Meal-induced changes in cardiometabolic peptides in the context of acute cardiovascular diseases and glucose metabolism

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Research Article

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Abstract

Background: Natriuretic peptides (NPs) and copeptin are established cardiovascular biomarkers which can partly affect human metabolism. However, impact of human metabolism on their postprandial regulation in high-risk patients remains insufficiently understood. We aimed to characterize meal-induced changes in MR-proANP, NT-proBNP, and copeptin and to assess how these dynamics relate to acute cardiovascular (CV) events, type 2 diabetes (T2D) and insulin resistance (IR).

Methods: Within the BeLOVE cohort, a subset of 373 participants with acute coronary syndrome, stroke or very high chronic CV risk underwent standardized study-specific mixed-meal testing 90 days after acute event or enrollment. Fasting and 120-min postprandial concentrations of MR-proANP, NT-proBNP, copeptin, insulin and glucose were assessed. Structural equation models were used to evaluate direct and indirect associations of acute CV events, T2D, and IR (HOMA-IR) with fasting levels and postprandial changes, adjusted for age, sex and BMI.

Results: MR-proANP showed the most pronounced meal-induced suppression. NT-proBNP demonstrated only minor and inconsistent postprandial changes. For all peptides, fasting concentrations were the main determinant of their postprandial changes. Acute CV events were associated with higher fasting NP levels (MR-proANP: β=0.22 [0.09; 0.34]; NT-proBNP: β=0.28 [0.15; 0.41]), while postprandial dynamics were not after accounting for baseline levels (MR-proANP: β=0.09 [−0.08; 0.25]; NT-proBNP: β=0.09 [−0.08; 0.26]). T2D showed no consistent association with fasting (MR-proANP: β=−0.02 [−0.13; 0.09]; NT-proBNP: β=0.08 [−0.04; 0.20]) or postprandial NP responses (MR-proANP: β=0.00 [−0.14; 0.14]; NT-proBNP: β=−0.05 [−0.18; 0.08]). Higher HOMA-IR was independently associated with lower fasting MR-proANP (β=−0.19 [−0.30; −0.07]) and a stronger postprandial decline (β=−0.18 [−0.33; −0.04]). Similarly, higher HOMA-IR was linked to lower fasting NT-proBNP (β=−0.15 [−0.28; −0.03]) but not to its postprandial change (β=−0.09 [−0.24; 0.05]). Copeptin dynamics were largely independent of acute CV disease (fasting: β=0.04 [−0.12; 0.20]; postprandial: β=−0.13 [−0.29; 0.02]) and metabolic status (fasting: β=0.07 [−0.06; 0.20], β=0.11 [−0.03; 0.24]; postprandial: β=0.06 [−0.08; 0.19], β=0.01 [−0.13; 0.15] for T2D and HOMA-IR, respectively).

Conclusions: In patients with an acute CV event, postprandial MR-proANP dynamics are linked to insulin resistance. This association suggests that impaired ANP regulation may represent a key component of metabolic inflexibility in high-risk individuals. In contrast, the limited postprandial variability of NT-proBNP and the largely fasting-related changes of copeptin make these markers less suited for capturing short-term metabolic responses. Together, these findings highlight the value of postprandial phenotyping for understanding cardiometabolic regulation and position MR-proANP as a potential biomarker of metabolic adaptability in future prospective studies with cardiovascular outcomes across subjects with and without acute CV event.

Trail registration: The study protocol was approved by the institutional ethics committee (EA1/066/17) and registered in the German Clinical Trials Register (DRKS00016852).

Research Insights

What is currently known about this topic? Natriuretic peptides are well-established cardiometabolic hormones that both influence and are regulated by human metabolism. However, postprandial NP dynamics and the impact of metabolism in high-risk patients are poorly characterized.
What is the key research question? How do acute cardiovascular events, type 2 diabetes and insulin resistance shape postprandial MR-proANP, NT-proBNP and copeptin dynamics?
What is new? Insulin resistance, rather than diabetes status, is associated with postprandial MR-proANP suppression in a high-risk population. Acute CV events elevate fasting NP levels but are not independently linked to postprandial responses. Copeptin dynamics appear independent of metabolic status.
How might this study influence clinical practice? Postprandial MR-proANP phenotyping may improve cardiometabolic risk stratification in high-risk populations.

1. Introduction

Natriuretic peptides (NPs) and copeptin are well-established biomarkers in cardiovascular disease (CVD). Atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP), released by cardiomyocytes in response to volume and pressure load, provide robust diagnostic and prognostic information in heart failure and other cardiovascular conditions[1]. Elevated copeptin, a stable surrogate of arginine vasopressin secretion, has also been consistently associated with adverse outcomes and higher mortality risk in various cardiovascular conditions[2]. In patients with heart failure after acute myocardial infarction, copeptin was a strong predictor of mortality and recurrent events, even outperforming BNP/NT-proBNP in the OPTIMAAL study[2], underscoring its value as a marker of CV risk.

Beyond hemodynamic stress, metabolic factors have also been proven to modulate NP physiology[3]. In fact, dysregulation of NPs has been associated with obesity, insulin resistance (IR) and related conditions like hypertension and impaired glucose tolerance. Large cohort studies indicate that IR, more than obesity per se, is associated with 10-30% lower NT-proBNP levels[4–6]. Mechanistically, an upregulation of natriuretic clearance receptor (NPR-C) by hyperinsulinemia was demonstrated in human subcutaneous adipose tissue[7]. In obesity, this receptor is often overexpressed and the ratio between its active form (NPRA) and clearance form (NPR-C) is shifted, leading to weaker natriuretic peptide signaling, a phenomenon referred to as the “natriuretic handicap” of obesity[8–10]. In parallel, copeptin is likewise associated with adverse metabolic profiles, showing links with IR, predicting incident diabetes and associating independently of insulin with metabolic syndrome components such as central obesity, hypertension and dysglycemia[11,12]. Notably, visceral adiposity and obesity are linked to higher copeptin, underscoring its role as a marker of adverse cardiometabolic risk.

The dynamic regulation of NPs and copeptin in response to nutrient intake is an emerging, but still poorly characterized dimension of this system. This might be particularly relevant, as the postprandial state of other cardiometabolic markers such as glucose or triacylglycerol levels, is more predictive for future cardiovascular events than the respective fasting levels[13,14]. Prior research indicates that glucose intake suppresses MR-proANP secretion, an effect observed in both lean and obese individuals[10,15], suggesting a functional link between nutrient metabolism and cardiac endocrine function. Postprandial BNP responses

are less reproducible and show rather mixed patterns following nutrient intake[16–19]. In the CoMEAL study, copeptin concentrations showed a modest postprandial decrease in healthy volunteers after both glucose and mixed meals, without differences between nutrient types[20]. This supports the view that higher copeptin levels may reflect chronic metabolic stress rather than an acute meal-induced response. Nonetheless, elevated copeptin is also linked to insulin resistance and abdominal obesity[21], making its regulation in cardiometabolic diseases interesting.

While the interplay between metabolism and cardiac peptides is increasingly recognized, key clinical modifiers of these dynamics remain unclear. Most prior studies of postprandial NPs or copeptin changes were done in healthy or obese individuals without acute CVD [20,22–24]. It is unknown to what extent diabetes status or the degree of insulin resistance might alter meal-induced NP/copeptin responses in such high-risk populations - gaps that limit translation of peptide physiology into actionable cardiometabolic phenotyping.

The Berlin Long-term Observation of Vascular Events (BeLOVE) study offers a unique opportunity to address these knowledge gaps in a high-risk population. By analyzing changes in MR-proANP, NT-proBNP, and copeptin after a standardized mixed meal test and modeling their associations with diabetes, insulin resistance, and acute cardiovascular event history, we aim to provide novel insights into how metabolic state is linked to cardiometabolic peptide dynamics. Establishing these links could refine biomarker interpretation in CVD and motivate the use of postprandial peptide responses as candidate tools for cardiometabolic risk stratification.

2. Methods

2.1. Study Design and Population

BeLOVE (Berlin Long-term Observation of Vascular Events) is a prospective observational cohort study of adult participants with high-cardiovascular-risk conducted at the Charité-Universitätsmedizin Berlin, the Max-Delbrück Center for Molecular Medicine and the Berlin Institute of Health. Participants are recruited at the clinical campuses of Charité-Universitätsmedizin Berlin and include both participants with acute CV events and participants without recent events but categorized as very high cardiovascular risk[25]. The study protocol was approved by the institutional ethics committee (EA1/066/17) and registered in the German Clinical Trials Register (DRKS00016852).

Inclusion criteria were age ≥ 18 years, ability to consent and willingness to participate and the presence of one of 5 conditions indicating high CV risk: acute coronary syndrome (ACS), acute cerebrovascular event, acute heart failure, acute kidney injury (AKI) or very-high-risk chronic CV condition (HR) without event in the preceding 12 months. For the acute ACS and stroke cohorts, participants were enrolled within 0-7 days after the index event. ACS was defined by typical chest pain, ECG changes consistent with myocardial infarction, and/or dynamic cardiac troponin. Stroke and TIA required an acute-onset neurological deficit with corresponding imaging findings or symptom duration for > 24 hours or, for TIA, full recovery within 24 hours. The very-high-risk chronic group included participants without a recent event but with established

CV disease or equivalent risk. For the sample used in this study, these were patients with diabetes mellitus type 2 with or without additional risk factors (arterial hypertension, hypercholesterolemia) or target organ damage (see Weber et. al for more details)[25].

Exclusion criteria included inability to give informed consent, pregnancy or breastfeeding, lack of health insurance, life expectancy < 6 months due to a non-cardiovascular condition, active malignancy or a history of solid organ transplantation.

Participants meeting all inclusion criteria and no exclusion criteria were eligible for enrollment. All participants provided written informed consent before the first study-related action was performed and could withdraw from study participation at any given time without the need for justification.

The present analysis represents a predefined sub-analysis of the BeLOVE cohort and was restricted to participants with ACS, stroke or very high chronic CV risk. Participants were excluded from the analysis if no meal test was performed at the deep phenotyping visit, if the meal test was not fully completed or if the predefined timing between meal ingestion and postprandial blood sampling was not met. In addition, participants with diabetes types other than type 2 diabetes (e.g. type 1 diabetes, MODY) were excluded.

2.2. Recruitment

Participants of the acute cardiovascular event groups were enrolled during the acute hospitalization at the Department of Neurology or Cardiology of the Charité-Universitätsmedizin Berlin, respectively (within 0-7 days after the event). Participants of the HR group were primarily recruited through the outpatient cardiology and endocrinology care units at Charité.

2.3. Phenotyping

Initial phenotyping (acute phase): All participants underwent an initial assessment soon after enrollment. For acute event participants, this was performed within 7 days of the index event, for the chronic high-risk participants within 14 days of study inclusion. This initial phenotyping included key measurements and biosamples (fasting blood and spot urine) collection. Demographic data, medical history, weight, height and acute clinical course were recorded, including comorbidities and treatments.

Deep phenotyping: Participants were reinvited for an extensive in-person evaluation at one of the dedicated BeLOVE clinical research units. This visit was scheduled around 3 months after inclusion for the HR cohort or after the acute index event respectively. The deep phenotyping protocol included data on socio-demographics and lifestyle, medical history, physical examination and vital signs, cardiovascular, neurological, and metabolic assessments as previously reported[25]. Participants were assigned to either a nutritional challenge with metabolic phenotyping or a physical challenge (ergometry). Randomization was performed when no contraindications for either of the two challenges were present. The present analysis focuses exclusively on participants who underwent the nutritional challenge, which included fasting blood samples as well as a mixed meal test.

2.4. Mixed Meal Test

A study-specific standardized Mixed Meal Test (MMT) was conducted as part of the metabolic phenotyping during the deep phenotyping visit to assess postprandial metabolic responses. Participants arrived at the Clinical Research Unit after an overnight fast of at least 8 hours, refraining from any caloric intake, caffeine, nicotine and alcohol. After collection of fasting blood samples for baseline glucose, insulin, NT-proBNP, MR-proANP, copeptin, lipids and other markers, participants consumed a liquid test meal specifically designed for the BeLOVE study.

In contrast to conventional mixed meal tests, which are typically carbohydrate-rich and lower in fat and protein[26–28], the BeLOVE meal challenge was deliberately designed as a fat-enriched liquid meal to better reflect the increasing dietary fat intake observed in the German population in recent decades[29,30]. The test provided approximately 500 kcal of energy, with a macronutrient composition of 15% protein, 45% fat, and 40% carbohydrates (Table 1). Participants were instructed to consume the meal within 5 minutes under supervision. A second blood sample was collected 120 minutes after meal completion. During the 120-minute postprandial period, participants were only allowed to drink a small amount of water. No other caloric intake, smoking or strenuous physical activity was allowed for the duration of the test.

Table 1. **Composition and nutritional content of the BeLOVE test meal**

Ingredient	Amount	Protein (g)	Fat (g)	Carbohydrates (g)	Energy (kcal)
Optifast Vanilla Drink	52 g	18.9	5.8	18.0	204.0
Rapeseed oil	20 g	0.0	18.3	0.0	163.8
Maltodextrin	32 g	0.0	0.0	30.7	122.9
Water	300 mL	-	-	-	-
Total:	-	**18.9 g**	**24.1 g**	**48.7 g**	**490.7 kcal**
Energy distribution:	-	**15%**	**45%**	**40%**	-

2.5. Laboratory analyses

All laboratory measurements were performed in a central certified laboratory in accordance with the manufacturers' protocols. Samples obtained at the inclusion as well as day 90 visits were processed immediately and analyzed or stored at -80°C for batch analysis of research biomarkers. Routine clinical chemistry (e.g. electrolytes, renal and liver function, high-sensitivity troponin, HbA1c) and hematology were measured on the day of collection using automated analyzers as per hospital standards. Laboratory personnel were blinded to clinical outcomes when performing batched analyses of study-specific biomarkers. Laboratory analyses are described in detail in the supplements.

2.6. Data storage

All baseline and follow-up data were captured in a pseudonymized form using a secure electronic case report form system (REDCap[31,32], hosted at Charité) in accordance with the study's data protection

concept[25].

2.7. Sample Size and Comparative Groups

For the present analysis, participants who underwent an MMT at the deep phenotyping visit were selected from the BeLOVE cohort. Participants from the AKI and heart failure cohorts were excluded, as only 13 and 20 participants, respectively, completed the nutritional challenge during the visit. These sample sizes would have been insufficient to support the multivariable SEM approach used in the analysis. The remaining participants were categorized into three predefined groups: a high-risk reference group (HR), participants with acute coronary syndrome (ACS), and participants with acute cerebrovascular disorder (stroke).

2.8. Statistical Analysis

Descriptive statistics for continuous variables are given as median with limits of the interquartile range (IQR, [25^{th};75^{th} percentile]). Categorical variables were summarized as counts and percentages. For descriptive comparisons of different inclusion entities, demographic characteristics as well as fasting values of metabolic parameters and cardiometabolic peptides were reported (Table 2). The postprandial response was descriptively presented as the ratio of postprandial to fasting concentrations (Table 3).

2.8.1. Structural Equation Modeling

We used Structural Equation Models (SEM) to explore whether specific cardiovascular (acute CV events) or metabolic conditions (T2D and IR) are directly associated with postprandial peptide change (conditional pathway), or through their relationship with baseline levels (indirect pathway), or both (Supplementary Fig. 1). This approach was chosen over conventional regression analyses because postprandial changes are inherently dependent on fasting concentrations, while fasting concentrations may already be influenced by the pre-existing conditions. In observational studies, simple baseline adjustment can lead to biased estimates when baseline variables lie on the causal pathway between the exposure and outcome[33].

Under the assumption that the acute CV event groups ACS and stroke share similar patterns of association to postprandial changes, both groups were pooled. In addition, separated results for each acute event type are also presented in Supplementary Fig. 3-5 and Supplementary Tables 2-3, 5-6, 8-9.

Associations of patient conditions (acute CV events, T2D, IR) with fasting peptide concentrations and postprandial changes were additionally adjusted for age, sex and BMI as potential confounders. Additionally, we performed sensitivity analyses including the presence of atrial fibrillation (AF) and renal function (eGFR) as further confounders, both recognized modulators of natriuretic peptides. Sensitivity analyses are reported in the Supplementary Material (Tables 1d-f, 4d-f, 7d-f).

Due to lognormal distributions, cardiometabolic peptides were transformed using the natural log before using SEMs. Postprandial changes were expressed as log-transformed ratios of postprandial to fasting concentrations. Insulin resistance was represented by the homeostatic model assessment of Insulin

Resistance Index (HOMA-IR) calculated from fasting glucose and fasting insulin concentrations as described previously by Matthews et al[34]. Similar to cardiometabolic peptides, HOMA-IR was log-transformed, as recommended for non-normally distributed HOMA estimates[35].

To investigate the association of a recent acute CV event (condition i) with postprandial changes of cardiometabolic peptides, the analyses were restricted to the T2D participants from the acute CV event groups (ACS or stroke) and the HR group. Correspondingly, we explored the association of T2D presence (condition ii) with postprandial peptide changes in participants only from the acute event groups, seeking to reduce confounding from acute events. To assess the link between insulin resistance (condition iii) and cardiometabolic peptides, we analyzed all participants from the acute event groups, irrespective of diabetes status.

As recommended by the American Statistical Association[36], this exploratory study focused on effect size estimation with 95% confidence intervals, rather than on null-hypothesis significance testing. Reported p-values are provided for descriptive purposes only and were not adjusted for multiple testing. Given our observational design and the unknown temporal relationship prior to the index event or study inclusion, these associations should not be interpreted as causal effects but rather as descriptive relationships that warrant further investigation. Analyses were based on the BeLOVE data export of 4 July 2025 and were performed using R version 4.2.2. SEM analyses were done using the lavaan package[37,38]. The reporting adheres to the STROBE guidelines[39].

3. Results

3.1. Baseline Characteristics

Between 15 July 2017 and 11 December 2020, a total of 1,897 individuals were enrolled in the BeLOVE study across five predefined clinical arms (high-risk diabetes, acute kidney injury, acute coronary syndrome, heart failure and stroke). Of these, 1,471 did not undergo the nutritional challenge at the deep phenotyping visit. A total of 426 participants completed the MMT. Of these, 33 were excluded due to limited sample size (see section 2.7). Further exclusions were applied for incomplete MMT, deviations from the predefined postprandial sampling interval, non-fasting status and missing fasting information (Fig. 1). The final analytical sample included 373 participants from three study arms: 81 had experienced ACS, 186 had a history of acute cerebrovascular disorder and 106 belonged to the HR group. The baseline demographic and metabolic characteristics of these groups are presented in Table 2.

Table 2. **Baseline demographic and metabolic characteristics of the cohort under investigation.** Data are presented as median and interquartile range of selected baseline parameters or absolute/relative frequencies.

Baseline parameters [IQR]	HR group n = 106	ACS n = 81	Stroke n = 186
Age (y)	64 [58;69]	64 [55;74]	66 [56;77]
Male/ Female (%)	63%/37%	81%/19%	68%/32%
Days since enrollment	39 [31;46]	100 [90;112]	99 [91;116]
T2D n (%)	106 (100)	26 (32)	32 (17)
Atrial fibrillation n (%)	19 (17.9)	14 (17.3)	46 (24.7)
w/o T2D	-	9 (16.4)	37 (24.2)
w T2D	19 (17.9)	5 (19.2)	9 (28.1)
Missing data	0	0	1
eGFR (mL/min/1.73 m²)	84.5 [69.2; 101.0]	81.0 [71.0; 95.0]	81.0 [69.0; 95.0]
w/o T2D	-	80.0 [71.0; 99.2]	82.0 [72.0; 96.0]
w T2D	84.5 [69.2; 101.0]	84.0 [68.0; 94.0]	71.5 [58.8; 88.8]
Missing data	6	4	5
Insulin treatment n (%)			
w/o T2D	-	-	-
w T2D	106 (43.4)	4 (15.4)	7 (21.9)
BMI (kg/m²)	33.2 [28.7;37.3]	27.2 [25.1;31.0]	27.1[23.8;29.8]
w/o T2D	-	27.0 [25.1;30.3]	26.4 [23.5;28.8]
w T2D	33.2 [28.7;37.3]	30.2 [26.0;32.9]	31.1 [28.2;35.0]
Waist circumference (cm)	113.5 [103.6;125.0]	102.3 [94.5; 111.3]	97.7 [90.3; 107.3]
w/o T2D	-	101.5 [93.5;106.0]	96.0 [88.0;104.0]
w T2D	113.5 [103.6;125.0]	106.0 [98.4;115.0]	110.2 [99.5;119.2]
Missing data	0	0	1
Fasting glucose (mg/dl)	137.0 [115.0;160.5]	98.0 [93.0;111.0]	93.0 [86.0;103.0]
w/o T2D	-	96.0 [91.0;100.0]	91.0 [85.0;98.0]
w T2D	137.0 [115.0;160.5]	125.0 [100.0;143.0]	117.5 [106.0;141.5]
Missing data	6	4	4
HbA1c (%)	6.8 [6.1;7.6]	5.8 [5.6;6.1]	5.6 [5.4;5.9]
w/o T2D	-	5.7 [5.5;5.9]	5.5 [5.3;5.7]

w T2D	6.8 [6.1;7.6]	6.3 [6.0;6.9]	6.5 [6.1;7.1]
Missing data	6	5	6
Fasting insulin (mU/l)	16.6 [9.1;24.2]	13.9 [9.9;19.6]	11.3 [7.7;16.3]
w/o T2D	-	13.3 [8.3;20.9]	10.7 [7.5;14.6]
w T2D	16.6 [9.1;24.2]	15.1 [12.2;18.3]	13.2 [10.7;30.1]
Missing data	7	4	5
HOMA-IR	5.50 [3.35;9.40]	3.4 [2.4;5.1]	2.7 [1.8;3.8]
w/o T2D	-	3.2 [2.0;4.5]	2.4 [1.7;3.4]
w T2D	5.50 [3.35;9.40]	4.4 [3.7;5.5]	4.8 [3.0;9.7]
Missing data	7	4	6
Fasting NT-proBNP (ng/l)	75 [30;130]	246 [83;422]	125 [51;317]
w/o T2D	-	242 [104;398]	112 [42;272]
w T2D	75 [30;130]	256 [62;892]	192 [122;490]
Missing data	7	4	4
Fasting MR-proANP (pmol/l)	67.0 [42.5;100.0]	126.0 [76.0;192.0]	109.5 [62.0;172.5]
w/o T2D	-	124.5 [70.2;177.8]	98.0 [59.5;169.8]
w T2D	67.0 [42.5;100.0]	126.0 [90.0;196.0]	136.5 [91.5;177.5]
Missing data	7	4	6
Fasting Copeptin (pmol/l)	7.6 [4.6;11.7]	8.2 [4.9;12.2]	6.4 [3.9;10.0]
w/o T2D	-	8.3 [5.0;12.4]	5.8 [3.6;9.1]
w T2D	7.6 [4.6;11.7]	7.2 [4.8;11.0]	9.4 [7.8;15.8]
Missing data	11	10	10

ACS, acute coronary syndrome; HR, high risk; IQR, interquartile range; T2D, type 2 diabetes; w/o T2D, without type 2 diabetes; w T2D, with type 2 diabetes; eGFR, estimated glomerular filtration rate; BMI, body mass index; HbA1c, glycated hemoglobin A1c; HOMA-IR, homeostatic model assessment of insulin resistance; NT-proBNP, N-terminal pro-B-type natriuretic peptide; MR-proANP, mid-regional pro-atrial natriuretic peptide.

All individuals in the HR group had type 2 diabetes, while the prevalence of diabetes was 32.1% in the ACS group and 17.2% in the stroke group. Not surprisingly, individuals with diabetes demonstrated numerically higher BMI and waist circumference compared to participants without diabetes. The median age was

similar between all groups, while gender was not. Male participants were predominant in all three cohorts, particularly within the ACS group (81%).

Markers of glycemic control such as HbA1c and fasting glucose, and insulin resistance (fasting insulin levels, HOMA-IR) were elevated in participants with diabetes across disease entities, with numerically highest values in the HR cohort (Table 2). 43.4% of the HR group required insulin therapy, compared with 15.4% of the diabetes patients in the ACS group and 21.9% of the diabetes patients in the stroke group (Table 2).

Baseline MR-proANP and NT-proBNP were substantially higher in the acute cardiovascular event groups compared with the HR reference group. Fasting copeptin levels were highest in stroke patients with diabetes compared with both, stroke patients without diabetes and the HR group.

3.2. Effects of meal challenge

All parameters related to glucose metabolism increased following consumption of the standardized test meal. An increase in blood glucose was observed in the HR group and in participants with diabetes in both event cohorts, whereas changes in the non-diabetic subgroups were smaller. The increase of glucose levels was accompanied by an increase in insulin postprandially in all groups. MR-proANP decreased consistently after the MMT across all groups. NT-proBNP showed little to no postprandial change, while copeptin showed a slight median decline with greater interindividual variability (Table 3).

Table 3. Changes in metabolic parameters, natriuretic peptides and copeptin after the mixed meal challenge. Data are presented as median and interquartile range of the postprandial response (postprandial/fasting ratio).

Parameters (factor of change)	HR group n = 106	ACS n = 81	Stroke n = 186
Glucose	1.38 [1.21;1.59]	1.20 [1.03;1.39]	1.16 [1.00;1.42]
w/o T2D	-	1.14 [1.01;1.37]	1.14 [0.98;1.39]
w T2D	1.38 [1.21;1.59]	1.25 [1.09;1.43]	1.23 [1.08;1.48]
Insulin	3.12 [2.03;4.54]	5.46 [3.20;7.40]	4.80 [3.11;7.96]
w/o T2D	-	5.80 [3.61;7.29]	4.94 [3.15;8.46]
w T2D	3.12 [2.03;4.54]	4.57 [2.77;7.74]	3.85 [2.13;5.92]
MR-proANP	0.87 [0.80;0.96]	0.85 [0.78;0.97]	0.89 [0.80;0.96]
w/o T2D	-	0.84 [0.78;0.99]	0.89 [0.80;0.97]
w T2D	0.87 [0.80;0.96]	0.89 [0.78;0.97]	0.90 [0.80;0.94]
NT-proBNP	1.00 [0.91;1.08]	1.01 [0.93;1.11]	1.03 [0.96;1.12]
w/o T2D	-	1.00 [0.93;1.09]	1.02 [0.95;1.12]
w T2D	1.00 [0.91;1.08]	1.02 [0.92;1.11]	1.05 [0.99;1.09]
Copeptin	0.97 [0.84;1.19]	0.91 [0.75;1.11]	0.93 [0.79;1.04]
w/o T2D	-	0.90 [0.73;1.10]	0.93 [0.79;1.04]
w T2D	0.97 [0.84;1.19]	0.95 [0.82;1.13]	0.92 [0.84;1.02]

ACS, acute coronary syndrome; ANP, atrial natriuretic peptide; BNP, B-type natriuretic peptide; HR, high risk; IQR, interquartile range; MR-proANP, mid-regional pro-atrial natriuretic peptide; NT-proBNP, N-terminal pro-B-type natriuretic peptide; T2D, type 2 diabetes; w/o T2D, without type 2 diabetes; w T2D, with type 2 diabetes.

3.3. Cardiometabolic peptide responses in relation to CV disease and metabolic state

3.3.1. Link between acute CV events and cardiometabolic peptide response in participants with T2D

To investigate how disease entity and clinical-metabolic parameters are associated with fasting concentrations and postprandial responses of cardiometabolic peptides, we used the SEM framework in patients with T2D across all disease entities. This allows separation of specific effects of T2D on cardiometabolic peptides.

A prior acute CV event was associated with higher fasting MR-proANP concentrations, and higher baseline levels were related to a more pronounced postprandial decline. However, there was no relevant direct link between prior CV event and the magnitude of postprandial MR-proANP decline (Fig. 2a).

Similarly, fasting NT-proBNP concentrations were elevated in participants with a history of acute CV event. However, the prior acute CV events showed no relevant association with postprandial changes. Fasting NT-proBNP levels indicated some negative association with ΔNT-proBNP (Fig. 2b).

For copeptin, we found no relevant associations between prior acute CV events and either fasting copeptin or its postprandial change. Only higher baseline levels were linked to a more pronounced decline of copeptin (Fig. 2c).

Similar findings as for copeptin could be observed for all three cardiometabolic markers when ACS and stroke subgroups were analyzed separately (Supplementary Fig. 3). Including AF and eGFR as covariates in our SEMs also did not alter the main associations of interest (Supplementary Tables 1d-f).

3.3.2. Link between T2D and cardiometabolic peptide response in participants with acute CV event

All patients with acute CV events, both with and without T2D, were analyzed to separate the effect of T2D. T2D was not associated with differences in fasting or postprandial changes of MR-proANP (Supplementary Fig. 2a) or NT-proBNP (Supplementary Fig. 2b). The only consistent relationship was found between fasting and postprandial peptide levels, where higher baseline concentrations were related to larger declines, particularly for MR-proANP. These patterns were similar when ACS and stroke cohorts were analyzed separately (Supplementary Fig. 4).

In contrast, diabetes was linked to higher fasting copeptin levels and a stronger postprandial decline only within the stroke subgroup (Supplementary Fig. 4c). No consistent associations were observed between participants with and without diabetes in the ACS subgroup (Supplementary Fig. 4c) or the pooled cohort (Supplementary Fig. 2c).

Adjusting the models for AF and renal function did not materially change the observed associations (Supplementary Tables 4d-f).

3.3.3. Link between insulin resistance and cardiometabolic peptide response in participants with acute CV event

Given the effect of acute events on changes of cardiometabolic peptides, we analyzed the relationship between insulin resistance and changes in cardiometabolic peptides in all participants with a recent acute CV event using SEMs. This was in line with previous analyses regarding the effect of T2D. We revealed that higher HOMA-IR was associated with lower fasting MR-proANP and a stronger postprandial decline of MR-proANP (Fig. 3a).

For NT-proBNP, a similar but weaker pattern was observed. Higher HOMA-IR values were associated with lower fasting concentrations of NT-proBNP, but the direct association between HOMA-IR and postprandial NT-proBNP change was minor (Fig. 3b).

In contrast, neither fasting nor postprandial copeptin levels showed any relevant direct association with HOMA-IR (Fig. 3c).

Using separate SEMs for stroke and ACS participants showed similar patterns, with a stronger association between HOMA-IR and lower fasting MR-proANP in the stroke cohort, but more pronounced associations with postprandial MR-proANP changes in the ACS cohort (Supplementary Fig. 5a). The results were similar after additional adjustment for AF and eGFR as potential confounders (Supplementary Tables 7d-f).

4. Discussion

Our study provides new insights into the postprandial dynamics of cardiometabolic peptides in a deeply phenotyped cohort of participants with high cardiovascular risk. By assessing responses of MR-proANP, NT-proBNP, and copeptin to a standardized study-specific mixed meal test, we demonstrated that a postprandial suppression of MR-proANP and copeptin can be induced by a fat-enriched meal, while NT-proBNP seems not to be substantially affected by meal intake. Across all three peptides, postprandial concentrations were primarily associated with fasting peptide concentrations, with no significant association observed for acute CV events or T2D. Interestingly, the postprandial MR-proANP levels were additionally associated with IR rather than diabetes per se.

Mechanistically, the postprandial decline of MR-proANP likely reflects physiological suppression of ANP-mediated lipolysis, as ANP activates adipocyte triglyceride hydrolysis via the NPR-A/cGMP/PKG pathway, a metabolic effect largely absent for BNP[3,40,41]. This transient reduction after food intake contributes to the normal postprandial shift from lipid oxidation in the fasting state toward carbohydrate utilization in the fed state. Prior acute CV event did not blunt the known postprandial drop in MR-proANP, suggesting that nutritional state could override acute post-ischemic alterations in short-term NP regulation. These findings did not support a relevant impact of acute cardiovascular events on drivers of metabolic flexibility, which is frequently impaired in type 2 diabetes.

Recent acute CV events were associated with higher fasting levels of both natriuretic peptides, but not substantially with their postprandial responses. Elevated fasting MR-proANP after ACS or stroke likely reflects hemodynamic stress and may increase basal lipolysis and lipid oxidation, providing additional fatty acid substrates for myocardial energy metabolism[42–44]. However, there is currently no direct evidence from human studies linking increased ANP to cardiac lipid utilization after ischemic injury. Animal data suggest that natriuretic peptide infusion can increase cardiac triglyceride content after ischemia-reperfusion, but this has not been confirmed in humans[45]. Additionally, elevations in peptides are accompanied by rises in plasma non-esterified fatty acids, which have been associated with impaired outcomes such as arrhythmias and oxidative injury[46–48]. Thus, whether this is potentially beneficial under emergency energy demand or harmful under conditions of lipid overload remains unclear.

In contrast to the minimal relationship of acute CV events and postprandial NP responses, higher HOMA-IR was associated not only with lower fasting MR-proANP but also with a stronger postprandial suppression, even in participants with well-controlled diabetes. The lower fasting levels indicate reduced ANP-mediated lipid mobilization capacity, potentially driven by chronic insulin-mediated upregulation of the NPR-C clearance receptor[7]. Although postprandial MR-proANP suppression per se favors carbohydrate oxidation, the combination of chronically low fasting levels and a stronger postprandial drop suggests a persistently dysregulated system rather than genuine metabolic flexibility. Further studies are required to address the mechanisms underlying the stronger postprandial suppression in participants with higher IR. Vice versa it is tempting to speculate that the stronger postprandial decline of MR-proANP could also reflect a potential counterregulatory mechanism to maintain metabolic flexibility. However, this clearly needs further investigation.

In contrast to the NPs, neither IR, T2D, nor prior acute CV events were substantially associated with fasting copeptin or its postprandial change. The only consistent relationship was between fasting copeptin and its own postprandial response, where higher baseline concentrations were linked to a larger subsequent decline. This suggests that copeptin dynamics are primarily driven by individual baseline regulation rather than metabolic or cardiovascular status. The postprandial reduction observed across our cohort may reflect transient suppression of vasopressin release secondary to fluid ingestion and changes in plasma osmolality, rather than a specific nutrient-driven effect. This is supported by studies demonstrating that oral fluid intake, even in small amounts, can significantly decrease copeptin levels, while food intake without accompanying fluid does not show a substantial effect[20,53].

Methodologically, the explained variance in postprandial peptide changes was modest across all models (R^2 ranging from 0.05 to 0.17), which is not unexpected given the complexity of postprandial regulation in clinically heterogeneous, high-risk cohorts.

5. Strengths and limitations

In this study, we used a standardized, study-specific mixed-meal test, developed to better reflect typical Western dietary patterns with a higher proportion of fat compared to conventional carbohydrate-rich test meals. The integration of detailed clinical and metabolic phenotyping, together with the direct comparison of ACS and stroke subgroups as well as diabetic vs non-diabetic individuals, provides an overview of cardiometabolic regulation across different contexts. While our study design points out limitations of static fasting assessments and demonstrates the feasibility of standardized postprandial phenotyping in high-risk patients, the value of dynamic peptide responses as potential risk markers requires further validation. The planned longitudinal follow-up within BeLOVE will be critical to determine whether postprandial peptide responses provide prognostic information for cardiovascular outcomes and can inform therapeutic strategies[54–57].

However, several limitations should also be considered. First, the study population is characterized by high cardiovascular risk and predominance of well-controlled diabetes (median HbA1c 6.3% - 6.8%), which may limit generalizability to broader or less well-managed populations. The relatively low glycemic variability

within this cohort could weaken observed associations between diabetes status and peptide responses, potentially underestimating the impact of poorly controlled diabetes. Second, participants included in the present analysis were required to have completed the MMT after the index event. This may have introduced a selection bias toward clinically more stable individuals, as participants with more severe acute presentations or greater frailty may have been less likely to attend or complete the deep phenotyping visit. Consequently, our findings may not fully represent cardiometabolic peptide dynamics in more severely ill patients. Third, although the overall sample size is substantial, subgroup analyses (by acute event type and diabetes status) involved smaller sample sizes resulting in more uncertainty in estimates. Additionally, a minority of participants with T2D in the acute event groups were receiving insulin therapy at the time of the mixed meal test (11 out of 267 participants), which may affect HOMA-IR estimates due to exogenous insulin elevating fasting insulin concentrations. However, given the small proportion affected, this is unlikely to have materially influenced the observed associations. Fourth, our observational design does not allow for a causal inference, restricting the interpretation to associations. Finally, the prognostic relevance of postprandial peptide changes remains to be established and requires longitudinal analyses with careful adjustment for baseline comorbidities.

In summary, this study identified distinct postprandial dynamics for MR-proANP, NT-proBNP, and copeptin in participants with high cardiovascular risk. MR-proANP emerges as the most metabolically responsive peptide, with insulin resistance as a central determinant of its regulation, whereas NT-proBNP remains largely insensitive to short-term metabolic stimuli. The consistent association of lower fasting MR-proANP and stronger postprandial decline with higher insulin resistance indicates impaired hormonal adaptability and reduced metabolic flexibility. In contrast, copeptin dynamics appear independent of metabolic or cardiovascular status and may reflect individual fluid- or osmoregulatory effects rather than nutrient-driven mechanisms. Together, these findings highlight the complex interplay between metabolic status and cardiac function and support the need for future longitudinal analyses to determine whether dynamic hormone responses can serve as prognostic markers and therapeutic targets in cardiovascular diseases.

Abbreviations

ACS	Acute Coronary Syndrome
AF	Atrial Fibrillation
AKI	Acute Kidney Injury
ANP	Atrial Natriuretic Peptide
BMI	Body Mass Index
BNP	B-type Natriuretic Peptide
CI	Confidence Interval
CV	Cardiovascular
CVD	Cardiovascular Disease
eGFR	Estimated Glomerular Filtration Rate
HOMA-IR	Homeostatic Model Assessment of Insulin Resistance
HR	High Risk (reference group)
MMT	Mixed Meal Test
MR-proANP	Mid-Regional Pro-Atrial Natriuretic Peptide
NT-proBNP	N-Terminal pro-B-Type Natriuretic Peptide
NP	Natriuretic Peptide
NPR-A	Natriuretic Peptide Receptor A
NPR-C	Natriuretic Peptide Clearance Receptor
SEM	Structural Equation Model
T2D	Type 2 Diabetes
TIA	Transient Ischemic Attack

Declarations

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Competing Interests

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Author Contributions

G.L., J.G. and K.M. analyzed and interpreted the data, and drafted the manuscript. O.S. contributed to processing and provision of the data, and statistical analyses. K.M. provided study conception, clinical oversight and supervision, and critically revised the manuscript. L.L. contributed to the conception and design of the meal challenge and provided data. K.v.R. contributed to participant recruitment. U.L., F.E., K.U.E., M.E., J.E.W., T.P., J.S.M., D.N.M., J.S. contributed to study conception and design. All authors reviewed the manuscript and approved the final version.

Data Availability

Data from the BeLOVE study are available upon reasonable request. Researchers may apply for access through the study's Use and Access Committee, with the involvement of a collaborator from the BIH research community. Requests are evaluated for scientific merit and feasibility. Data sharing is conducted in accordance with General Data Protection Regulation (GDPR) requirements.

Ethics approval:

The study was approved by the Charité - Universitätsmedizin Berlin ethics committee (Date of vote: 2017-07-06; Ethic committee number EA1/066/17).

Study registration:

Approved WHO primary register: German Clinical Trials Register: https://drks.de/search/de/trial/DRKS00016852; WHO International Clinical Registry Platform: http://apps.who.int/trialsearch/Trial2.aspx?TrialID=DRKS00016852. Recruitment started on July 18, 2017.

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Figures

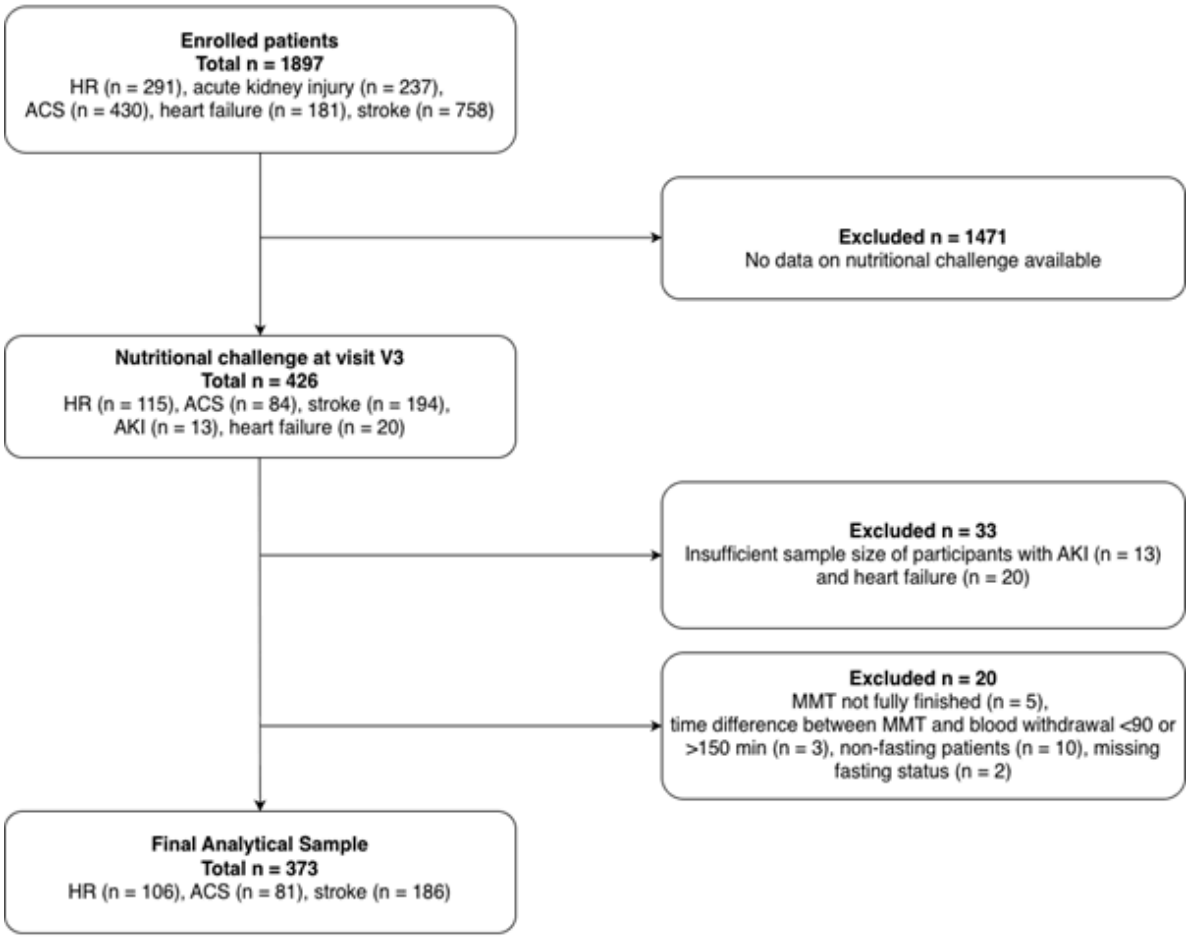

Figure 1

STROBE flow chart of the study.

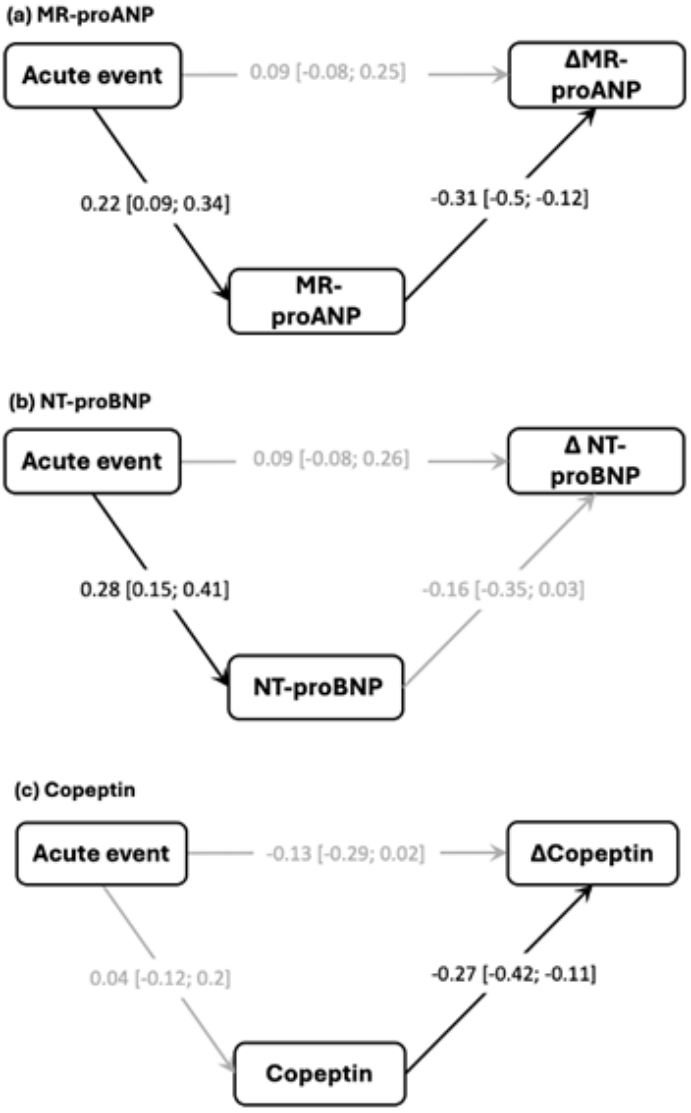

Figure 2

SEMs linking acute CV events to fasting and postprandial levels of (a) MR-proANP, (b) NT-proBNP and (c) copeptin. The reference category for the binary exposure variable was the high CV risk group without acute cardiovascular event. Group sizes per model: (a, b) n=57 with acute CV event (stroke/ACS) and diabetes vs. n=98 high CV risk group; (c) n=56 vs. n=94, respectively. Numbers at arrows indicate standardized regression coefficients with 95% CI. (a) R^2=0.11 for ΔMR-proANP and R^2=0.43 for fasting MR-proANP. (b) R^2=0.07 for ΔNT-proBNP and R^2=0.36 for NT-proBNP. (c) R^2=0.16 for Δcopeptin and R^2=0.15 for fasting copeptin. Fasting and postprandial levels were adjusted for age, sex and BMI. Full models, including unstandardized regression coefficients, are shown in the Supplements (Tables 1a-c).

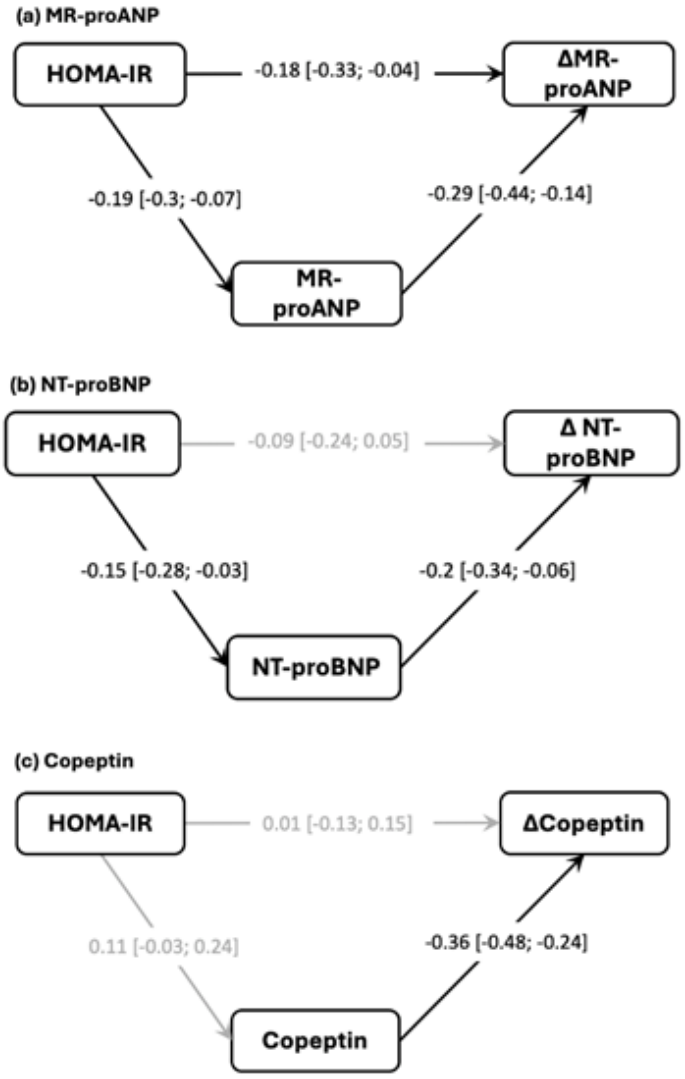

Figure 3

SEMs linking HOMA-IR to fasting and postprandial levels of (a) MR-proANP, (b) NT-proBNP and (c) copeptin. Numbers at arrows indicate standardized regression coefficients with 95% CI. (a) n=245; R^2=0.07 for ΔMR-proANP and R^2=0.39 for fasting MR-proANP. (b) n=248; R^2=0.10 for ΔNT-proBNP and R^2=0.27 for NT-proBNP. (c) n=235; R^2=0.13 for Δcopeptin and R^2=0.16 for fasting copeptin. Fasting and postprandial levels were adjusted for age, sex and BMI. Full models, including unstandardized regression coefficients, are shown in the Supplements (Tables 7a-c).

Supplementary Files

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