Epidemiological Study of Myocardial Infarction (PRIME) Belfast	Frank Kee (Principal Investigator), Alun Evans (former Principal Investigator), John Yarnell, Angela Scott, Evelyn Gardner (up to 2008).
Kooperative Gesundheits-forschung in der Region Augsburg (KORA)	Barbara Thorand (Principal Investigator), Annette Peters (former Principal Investigator), Angela Döring (former Principal Investigator), Christa Meisinger, Margit Heier, Andrea Schneider.
BiomarCaRE Laboratory	Tanja Zeller (Head), Stefan Blankenberg, University Medical Center Hamburg-Eppendorf, Hamburg, Germany.
MORGAM Data Centre	Kari Kuulasmaa (Head), Ari Haukijärvi, Teemu Niiranen, Tarja Palosaari, Jaakko Reinikainen, Finnish Institute for Health and Welfare (THL), Helsinki, Finland
MORGAM/BiomarCaRE Steering Group	Stefan Blankenberg, Alun Evans, Licia Iacoviello, Frank Kee, Wolfgang Koenig, Kari Kuulasmaa, Teemu Niiranen, Markus Perola, Veikko Salomaa, Renate Schnabel, Hugh Tunstall-Pedoe, Giovanni Veronesi, Tanja Zeller.

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List of all scientific publications to date

Sujana, C.; Salomaa, V.; Kee, F.; Jousilahti, P.; Neville, C.; Koenig, W.; Kuulasmaa, K.; Reinikainen, J.; Blankenberg, S.; Zeller, T.; Herder, C.; Mansmann, U.; Peters, A.; and Thorand, B. Associations of the Vasoactive Peptides CT-proET-1 and MR-proADM with Incident Type 2 Diabetes: Results from the BiomarCaRE Consortium. Cardiovascular Diabetology (in press).

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