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ORIGINAL ARTICLE

Clinical Trials and Investigations

Proteomic biomarkers related to obesity in heart failure with reduced ejection fraction and their associations with outcome

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Abstract

Objective: Heart failure (HF) pathophysiology in patients with obesity may be distinct. To study these features, we identified obesity-related biomarkers from 4210 circulating proteins in patients with HF with reduced ejection fraction (HFrEF) and examined associations of these proteins with HF prognosis and biological mechanisms.

Methods: In 373 patients with trimonthly blood sampling during a median follow-up of 2.1 (25th–75th percentile: 1.1–2.6) years, we applied an aptamer-based multiplex approach measuring 4210 proteins in baseline samples and the last two samples before study end. Associations between obesity (BMI > 30 kg/m^2) and baseline protein levels were analyzed. Subsequently, associations of serially measured obesity-related proteins with biological mechanisms and the primary endpoint (PEP; composite of cardiovascular mortality, HF hospitalization, left ventricular assist device implantation, and heart transplantation) were examined.

Results: Obesity was identified in 26% (96/373) of patients. A total of 30% (112/373) experienced a PEP (with obesity: 26% [25/96] vs. without obesity: 31% [87/277]). A total of 141/4210 proteins were linked to obesity, reflecting mechanisms of neuron projection development, cell adhesion, and muscle cell migration. A total of 50/141 proteins were associated with the PEP, of which 12 proteins related to atherosclerosis or hypertrophy provided prognostic information beyond clinical characteristics, N-terminal pro-B-type natriuretic peptide, and high-sensitivity troponin T.

Conclusions: Patients with HFrEF and obesity show distinct proteomic profiles compared to patients with HFrEF without obesity. Obesity-related proteins are independently associated with HF outcome. These proteins carry potential to improve management of obesity-related HF and could be leads for future research.

INTRODUCTION

Heart failure (HF) is a heterogeneous condition with high mortality rates, and its prevalence is projected to increase globally [1, 2]. At the same time, obesity, a major risk factor for HF, has seen spectacular growth over the last decades [3]. Obesity is associated with many factors that could influence heart health such as changes in cardiac structure and function (increased prevalence of left ventricular hypertrophy, diastolic dysfunction, and impaired left ventricular contractility), altered adipokine profiles, systemic inflammation, oxidative stress, hemodynamic factors (increased blood volume and cardiac output), and metabolic factors (insulin resistance, dyslipidemia, and endothelial dysfunction) [4]. As such, the pathophysiology of HF in patients with and without obesity may be distinct.

Identifying HF subgroups that may benefit from specific treatment is essential for developing effective strategies for HF management. An obesity phenotype in HF with preserved ejection fraction (HFpEF) has been a much-studied subject in recent literature [5, 6]. However, research on the role of obesity in the pathophysiology of HF with reduced ejection fraction (HFrEF) remains scarce.

The aim of this study is to identify and describe distinct pathophysiological features of patients with HFrEF and obesity. Identifying circulating proteins related to obesity in a population with HFrEF may uncover underlying biological pathways and provide additional insights into the obesity-HFrEF phenotype. To find differences in the pathophysiology of HFrEF between patients with and without obesity (body mass index [BMI] > 30 kg/m^2), we identified proteins associated with obesity from a set of 4210 serially measured proteomic biomarkers in a population of 373 patients with chronic HFrEF in Rotterdam and Alkmaar, the Netherlands. These selected proteins were subsequently studied for their effects on adverse clinical events and association with underlying biological mechanisms.

Study Importance

What is already known?

- Heart failure (HF) is a heterogeneous condition with high mortality rates and increasing prevalence.
- Obesity is a risk factor for HF.
- Plasma levels of proteins linked to HF such as N-terminal pro-B-type natriuretic peptide (NT-proBNP) and C-reactive protein can be affected by obesity.

What does this study add?

- Circulating proteomic profiles of patients with both HF with reduced ejection fraction (HFrEF) and obesity are distinct compared to patients with HFrEF without obesity.
- These differing proteins are related to mechanisms of neuron projection development, synapse assembly, cell adhesion, and muscle cell migration and are associated with HF outcome.
- Trajectories of proteins related to atherosclerosis or hypertrophy provided prognostic information beyond clinical characteristics, NT-proBNP, and high-sensitivity troponin T.

How might these results change the direction of research or the focus of clinical practice?

- This overview of proteomic profiles of patients with HFrEF with obesity helps us understand the pathophysiology of HF.
- Described differences carry potential to improve management of obesity-related HF.
- Distinct proteins can serve as leads for future research in stratified HF treatment.

METHODS

Study design

We performed the investigation within the Serial Biomarker Measurements and New Echocardiographic Techniques in Chronic Heart Failure Patients Result in Tailored Prediction of Prognosis (Bio-SHiFT) study, conducted at Erasmus MC in Rotterdam and Northwest Clinics in Alkmaar, the Netherlands. This was a prospective cohort study of stable patients with chronic HF (CHF) [7]. The study was approved by the medical ethics committee of the Erasmus MC in Rotterdam and complied with the Declaration of Helsinki. All included patients provided written informed consent.

Inclusion criteria

Patients were recruited during regular outpatient visits and included if they were 18 years or older, able to understand and sign the informed consent form, diagnosed with CHF at least 3 months ago according to the guidelines of the European Society of Cardiology [8–10], and had not been hospitalized for HF less than 3 months prior to inclusion. Detailed inclusion and exclusion criteria are provided in Figure S1. Eligibility assessment included retrieval of necessary parameters from echocardiographic reports that were available from routine clinical care up to the moment of eligibility assessment, including quantitative left ventricular ejection fraction (LVEF).

Exclusion criteria

Patients were excluded based on the following criteria: 1) HF was secondary to circulatory high output conditions; 2) they were scheduled for surgery or intervention for both coronary and noncoronary indication within 6 months of inclusion; 3) they had severe renal failure for

which dialysis is needed; 4) they had known moderate or severe liver disease; 5) they had chronic obstructive pulmonary disease gold stage IV; 6) they had a coexistent condition with life expectancy ≤ 1 year; or 7) they had congenital heart disease.

Patient population and follow-up

A total of 398 patients with CHF were enrolled between August 2011 and January 2018. The current investigation concerns the 373 patients with HFrEF and known baseline BMI > 18.5 kg/m^2. Information about the patients was recorded at baseline and at predefined follow-up visits, which were scheduled every 3 (±1) months. These visits included collection of blood samples, short medical examinations, and documentation of adverse cardiovascular events since the last visit.

Clinical assessment at baseline

All patients were evaluated at baseline by a research physician or research nurse, who performed a physical examination and recorded HF-related symptoms and New York Heart Association (NYHA) class. Medical history, HF etiology, cardiovascular risk factors, and medication use were primarily retrieved from hospital records and checked in case of ambiguities. Electro- and echocardiography were performed. Specifically, for echocardiography, two-dimensional gray-scale harmonic images were obtained in the left lateral decubitus position using a commercially available ultrasound system (iE33, Philips, Best, the Netherlands), equipped with a broadband (1–5 MHz) S5-1 transducer (frequency transmitted 1.7 MHz, received 3.4 MHz) and stored in the echo core lab of Erasmus MC. Using specialized software (TomTec imaging, Unterschleissheim, Germany), parameters including LVEF were measured. LVEF was measured using the two-dimensional (2D) triplane method. The 2D triplane method extends the biplane approach by incorporating an additional apical view, usually the apical long-axis view. It aims to provide a more comprehensive assessment by including three planes, potentially reducing geometric assumptions and capturing more of the LV shape and size compared to Simpson's biplane method.

Outcome definitions

A clinical event committee determined the study's endpoints based on hospital records, discharge letters, and event definitions, without knowledge of the proteomic measurements. In patients with multiple endpoints, only the first one was considered for analysis. The primary endpoint (PEP) was predefined as the composite of cardiovascular death, left ventricular assist device (LVAD) implantation, heart transplantation, and hospitalization for acute or worsened HF because these clinical outcomes together cover the most important manifestations of HF exacerbation. Patients were considered hospitalized for acute or worsened HF when hospitalized for an exacerbation of HF symptoms together with two of the following conditions: N-terminal pro-B-type natriuretic peptide (NT-proBNP) or BNP exceeding three times the normal upper limit; signs of worsening HF such as pulmonary rates, raised jugular venous pressure, or peripheral edema; increased dose or intravenous administration of diuretics; or administration of positive inotropic agents.

Proteomic measurements

Blood samples were collected at baseline and every 3 months thereafter. Blood samples used for this investigation included samples drawn at baseline and the last two samples drawn before a PEP or censoring. Previous investigations in this cohort using all samples have shown that the most prominent changes in levels of several plasma and urine biomarkers occur in the months before an adverse event [7, 11]. We capture these biomarker changes while minimizing the number of measurements by using the first and the last two samples. Blood samples were processed within 2 h after collection, after which they were stored at −80°C.

Proteomic analyses were performed using 55 μL of EDTA plasma, using the aptamer-based proteomic SOMAscan platform [12]. In total, 5284 aptamers were applied in the samples. We excluded 300 aptamers with non-validated or non-human targets. Furthermore, when aptamers targeted the same protein, we kept the aptamer with the highest binding affinity and excluded the others. This procedure left us with 4210 aptamers corresponding to an equal number of proteomic biomarkers. Additional information is provided in the Supplemental methods, and analytical performance of the SOMAscan assay can be found in Table S1.

Statistical analysis

Differences in the clinical characteristics between patients with and without obesity were investigated using Wilcoxon rank sum tests, χ^2 tests, or Fisher exact tests where appropriate. Differences in HF prognosis between these groups were investigated via Kaplan–Meier curves and a log-rank test.

The protein levels were log-transformed for all analyses. Associations between standardized baseline protein levels (dependent variable) and obesity (independent variable; BMI > 30 kg/m^2) were determined using separate linear regression models adjusted for age; sex; kidney function (estimated glomerular filtration rate [eGFR] Chronic Kidney Disease Epidemiology Collaboration [CKD-EPI] formula); and history of hypertension, diabetes mellitus, and hypercholesterolemia. Proteins significantly associated with obesity (false discovery rate [FDR] < 0.05) were selected for further analysis. The stability of these selected proteins was evaluated by internal validation via a bootstrap analysis [13]. The sets of proteins associated positively and negatively with obesity were analyzed both separately and jointly for their associations with biological processes of the Gene Ontology: Biological Processes [14] database by an enrichment analysis conducted using the Toppgene suite, with the full set of 4210 proteins taken as reference [15].

To account for the dynamic nature of HF, associations between repeated protein measurements and the PEP were evaluated using

separate joint models for longitudinal and time-to-event data for every single protein [16, 17]. Joint models combine linear mixed models for the temporal evolution of the protein level, with proportional hazard models for the time-to-event data. We summarized the protein-level trajectories over time per patient using random intercepts and slopes and incorporated the estimated trajectory in the jointly fitted survival model. Results are presented as hazard ratios (HR) with 95% confidence intervals (CI). The model was fitted twice, using two levels of adjustment for the time-to-event part, first for obesity and for the Meta-Analysis Global Group in Chronic (MAGGIC) Heart Failure Risk Score [18] and second additionally adjusted for time-varying NT-proBNP and high-sensitivity (hs)-troponin T levels [9]. The MAGGIC score is an HF risk score consisting of the following 13 clinical variables: age, sex, diabetes mellitus, chronic obstructive pulmonary disease, smoking status, HF duration, NYHA class, β blocker use, angiotensin-converting enzyme inhibitor/angiotensin receptor blocker use, systolic blood pressure, BMI, serum creatinine, and ejection fraction.

Proteins of interest were placed in clinical context using hierarchical clustering methods. Clinical characteristics and selected proteins were clustered via complete-linkage clustering, using one minus the absolute value of the pairwise Spearman correlation coefficient as distance measure ($1 - |\text{cor}(x,y)|$).

R version 4.2.1 was used for all analyses, R-package JMbayes2 [19] was used to fit the joint models of longitudinal and time-to-event data, and two-sided $p < 0.05$ was considered statistically significant.

RESULTS

Baseline characteristics

Table 1 illustrates the baseline characteristics of our cohort. The median (25th, 75th percentile) age was 64 years (56, 72), 73% were male, the median BMI was 26.5 (24.1, 30.1), and 26% had obesity. Patients with obesity were younger (median age 60 [54, 69] vs. 65 [56, 74] years), had a longer duration of HF at inclusion (5.3 [2.3, 11.2] vs. 3.8 [1.1, 8.9] years), had higher systolic (120 [107, 130] vs. 112 [100, 128] mm Hg) and diastolic blood pressure (72 [68, 80] vs. 70 [60, 76] mm Hg), and had lower NT-proBNP (71 [26, 181] vs. 169 [70, 316] pmol/L) and hs-troponin T (14 [9, 23] vs. 19 [10, 36] ng/L) levels at baseline but had higher C-reactive protein (CRP) levels (3.1 [1.4, 6.3] vs. 1.7 [0.7, 4.2] mg/L) than their counterparts without obesity. Furthermore, patients with obesity more often had a history of diabetes mellitus (45% vs. 19%), known hypercholesterolemia (53% vs. 40%), and hypertension (61% vs. 39%).

Impact of obesity on proteomic profile in patients with HFrEF

Figure 1 and Table S2 illustrate associations between obesity and standardized baseline protein values adjusting for age; sex; kidney function (eGFR CKD-EPI); and history of hypertension, diabetes mellitus, and hypercholesterolemia. These estimates can be interpreted as standard deviation differences in log-protein value between patients with and without obesity. After correction for multiple testing, we found 35 proteins positively and 106 proteins negatively associated with obesity. The top three most positively associated proteins were γ-glutamyl hydrolase (standardized coefficient [95% CI]: 0.71 [95% CI: 0.46 to 0.96], FDR < 0.001), growth hormone receptor (0.70 [95% CI: 0.47 to 0.94], FDR < 0.001), and galectin-3-binding protein (0.63 [95% CI: 0.38 to 0.88], FDR < 0.001). The top three most negatively associated proteins (all FDR < 0.001) were SLIT and NTRK-like protein 3 (standardized coefficient [95% CI]: −0.77 [95% CI: −1.01 to −0.53]), insulin-like growth factor-binding protein 1 (IGFBP1; −0.74 [95% CI: −0.99 to −0.50]), and neural cell adhesion molecule L1-like protein (−0.73 [95% CI: −0.99 to −0.48]). Other notable (clinically relevant) proteins included heart-type fatty acid binding protein (0.57 [95% CI: 0.37 to 0.78], FDR < 0.001), NT-proBNP (−0.63 [95% CI: −0.87 to −0.38], FDR < 0.001), and CRP (0.48 [95% CI: 0.22 to 0.74], FDR < 0.001).

The stability of this selection of proteins was assessed by repeating the analysis on 1000 bootstrap resampled data sets and calculating the fraction of times that every protein was included as significantly associated with obesity. These resulting bootstrap inclusion fractions are included in Table S2 and serve as estimates of the probability of finding an association between the protein and obesity. Because all proteins in our selection were included in the majority of bootstrap iterations, we consider this selection stable [13].

Figures S2 and S3 provide summaries of the biological processes associated with the differently expressed proteins. Notable associations include neuron projection development (FDR = 7.37E-05), synapse assembly (FDR = 2.05E-02), muscle cell migration (FDR = 3.22E-02), and cell adhesion (FDR = 4.37E-04). A full overview of the significant results of the enrichment analysis is provided in Table S3.

Associations of PEP and proteins

During a median (25th, 75th percentile) follow-up of 2.1 (1.1, 2.6) years, 112 patients experienced a PEP. These 112 patients either experienced cardiovascular death, were hospitalized for acute or worsened HF, underwent a heart transplantation, or received an LVAD, whichever occurred first. In total, individual components of the PEP occurred more frequently, with 32 patients experiencing cardiovascular death, 88 being hospitalized for acute or worsened HF, 16 undergoing a heart transplantation, and 13 receiving an LVAD. In patients with obesity, 26% (25/96) experienced a PEP versus 31% (87/277) in the group without obesity. Figure S4 illustrates the prognosis of patients with and without obesity. In this unadjusted figure, no significant difference in prognosis was detected.

The proteins were measured at a median of three time points per patient (time points per patient: one, 6%; two, 8%; three, 86%). Figure 2 illustrates the associations of the trajectories of differently expressed proteins with the PEP, while adjusting for clinical

TABLE 1 Baseline characteristics.

	Overall, $N = 373$[a]	With obesity, $n = 96$[a]	Without obesity, $n = 277$[a]	p value[b]
Demographics				
Age at baseline visit (y)	64 (56, 72)	60 (54, 69)	65 (56, 74)	**0.03**
Men	272 (73%)	71 (74%)	201 (73%)	0.8
Ethnicity: Caucasian	343 (93%)	85 (89%)	258 (94%)	0.2
Features of HF				
Duration of HF (y)	4.2 (1.6, 9.5)	5.3 (2.3, 11.2)	3.8 (1.1, 8.9)	**0.034**
NYHA class				0.5
I	90 (24%)	25 (26%)	65 (24%)	
II	180 (49%)	42 (44%)	138 (50%)	
III	98 (26%)	29 (30%)	69 (25%)	
IV	3 (0.8%)	0 (0%)	3 (1.1%)	
Systolic ejection fraction at baseline (%)	30 (23, 37)	29 (23, 39)	30 (22, 37)	0.5
Clinical characteristics				
BMI (kg/m^2)	26.5 (24.1, 30.1)	32.1 (30.9, 34.9)	25.2 (23.2, 27.1)	**<0.001**
eGFR CKD-EPI (mL/min/1.73 m^2)	57 (43, 74)	61 (47, 78)	55 (42, 72)	0.061
Systolic blood pressure (mm Hg)	115 (100, 130)	120 (107, 130)	112 (100, 128)	**0.034**
Diastolic blood pressure (mm Hg)	70 (60, 78)	72 (68, 80)	70 (60, 76)	**0.003**
Biomarker levels				
NT-proBNP (pmol/L)	126 (45, 273)	71 (26, 181)	169 (70, 316)	**<0.001**
hs-troponin T (ng/L)	18 (9, 32)	14 (9, 23)	19 (10, 36)	**0.027**
CRP (mg/L)	2.2 (0.9, 4.9)	3.1 (1.4, 6.3)	1.7 (0.7, 4.2)	**0.007**
Etiology of HF				
Ischemic heart disease	163 (44%)	42 (44%)	121 (44%)	>0.9
Hypertension	33 (8.8%)	11 (11%)	22 (7.9%)	0.3
Secondary to valvular heart disease	10 (2.7%)	1 (1.0%)	9 (3.2%)	0.5
Cardiomyopathy	120 (32%)	32 (33%)	88 (32%)	0.8
HCM	14 (3.8%)	2 (2.1%)	12 (4.3%)	0.5
DCM	96 (26%)	27 (28%)	69 (25%)	0.5
Restrictive	0 (0%)	0 (0%)	0 (0%)	>0.9
ARVC	1 (0.3%)	0 (0%)	1 (0.4%)	>0.9
Non compaction cardiomyopathy	4 (1.1%)	1 (1.0%)	3 (1.1%)	>0.9
Unclassified	7 (1.9%)	2 (2.1%)	5 (1.8%)	>0.9
Unknown	26 (7.0%)	5 (5.2%)	21 (7.6%)	0.4
Medical history				
Myocardial infarction	143 (39%)	36 (38%)	107 (39%)	0.8
PCI	124 (33%)	39 (41%)	85 (31%)	0.075
CABG	53 (14%)	8 (8.3%)	45 (16%)	0.056
Atrial fibrillation	133 (36%)	28 (29%)	105 (38%)	0.12
Stroke (CVA/TIA)	46 (12%)	7 (7.4%)	39 (14%)	0.086
Chronic renal failure	177 (48%)	46 (48%)	131 (48%)	>0.9
Diabetes mellitus	97 (26%)	43 (45%)	54 (19%)	**<0.001**
Known hypercholesterolemia	157 (43%)	50 (53%)	107 (40%)	**0.03**
Hypertension	165 (45%)	59 (61%)	106 (39%)	**<0.001**
Intoxication				
Smoking: Ever	264 (71%)	72 (76%)	192 (70%)	0.2
Smoking: Current	37 (10.0%)	10 (11%)	27 (9.8%)	0.8

(Continues)

TABLE 1 (Continued)

	Overall, $N = 373$[a]	With obesity, $n = 96$[a]	Without obesity, $n = 277$[a]	p value[b]
Medication				
ACE inhibitor	251 (67%)	63 (66%)	188 (68%)	0.7
ARB	106 (28%)	31 (32%)	75 (27%)	0.3
Aldosteron antagonists	287 (77%)	79 (82%)	208 (75%)	0.15
Loop diuretics	344 (92%)	91 (95%)	253 (91%)	0.3
Thiazide diuretics	12 (3.2%)	5 (5.2%)	7 (2.5%)	0.2
Other diuretics	5 (1.3%)	3 (3.1%)	2 (0.7%)	0.11
β blockers	342 (92%)	92 (96%)	250 (91%)	0.1
Aspirin	76 (20%)	25 (26%)	51 (18%)	0.11
Anticoagulants	273 (73%)	66 (69%)	207 (75%)	0.3

Note: Bold indicates statically significant values ($p < 0.05$).

Abbreviations: ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; ARVC, arrhythmogenic right ventricular; CABG, coronary artery bypass surgery; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; CRP, C-reactive protein; CVA, cerebral vascular accident; DCM, dilated cardiomyopathy; eGFR, estimated glomerular filtration rate; HCM, hypertrophic cardiomyopathy; HF, heart failure; hs, high-sensitivity; NT-proBNP, N-terminal pro-B-type natriuretic peptide; NYHA, New York Heart Association; PCI, percutaneous coronary intervention; TIA, transient ischemic attack.

[a]Median (IQR); *n* (%).

[b]Wilcoxon rank sum test; Pearson χ^2test; Fisher exact test.

characteristics using the MAGGIC score, and obesity. Out of the 141 selected proteins expressed differently between patients with and without obesity, 37 were positively associated with the PEP (FDR < 0.05) and 13 proteins negatively associated. The top three proteins with the strongest positive association were NT-proBNP, carbohydrate sulfotransferase 15 (CHST15), and neuropilin-1. The top three proteins with the strongest negative associations were neutral ceramidase (ASAH2), afamin, and anthrax toxin receptor 1 (ANTXR1).

Figures 3 and S5 show associations of the obesity-related proteins associated with the PEP while additionally adjusting for time-varying NT-proBNP and hs-troponin T. In total, 12 proteins remained significantly associated with the PEP, i.e., 4 with positive and 8 with negative associations. Three proteins were positively associated with the PEP and were elevated in patients with obesity, i.e., serine protease HTRA1, plasminogen activator inhibitor 1 (PAI-1; SERPINE1), and CRP. Seven proteins were negatively associated with the PEP and were downregulated in patients with obesity, i.e., tyrosine–protein kinase transmembrane receptor ROR2 (ROR2), transgelin, histone–lysine N − methyltransferase EHMT2 (EHMT2; G9a), ephrin type–A receptor 4 (EPHA4), neurogenic locus notch homolog protein 1 (NOTCH1), receptor-type tyrosine–protein phosphatase delta (PTPRD), and anthrax toxin receptor 1 (ANTXR1). One protein was negatively associated with PEP and was upregulated in patients with obesity, i.e., neutral ceramidase (ASAH2), and one protein was positively associated with PEP and was downregulated in patients with obesity, i.e., carbohydrate sulfotransferase 15 (CHST15).

Figure 4 and S6 show the results of hierarchical clustering of these 12 proteins and clinical characteristics based on pairwise correlations. Serine protease HTRA1 was clustered together with CRP, whereas transgelin and ROR2 were correlated with NT-proBNP and hs-troponin T. The other proteins clustered together.

DISCUSSION

Both HFrEF and obesity are complex conditions linked to many biological mechanisms. Our study uses a large panel of 4210 diverse proteomic biomarkers and provides a broad perspective on the mechanisms common to obesity and HF.

We found distinct proteomic profiles for patients with HFrEF and obesity compared to patients with HFrEF without obesity, in which 141 out of the 4210 measured proteins showed different levels. Specifically, we found 35 upregulated and 106 downregulated proteins in patients with obesity. Associations of established HF biomarkers were in line with the literature: NT-proBNP was negatively associated with obesity [20, 21]; and CRP, reflecting systemic inflammation, was positively associated with obesity [22, 23]. Our results are also in line with a previous study conducted on 6981 individuals enrolled in the Framingham Heart Study that identified several proteins associated with obesity from a smaller panel of 71 proteins, which found strong positive associations for leptin, CRP, and PAI-1 and strong negative associations with IGFBP1 and 2 [24].

Our enrichment analysis revealed that the set of differently expressed proteins was associated with biological processes such as neuron projection development, synapse assembly, cell adhesion, and muscle cell migration. Obesity has been associated with altered synaptic plasticity and impaired cognitive function, which is in line with the link with synapse assembly and neuron projection development [25, 26]. Cell adhesion has been described as a mechanism related to HFpEF [27], and circulating cell adhesion molecules have been shown to reflect and mediate obesity-related cardiovascular risk [28]. Accordingly, cell adhesion could be a pathophysiologic phenomenon in patients with HFrEF with obesity as well. Migration of muscle cells, particularly smooth muscle cells, is linked to the formation and progression of arterial plaque, a key component of

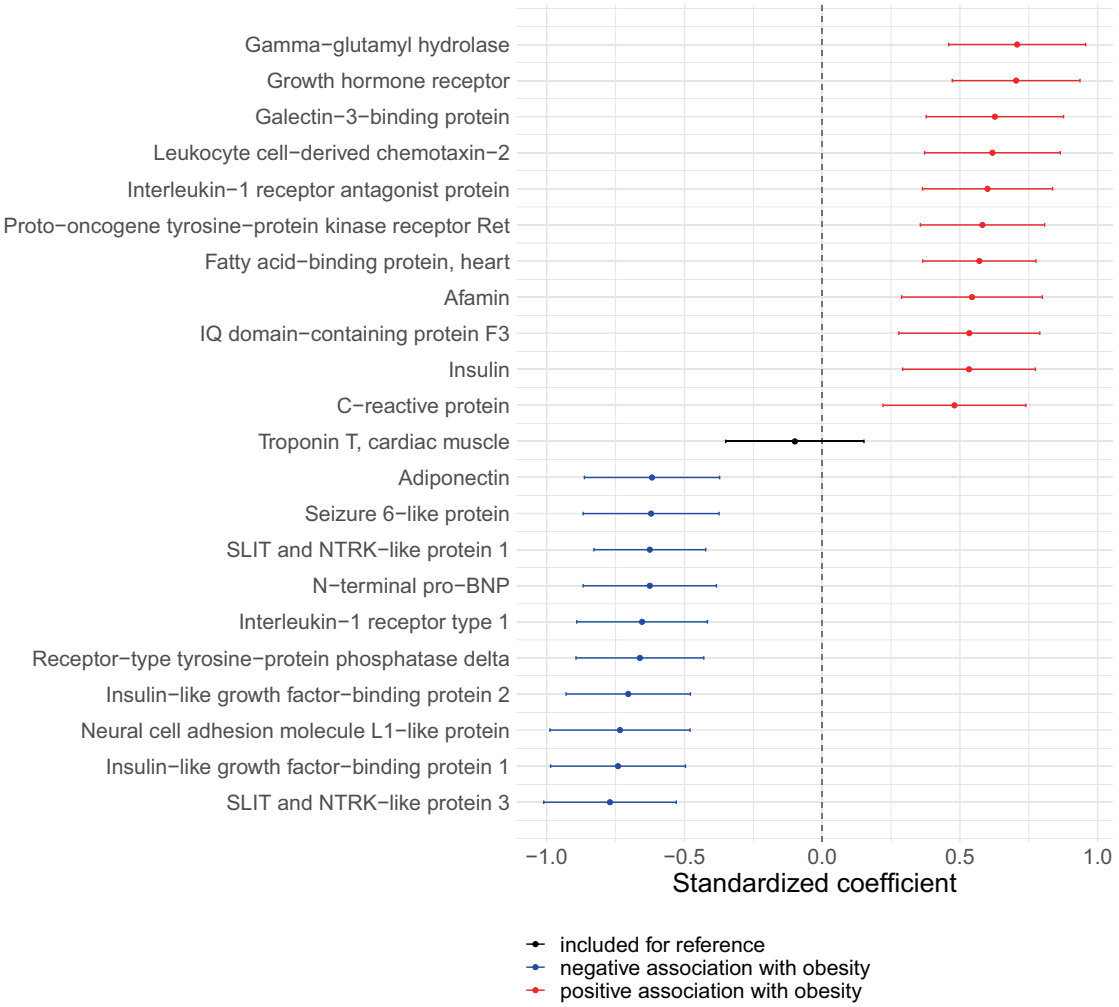

FIGURE 1 Forest plot of association between obesity and standardized baseline protein measurements, adjusted for age; sex; kidney function (estimated glomerular filtration rate Chronic Kidney Disease Epidemiology Collaboration formula); and history of hypertension, diabetes mellitus, and hypercholesterolemia. Top 10 proteins with largest positive and negative effect size are displayed in this figure, together with C-reactive protein and troponin T for reference. [Color figure can be viewed at wileyonlinelibrary.com]

atherosclerosis [29]. Obesity is considered a risk factor for atherosclerosis through various mechanisms (such as hypertension, increased glucose levels, atypical lipid profiles, and systemic inflammation). Furthermore, obesity can affect smooth muscle cells through abnormal regulation of various adipokines [29, 30].

Proteins that are both differently expressed in patients with HFrEF with and without obesity and associated with HF prognosis might be interesting targets for stratified or personalized monitoring and treatment. Of the 141 proteins differently expressed proteins, 50 were associated with HF prognosis. This set of 50 proteins contained both well- and lesser-known HF markers. Noteworthy proteins that are positively associated with both obesity and PEP include serine protease HTRA1, heart-type fatty acid binding protein (H-FABP), and PAI-1. Serine protease HTRA1 is known to regulate the availability of insulin-like growth factors (IGFs) by cleaving their binding proteins (IGFBPs) [31]. IGFs and IGFBPs such as IGF1 and IGFBP7 are associated with cardiac health through their role in cellular metabolism and aging [32, 33]. H-FABP is a well-studied protein in cardiovascular research that is known for its influence on lipid transport and myocyte metabolism. It is released upon ischemic myocardial damage and found to be a candidate myocardial injury marker that can provide prognostic value beyond hs-troponin T measurements [34]. It is considered especially of value in patients with obesity, in whom it can take over the predictive role of BNP measurements, as the same metabolic factors that reduce BNP levels tend to accelerate H-FABP leakage [35].

After including NT-proBNP trajectories in the model, we found that 12 proteins remained significantly associated with the PEP. These 12 proteins might provide valuable information for the prognosis of patients with HFrEFand obesity over established assessments using clinical characteristics, NT-proBNP, and hs-troponin T. Based on literature, these proteins can be divided roughly in two groups. The first group includes proteins that have known associations with atherosclerosis or coronary artery disease. CRP is an often-used marker for

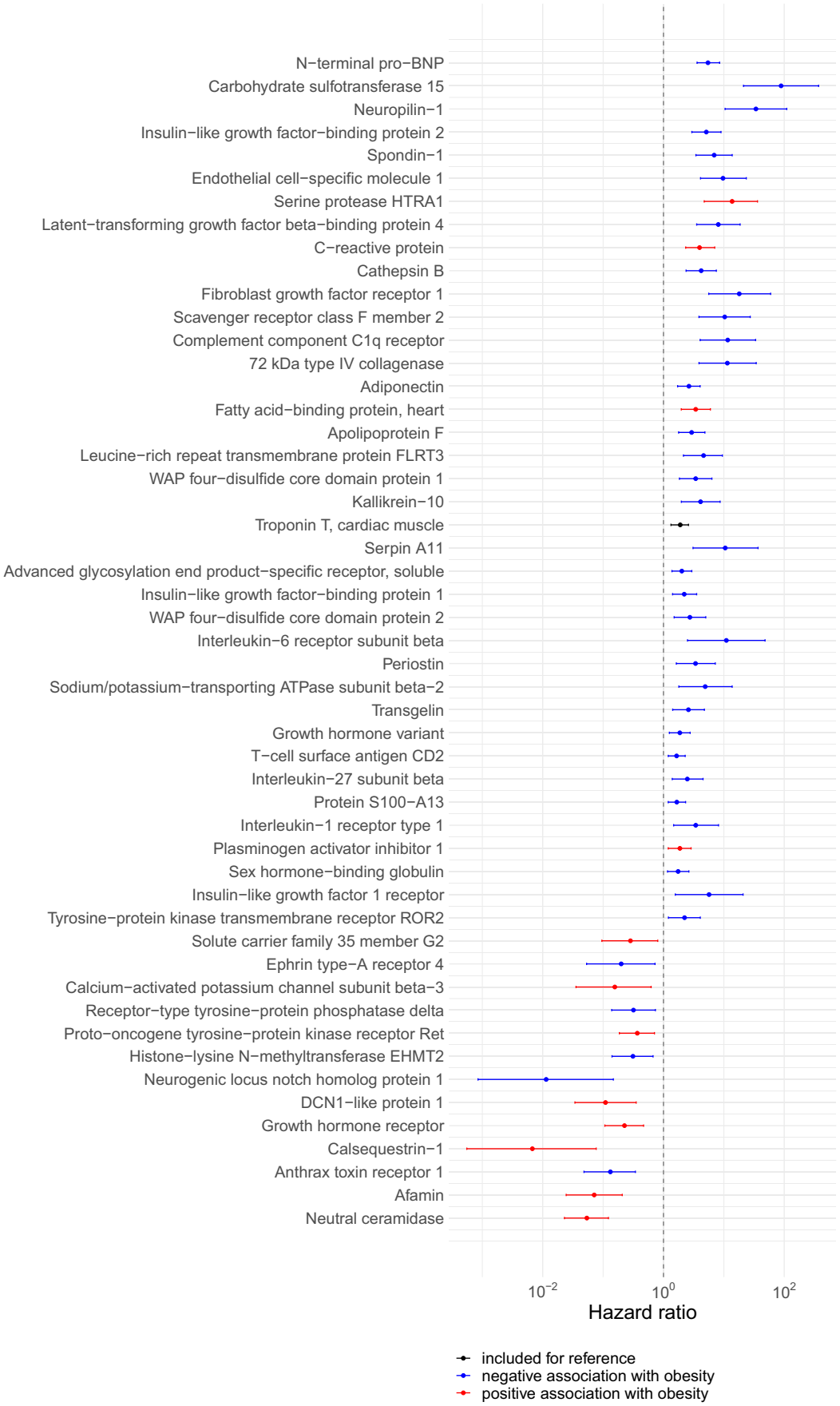

FIGURE 2 Legend on next page.

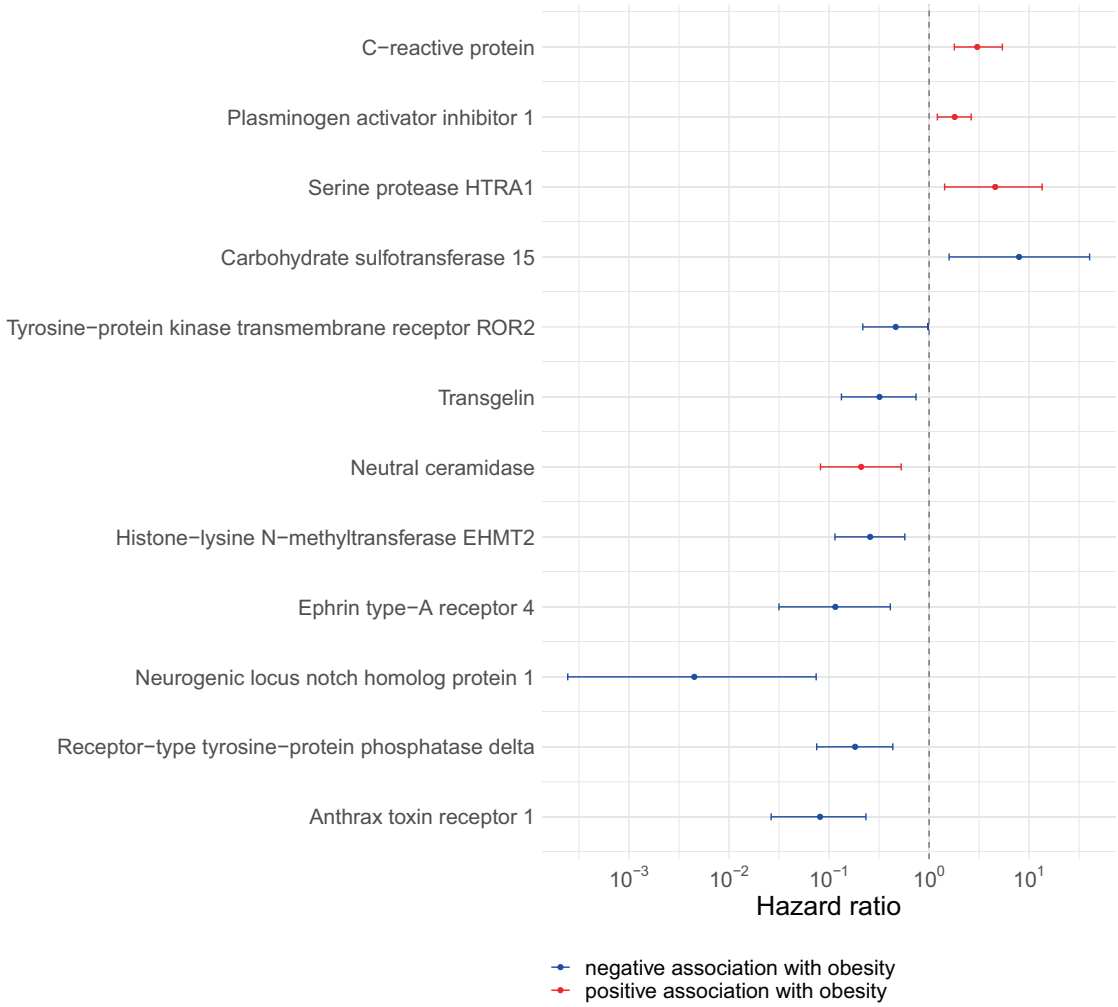

FIGURE 3 Associations of proteins differently expressed in patients with and without obesity, with prognosis after adjustment for time-varying N-terminal pro-B-type natriuretic peptide (NT-proBNP). Forest plot illustrating the hazard ratios of proteins significantly associated with the primary endpoint (PEP; composite of cardiovascular mortality, heart failure hospitalization, left ventricular assist device implantation, and heart transplantation) after adjustment for Meta-Analysis Global Group in Chronic (MAGGIC) score, obesity status, and time-varying NT-proBNP and high-sensitivity troponin T levels ($p < 0.05$). Proteins are ordered based on level of association with the PEP as expressed via their t statistic. [Color figure can be viewed at wileyonlinelibrary.com]

inflammation, which plays a key role in the pathogenesis of atherosclerosis and thrombosis [36]. PAI-1 is a well-studied protein in the context of obesity and cardiovascular disease related to fibrinolysis. Elevated PAI-1 levels are a risk factor for thrombosis and atherosclerosis [37]. PTPRD is a signaling protein that has shown protective properties for coronary artery disease [38]. The second group includes proteins related to cardiac remodeling or hypertrophy. EHMT2, a well-studied protein essential for cardiomyocyte homeostasis, is protective against cardiac hypertrophy and deemed a promising candidate for drug targeting [39]. Moreover, the inhibition of CHST15 has been shown to impede cardiac remodeling [40]. ROR2 can be associated with remodeling of the heart after pressure overload through its role in myofibroblast differentiation [41]. NOTCH1 is related to both atherosclerosis and hypertrophy. It antagonizes inflammation during atherosclerosis [42] and plays an important role in the healing process of an injured heart by controlling the balance between fibrotic and regenerative repair [43]. Because NOTCH1 is downregulated in patients with HFrEF with obesity, it may be an

FIGURE 2 Associations of proteins differently expressed between patients with and without obesity, with prognosis. Forest plot illustrating the hazard ratios (HR) of proteins significantly associated with the primary endpoint (PEP; composite of cardiovascular mortality, heart failure hospitalization, left ventricular assist device implantation, and heart transplantation) after adjustment for Meta-Analysis Global Group in Chronic (MAGGIC) score and obesity status (false discovery rate [FDR] < 0.05). Proteins are ordered based on level of association with the PEP as expressed via their t statistic. [Color figure can be viewed at wileyonlinelibrary.com]

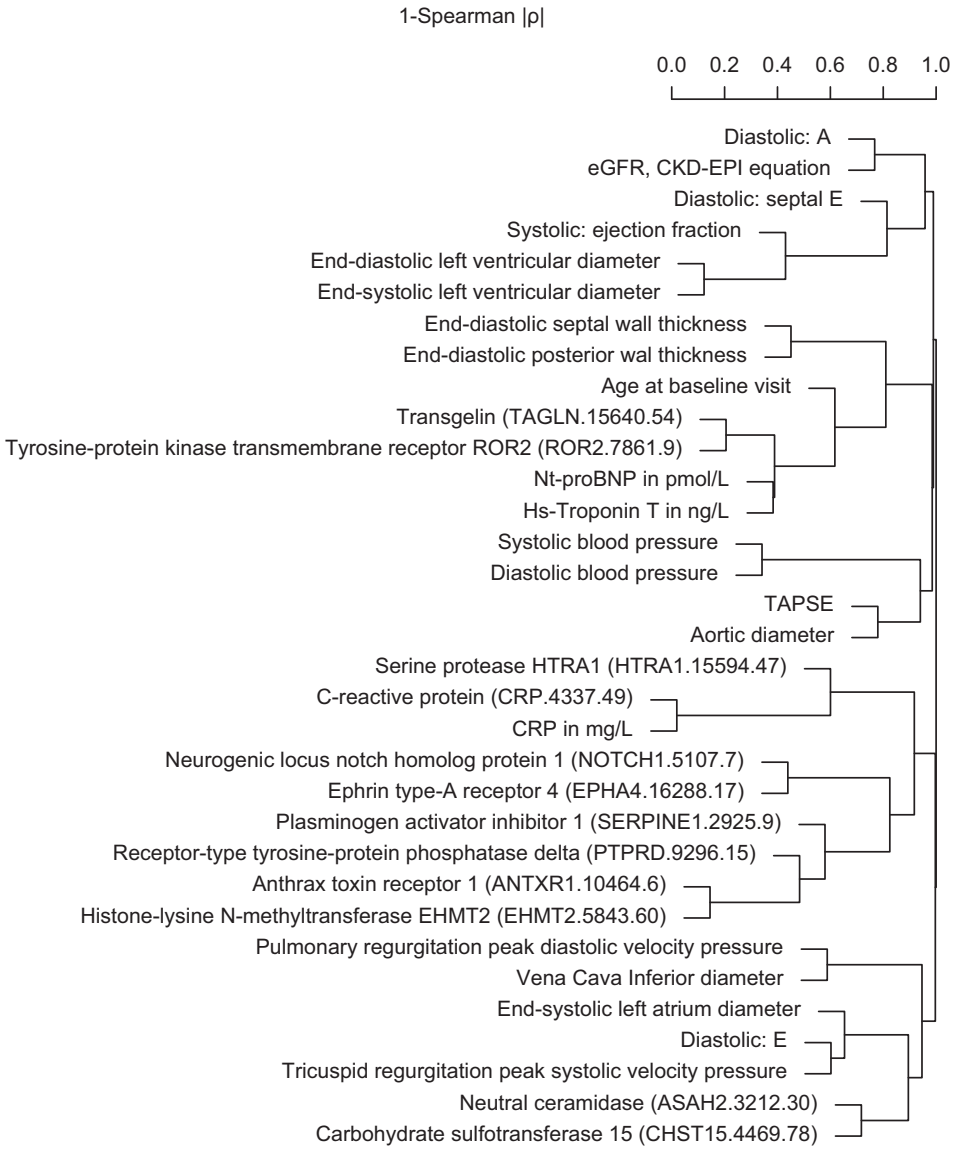

FIGURE 4 Dendogram of correlations between clinical characteristics and selected proteins. Hierarchical clustering of clinical characteristics and selected proteins via complete-linkage clustering, using one minus the absolute value of the pairwise Spearman correlation coefficient as distance measure ($1 - |\text{cor}(x,y)|$).

interesting therapeutic target to study for this group. A hierarchical cluster analysis of the proteins at baseline with clinical characteristics revealed that serine protease HTRA1 was correlated with CRP, ASAH2 was correlated with CHST15, and transgelin and ROR2 were correlated with NT-proBNP and hs-troponin T. The other proteins seemed to cluster among themselves. This may indicate a stronger correlation among themselves than with the included clinical characteristics.

Prior literature on proteomic differences in HF with and without obesity is generally based on small panels of circulating proteins measured at a single point in time, and a focus on HFrEF is still scarce [6]. In this study, we provide a comprehensive overview of pithe distinct proteomic profiles of patients with HFrEF and obesity by looking at a broad panel of 4210 proteins. Moreover, the associations of the distinct proteins with the PEP were examined while taking the dynamic nature of HF into account by incorporating full temporal protein trajectories. Enhanced knowledge on the distinct proteins and pathophysiological mechanisms at play in patients with both HFrEF and obesity carries several potential clinical and scientific implications. Our results could form the basis of more tailored management of obesity-related HF in the long term. Such tailored management could, for example, include biomarker-based timing of treatment or biomarker-based choices for particular treatment modalities. For development of such tailored management options, clinical utility of the implicated biomarkers should be further investigated. Moreover, our results could provide opportunities for retro-translations; specifically, they could provide leads for research on proteins that could serve as therapeutic targets for medication. Further studies should look into these personalized approaches to obesity-HFrEF management.

Limitations of this study include the relatively small fractions of patients with a non-Caucasian ethnicity (7%), patients with NYHA class III/IV (26.8%), and women (27%). The method of protein measurement also warrants some consideration. SOMAmer reagents were selected against proteins in their native folded conformations. Therefore, unfolded or denatured proteins are not detected. Furthermore, normalized relative fluorescent units are returned by the SOMAscan assay rather than absolute concentrations. These can be used to compare patients and changes over time within a patient. However, they are not recommended to be used to inform clinical decisions requiring absolute concentrations. The clinical outcome comprised a composite endpoint. Although components such as hospitalization for HF are "softer" endpoints compared to, for example, mortality, they have direct relevance for the patient. Moreover, given the relatively modest sample size of the study, composite endpoints provide benefits with regard to statistical power. As the median duration of follow-up was 2.1 years, we considered the effect of the proteins on prognosis in the medium term. Long-term follow-up could add further understanding of the relevance of our findings, also considering that circulating proteins such as CRP have previously been associated with ventricular function during longer-term follow-up [44, 45]. Finally, we were unable to assess leptin levels because its aptamer did not pass our quality criteria.

In conclusion, there are striking differences in the pathophysiological features of HFrEF in patients with obesity. In this study, we found a distinct proteomic profile for these patients related to several specific biological mechanisms. Furthermore, we found 12 proteins that were both differently expressed according to obesity status and significantly associated with HF prognosis after adjustment for clinical characteristics and NT-proBNP and hs-troponin T levels. These proteins carry potential to improve the management of obesity-related HF and could serve as potential leads for future research.O

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CONFLICT OF INTEREST STATEMENT

K. Martijn Akkerhuis reports membership of Cardialysis Cooperatief U.A. until 2023. Rudolf A. de Boer reports personal fees from Abbott Laboratories, AstraZeneca plc, Bristol Myers Squibb, Cardior Pharmaceuticals GmbH, Novo Nordisk, and Roche outside the submitted work and is the current President of the Dutch Cardiac Society. Isabella Kardys reports travel reimbursement from SomaLogic, Inc. The other authors declared no conflict of interest.

CLINICAL TRIAL REGISTRATION

ClinicalTrials.gov identifier: NCT01851538.

DATA AVAILABILITY STATEMENT

Anonymized data that support the findings of this study will be made available to other researchers for the purposes of reproducing the results upon reasonable request and in accordance with a data-sharing agreement.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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