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ORIGINAL RESEARCH

Impaired High-Density Lipoprotein Function in Patients With Heart Failure

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BACKGROUND: We recently showed that, in patients with heart failure, lower high-density lipoprotein (HDL) cholesterol concentration was a strong predictor of death or hospitalization for heart failure. In a follow-up study, we suggested that this association could be partly explained by HDL proteome composition. However, whether the emerging concept of HDL function contributes to the prognosis of patients with heart failure has not been addressed.

METHODS AND RESULTS: We measured 3 key protective HDL function metrics, namely, cholesterol efflux, antioxidative capacity, and anti-inflammatory capacity, at baseline and after 9 months in 446 randomly selected patients with heart failure from BIOSTAT-CHF (A Systems Biology Study to Tailored Treatment in Chronic Heart Failure). Additionally, the relationship between HDL functionality and HDL proteome composition was determined in 86 patients with heart failure. From baseline to 9 months, HDL cholesterol concentrations were unchanged, but HDL cholesterol efflux and anti-inflammatory capacity declined (both $P<0.001$). In contrast, antioxidative capacity increased ($P<0.001$). Higher HDL cholesterol efflux was associated with lower mortality after adjusting for BIOSTAT-CHF risk models and log HDL cholesterol (hazard ratio, 0.81; 95% CI, 0.71–0.92; $P=0.001$). Other functionality measures were not associated with outcome. Several HDL proteins correlated with HDL functionality, mainly with cholesterol efflux. Apolipoprotein A1 emerged as the main protein associated with all 3 HDL functionality measures.

CONCLUSIONS: Better HDL cholesterol efflux at baseline was associated with lower mortality during follow-up, independent of HDL cholesterol. HDL cholesterol efflux and anti-inflammatory capacity declined during follow-up in patients with heart failure. Measures of HDL function may provide clinical information in addition to HDL cholesterol concentration in patients with heart failure.

Key Words: cholesterol efflux ■ functionality ■ high-density lipoprotein ■ outcome ■ proteome

Recently, we showed that low high-density lipoprotein (HDL) cholesterol was among the strongest predictors of death or hospitalization for heart failure (HF) in 2 large European HF cohorts.[1] In addition, low concentrations of HDL cholesterol have been associated with a higher incidence of HF[2,3] and worse outcomes in patients with established HF.[3–6] In contrast, HDL cholesterol–increasing therapies have failed to show clear benefit, casting doubt on whether HDL cholesterol, as conventionally measured, can be ascribed a causal protective role.[7] However, HDL has high proteomic complexity and a wide range of biological effects,[8,9] including antioxidative, cholesterol efflux–promoting, anti-inflammatory, antiapoptotic, and endothelial-protective effects.[6] Since inflammation and oxidative stress are common in HF,[10] the antioxidative

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CLINICAL PERSPECTIVE

What Is New?

- High-density lipoprotein (HDL) cholesterol efflux and anti-inflammatory capacity significantly decreased over time in patients with HF, possibly signifying a "state of no return" for HDL functionality.
- Better HDL cholesterol efflux at baseline is associated with lower mortality during follow-up in heart failure, independent of HDL cholesterol and other relevant clinical factors.
- Apolipoprotein A1 emerged as an important protein that is associated with all 3 measures of HDL functionality.

What Are the Clinical Implications?

- Measures of HDL function (HDL quality), may provide clinical information beyond static HDL cholesterol concentration (HDL quantity) in patients with heart failure.
- In patients with heart failure, HDL cholesterol efflux might be a clinically relevant target aspect of HDL functionality for future therapeutic intervention.

Nonstandard Abbreviations and Acronyms

BIOSTAT-CHF	A Systems Biology Study to Tailored Treatment in Chronic Heart Failure

and anti-inflammatory properties of HDL might be an endogenous defense mechanism. These attributes of HDL are not captured by simply measuring conventional HDL cholesterol concentrations.

We have shown that HDL proteome composition is strongly associated with prognosis in patients with HF. The biggest differences were seen in proteins reflecting crosslinking of actin filaments, alveolar-capillary membrane function, inflammation, and oxidative stress.[11] HDL function might also have prognostic implications.[12–15] Previous studies included relatively few patients (ranging from 23 to 320 patients), did not have follow-up measurements, and did not correct for many potential confounders. Also, changes in HDL functionality as patients recover from an episode of worsening HF have not been studied. Finally, whether shifts in HDL proteome composition are associated with changes in function is largely unexplored.

We investigated 3 measures of HDL function (HDL cholesterol efflux, antioxidative capacity, and anti-inflammatory capacity) in patients with worsening HF, their changes after 9 months of treatment, and their associations with prognosis.

METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Patient Population

For the present study, 446 patients were randomly selected from the index cohort of BIOSTAT-CHF (A Systems Biology Study to Tailored Treatment in Chronic Heart Failure), which has been described in detail before.[16] A comparison between the present study cohort and the overall BIOSTAT-CHF cohort is provided in Table S1. In brief, BIOSTAT-CHF was an investigator-driven, multicenter clinical study consisting of 2516 patients with HF, which aimed to identify patients with a poor outcome despite currently recommended treatment. Patients who presented with either new-onset or worsening HF, which was defined as left ventricular ejection fraction ≤40% or B-type natriuretic peptide >400 pg/mL or NT-proBNP (N-terminal pro-B-type natriuretic peptide) >2000 pg/mL, were included. Patients were encouraged to be up-titrated to recommended treatment doses.[17] All patients enrolled in BIOSTAT-CHF provided written informed consent. BIOSTAT-CHF was conducted in compliance with the Declaration of Helsinki, national ethics and legal requirements, and relevant EU legislation. The study also received approval by national and local ethics committees of all involved centers.

Additionally, 86 patients from BIOSTAT-CHF with available data on proteomic analysis of HDL-associated proteins at baseline were included in the present study (baseline characteristics in Table S2, including differences between this cohort and the HDL functionality cohort and BIOSTAT-CHF patients without proteomic data available), for which detailed methodology (including selection procedure) and results have been published previously.[11] In brief, lipoproteins were isolated from whole plasma using calcium silicate matrix, reduced using tris (2-carboxyethyl) phosphine, alkylated by iodoacetamide, denatured using ammonium deoxycholate, and trypsin digested. Solid-phase extraction was performed, and samples were reconstituted and spiked with an internal standard to allow for absolute quantification. Data were normalized to the volume of plasma by injecting a constant amount of 1 µL of plasma for each sample. Samples were then analyzed in triplicate on nanoscale liquid chromatography coupled with mass spectrometry, with quality

controls after every 10 samples. Raw data were interrogated by Progenesis QI software (Nonlinear Dynamics, Gateshead, UK), which executed label-free quantification of the identified proteins using the Hi-N3 method. Analyses were focused on Hi-N relative quantification, with the Hi-N absolute quantification method serving as a verification backup if needed. The proteins that were previously found to have the strongest association with outcome in this cohort were updated according to available newly reviewed data.[18]

Sensitivity analyses were performed in patients with HF with preserved ejection fraction (left ventricular ejection fraction > 45%) and patients with chronic kidney disease (defined as estimated glomerular filtration rate <60 mL/min per 1.73 m^2). Treatment optimization with angiotensin-converting enzyme inhibitors and beta blockers was graded according to achievement of 4 different levels of treatment dose, as described before.[17] The influence of statin dose on HDL functionality was studied by calculating statin dose in simvastatin equivalent, where 40 mg of simvastatin corresponded to 20 mg of atorvastatin, 5 mg of rosuvastatin, and 80 mg of pravastatin.[19]

Study Design and Laboratory Measurements

The blood samples were collected by venipuncture and stored at –80˚C. Blood measurements were performed directly on the basis of standardized international methods if possible; otherwise, they were performed by a central laboratory. Interleukin-6 and endothelin-1 were measured in frozen plasma by Singulex Inc. (Alameda, CA) using high-sensitivity single molecule counting technology (Research Use Only, Erenna Immunoassay System; Singulex). Measurement of additional blood biomarkers was performed as previously described.[20,21]

In both study cohorts, 3 metrics of HDL functionality were measured at baseline and also after 9 months for the larger study cohort of 446 patients. The assays for cholesterol efflux, antioxidative capacity, and anti-inflammatory capacity are established assays that have been used in large cohorts before[22,23] and have been described in detail previously.[24] Each measurement was carried out at the same time for all patient samples to reduce potential experimental variation attributable to differing assay conditions. In brief, HDL was isolated from EDTA plasma by precipitation of apolipoprotein B–containing lipoproteins as described.[22]

HDL cholesterol efflux was measured by differentiating THP-1 human monocytes (ATCC via LGC Promochem, Teddington, UK) into macrophages and loading them with 50 µg/mL acetylated low-density lipoprotein (LDL) and 1 µCi/mL ^{3}H-cholesterol (Perkin Elmer, Boston, MA) for 24 hours. Afterwards, the macrophages were equilibrated for 18 hours in Roswell Park Memorial Institute 1640 medium followed by addition of 2% of individual apolipoprotein B–depleted plasma samples for 5 hours. Then, an aliquot of the medium was counted for quantification of effluxed cholesterol label (1600CA Tri-Carb; Packard, Meriden, CT). After washing and incubation with 0.1 M NaOH for 30 minutes, the radioactivity remaining within the cells was also determined with liquid scintillation counting. Efflux was expressed as the percentage of counts released into the medium relative to the total dose of initial radioactivity present. Values were corrected for nonspecific non–HDL-mediated efflux and corrected for potential plate-to-plate variations by a standard curve constructed using different HDL concentrations from pooled plasma of healthy donors. The intra-assay coefficient of variation of this assay is 5.7%.

The antioxidative capacity of HDL was assessed by measuring the capacity of HDL to inhibit native LDL oxidation. LDL was isolated from plasma of a fasted healthy male donor using sequential ultracentrifugation and oxidized with 5-mM AAPH for 24 hours either in the presence of 2% of the individual HDL preparations or a PBS control. After protein precipitation by using 10% trichloroacetic acid, the accumulation of thiobarbituric acid reactive substances was determined as a measure for oxidative modification.[24] Antioxidative capacity of HDL was expressed as the number of thiobarbituric acid reactive substances accumulated relative to control LDL oxidized in the absence of HDL. The intra-assay coefficient of variation of this assay is 7.1%.

The anti-inflammatory capacity of HDL was assessed using human umbilical vein endothelial cells (Endothelial Cell Core Facility of the University Medical Center Groningen). Human umbilical vein endothelial cells s were preincubated with either 2% of HDL or a PBS control for 30 minutes. Subsequently, 10 ng/mL tumor necrosis factor-α (R&D Systems, Abingdon, UK) was added and incubated for 5 hours. Then, RNA was isolated with Trizol (Invitrogen, Carlsbad, CA), and vascular cell adhesion molecule-1 mRNA expression levels were determined by quantitative real-time polymerase chain reaction using cyclophilin as a housekeeping gene essentially as published.[23,24] Values are expressed as percent reduction achieved by the addition of HDL as compared with the full vascular cell adhesion molecule-1 induction by tumor necrosis factor-α. The intra-assay coefficient of variation of this assay is 7.2%.

All analyses were performed at least in duplicate. For all HDL function assays, we demonstrated previously that the results are identical when comparing fresh with frozen plasma.[24] Further, we demonstrated dependency of the results on the presence of HDL in

apolipoprotein B–depleted plasma; in all 3 assays apolipoprotein B–depleted plasma from which HDL had been additionally removed by ultracentrifugation had no significantly different biological activity from the respective control conditions.[24] In addition, for all HDL function assays, the chosen concentrations are within the linear range of the respective assays.

Study End Points

The relation of HDL functionality with 3 clinical outcomes was investigated: all-cause mortality, unscheduled HF hospitalization, and a composite outcome of all-cause mortality and HF hospitalization. The end points were adjusted for the BIOSTAT-CHF risk models created for each specific outcome in this cohort. The BIOSTAT-CHF risk models included age, log blood urea nitrogen, log NT-proBNP, hemoglobin, and beta-blocker use at baseline for all-cause mortality; age, HF hospitalization in the previous year, peripheral edema, systolic blood pressure, and estimated glomerular filtration rate for HF hospitalization; and age, HF hospitalization in the previous year, systolic blood pressure, log NT-proBNP, hemoglobin, HDL cholesterol, sodium, and beta-blocker use at baseline for the combined end point.[1]

Statistical Analysis

Descriptive statistics were used to examine the baseline characteristics of the study population at baseline and 9 months. Data are presented as means±SD when continuous normally distributed, as medians (interquartile range) when skewed, and as frequencies (percentage) when categorical. Continuous normally distributed variables were tested with the Student independent t-test, skewed variables using the Mann-Whitney U test, and categorical variables using chi-squared tests (Tables S1 and S2). Paired testing was applied to Table 1 using paired t-test, Wilcoxon test for paired samples, or McNemar test for paired samples when variables were continuous normally distributed, skewed, or categorical, respectively. Adjustments for multiple testing were applied using the Bonferroni method. Predictors of HDL functionality were analyzed using univariable and multivariable regression analyses, in which all variables with $P<0.1$ in univariable analysis were included in multivariable analysis and subjected to the backward elimination method. Variables with $P<0.05$ were included in the final multivariable regression model. Before linear regression analysis, the assumption of normal distribution of residuals and linear relationship was checked, as well as checks for outliers. If appropriate, variables were logarithmically transformed (using natural logarithm). Cox proportional hazard models were constructed for all 3 end

Table 1. Baseline Characteristics of the HDL Functionality Cohort at Baseline and After 9 Months*

	Baseline	Change From Baseline to 9 Months	*P* Value
Clinical characteristics			
Age, y	68±12	0.75±0	NA
Sex (male), n (%)	283 (74)		NA
NYHA classification (III/IV), n (%)	199 (53)	−117 (−31)	<0.001
Systolic blood pressure, mm Hg	124±20	−0.06±22.9	0.961
Diastolic blood pressure, mm Hg	75±13	−0.79±15.1	0.308
Heart rate, bpm	81±21	−5±24	<0.001
LVEF, n (%)	30 (25–35)	5 (0 to 11)	<0.001
HFpEF/LVEF >45%, n (%)	6 (3)	18 (10)	<0.001
Diabetes mellitus, n (%)	103 (27)		NA
Smoking (past or current), n (%)	250 (65)		NA
Primary heart failure etiology, n (%)			
Ischemic heart disease	168 (44)		NA
Hypertension	38 (10)		NA
Cardiomyopathy	92 (24)		NA
Valvular disease	28 (7)		NA
Medication, n (%)			
ACE inhibitor/ARB	286 (75)	+52 (+13)	<0.001
≥50% target dose†	160 (42)	+68 (+18)	<0.001
100% target dose†	52 (14)	+55 (+14)	<0.001
Beta blocker	330 (86)	+26 (+13)	<0.001
≥50% target dose†	116 (30)	+50 (+13)	<0.001
100% target dose†	52 (14)	+5 (+1)	<0.001
Loop diuretics	382 (99)	−34 (−8)	<0.001
MRA	206 (54)	+19 (+5)	0.005
Statins (total)	200 (52)	−11 (−3)	0.127
Simvastatin	84 (42)	−2 (+1)	
Atorvastatin	80 (40)	−5 (0)	
Rosuvastatin	19 (10)	−2 (−1)	
Pravastatin	15 (8)	−1 (−1)	
Fluvastatin	2 (0)	−1 (0)	
Laboratory values			
Hemoglobin, g/dL	13.5±1.8	−0.2±1.5	0.078
Sodium, mmol/L	140 (137–142)	0 (−2 to 2)	0.735
Potassium, mmol/L	4.2 (3.9–4.5)	0.1 (−0.2 to 0.5)	<0.001
ASAT, U/L	26 (20–39)	−2 (−8.9 to 2.5)	<0.001
ALAT, U/L	27 (18–45)	−3 (−16 to 2)	<0.001
HDL cholesterol, mmol/L	1.1 (0.9–1.3)	0.04 (−0.08 to 0.2)	0.053
LDL cholesterol, mmol/L	2.5 (2.0–3.2)	0.1 (−0.5 to 0.4)	0.451
Total cholesterol, mmol/L	4.1 (3.4–5.0)	0.1 (−0.3 to 0.7)	0.080
Renal function			
Creatinine, µmol/L	98 (83–122)	4 (−8 to 21)	<0.001

(*Continued*)

Table 1. Continued

	Baseline	Change From Baseline to 9 Months	*P* Value
eGFR, mL/min/1.73 m^2	63±22	−4±16	<0.001
Urea, mmol/L	10.7 (7.3–16.8)	0.2 (–1.9 to 2.7)	0.093
Plasma NGAL, ng/mL	53 (35–81)	31 (5–68)	<0.001
Natriuretic peptides			
NT-proBNP, pg/mL	3676 (2185–7059)	−983 (–3140 to 85)	<0.001
BNP, pg/mL	222 (99–447)	–27 (–199 to 60)	<0.001
ANP, ng/mL	20 (12–39)	5 (–2 to 14)	<0.001
Inflammatory markers			
CRP, µg/mL	12.1 (4.8–24.4)	−6 (–18 to –2)	<0.001
Myeloperoxidase, ng/mL	29 (23–34)	−0.03 (–5 to 5)	0.958
TNFR1A, ng/mL	1.0 (0.5–1.5)	0.2 (–0.2 to 0.5)	<0.001

N=383.

ACE indicates angiotensin-converting enzyme; ANP, atrial natriuretic peptide; ALAT, alanine aminotransferase; ARB, angiotensin receptor blocker; ASAT, aspartate aminotransferase; BNP, B-type natriuretic peptide; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; HFpEF, heart failure with preserved ejection fraction; LDL, low-density lipoprotein; LVEF, left ventricular ejection fraction; MRA, mineralocorticoid receptor antagonist; NGAL, neutrophil gelatinase-associated lipocalin; NT-proBNP, N-terminal pro-B-type natriuretic peptide; NYHA, New York Heart Association; and TNFR1A, tumor necrosis factor receptor 1 alfa.

*Paired differences; patients who died before the 9-month measurement were excluded.

†Based on target guideline-recommended treatment doses.[25]

points and 3 HDL functionality modalities to evaluate the prognostic utility of measures of HDL function and adjusted for their respective BIOSTAT-CHF risk models. The assumption of proportionality of hazards and linearity were checked in all analyses. Results are expressed as hazard ratios with 95% CIs. The spline was constructed using the R packages Greg, rms, and splines. The correlation plot was constructed using the corrplot package. Additionally the R packages car, DescTools, foreign, ggplot2, ggpubr, Hmisc, lm.beta, psych, reshape2, sqldf, survival, and survminer were used in statistical analysis. A 2-tailed *P*-value <0.05 was considered statistically significant. Statistical analyses were performed with SPSS Statistics version 23 (IBM, Armonk, NY) and R version 3.4.3 (R Foundation for Statistical Computing, Vienna, Austria; www.r-project.org).

RESULTS

Study Population Characteristics and HDL Functionality Over Time

Clinical characteristics of the study population at baseline and after 9 months (paired differences; excluding patients who died before the 9-month measurement; n=383) are displayed in Table 1. Compared with baseline, at 9 months patients had less advanced HF (less often New York Heart Association class III/IV, lower NT-proBNP concentrations, and higher left ventricular ejection fraction), worse renal function (lower estimated glomerular filtration rate and a higher degree of tubular damage, as measured by plasma neutrophil gelatinase-associated lipocalin concentrations), and more often used angiotensin-converting enzyme inhibitors and beta blockers (all *P*<0.001). HDL, LDL, and total cholesterol concentrations and statin use did not significantly change. Clinical characteristics of the study population including all patients at baseline (N=446) are shown in Table S3.

Measures of HDL functionality at baseline and after 9 months are displayed in Figure 1. HDL cholesterol efflux had decreased after 9 months compared with baseline (baseline median, 5.8%; 9 months, 4.6%; *P*<0.001). HDL anti-inflammatory capacity had also decreased (baseline median, 17.6% reduction; 9 months, 15.2%; *P*<0.001), as expressed by less reduction of vascular cell adhesion molecule-1 expression by HDL. In contrast, a greater reduction of thiobarbituric acid reactive substances accumulation was observed, reflecting better antioxidative capacity of HDL (baseline median, 49.3% reduction; 9 months, 53.3%; *P*<0.001). Unpaired differences are shown in Figure S1.

Compared with patients who were still alive at 9 months, patients who died before the 9-month follow-up measurement had worse baseline HDL cholesterol efflux (5.3% versus 5.8%; *P*=0.005, after Bonferroni correction *P*=0.015), but had similar antioxidative capacity (51.0% reduction versus 49.3%; *P*=0.439) and anti-inflammatory capacity (17.5% reduction versus 17.6% reduction; *P*=0.614).

There were no differences in HDL functionality and HDL cholesterol at baseline between patients with HF with reduced ejection fraction and HF with preserved ejection fraction (Figure S2), between patients with and without chronic kidney disease, and between patients with and without an ischemic etiology of heart failure (data not shown). Measures of HDL functionality or their changes were also not associated with successful treatment optimization (data not shown). There were also no differences in HDL functionality between patients who used a statin and those who did not after applying Bonferroni correction.

Association Between HDL Functionality Measures and Clinical Variables

Correlation plots showing associations among the 3 measures of HDL functionality are displayed in Figure S3. Baseline HDL cholesterol efflux significantly

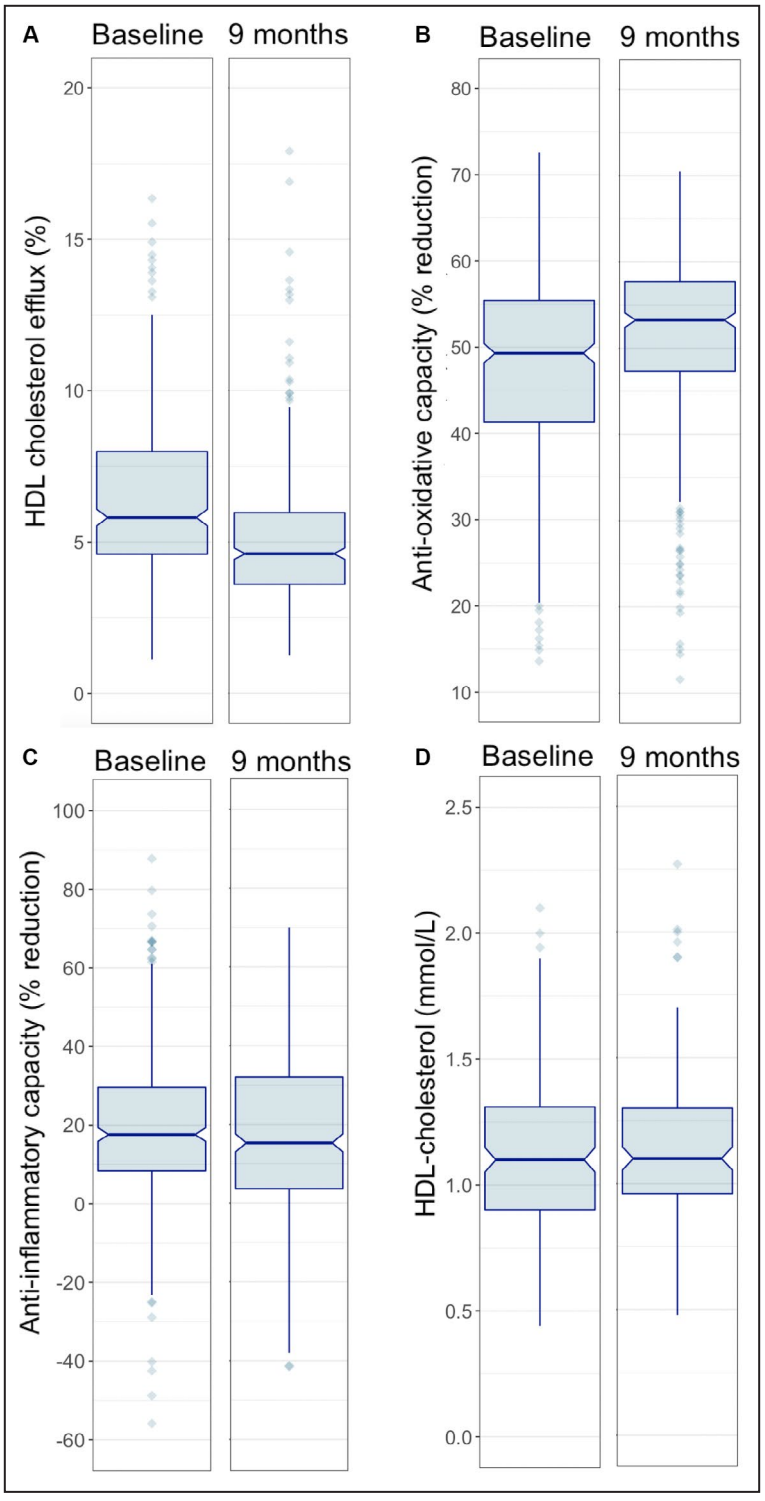

Figure 1. Differences in HDL functionality (relative to control) between baseline and 9 months.

Paired differences, N (patients) = 383. *P* values resulting from the Wilcoxon rank-sum test for paired samples. **A,** HDL cholesterol efflux at baseline (median, 5.8%) and 9 months (median, 4.6%), respectively; *P*<0.001. **B,** Antioxidative capacity at baseline (median, 49.3% reduction) and 9 months (median, 53.3% reduction), respectively; *P*<0.001. **C,** Anti-inflammatory capacity at baseline (median, 17.6% reduction) and 9 months (median, 15.2% reduction), respectively; *P*=0.046. **D,** HDL cholesterol concentration at baseline (median, 1.1 mmol/L) and 9 months (median, 1.1 mmol/L), respectively; *P*=0.053. HDL indicates high-density lipoprotein.

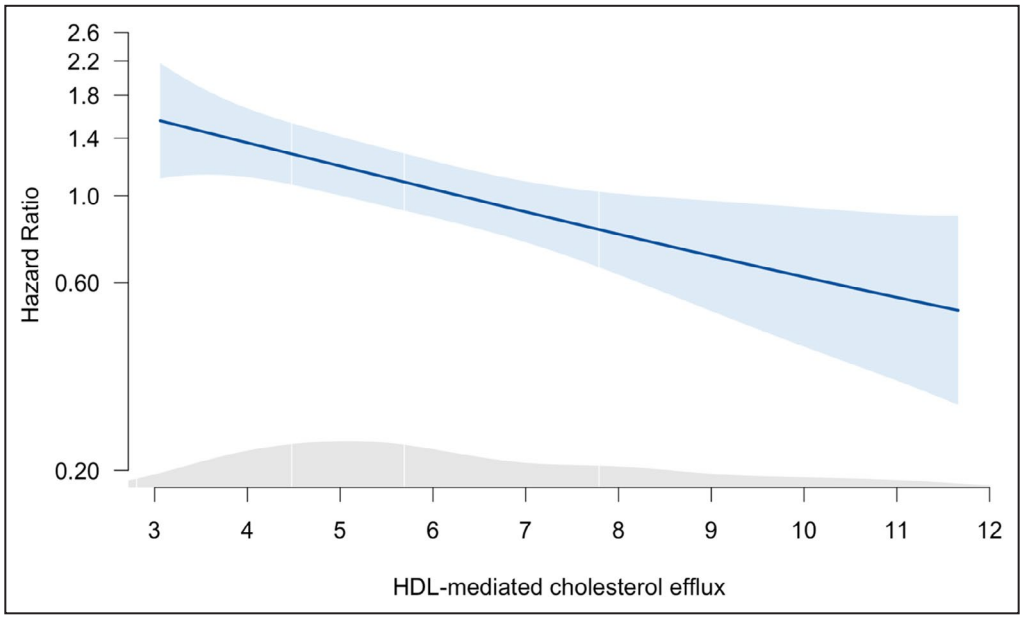

Figure 2. Hazard ratios for mortality on a continuum of HDL cholesterol efflux. *N* (patients) = 446. The thick blue line represents the hazard ratio for mortality on a continuum of HDL cholesterol efflux with its corresponding 95% CI in lighter blue. In gray, the density of the population along the values of HDL cholesterol efflux is represented. The spline was truncated at the lower 5% and upper 5% of HDL cholesterol efflux. HDL indicates high-density lipoprotein.

correlated with both baseline HDL antioxidative capacity and anti-inflammatory capacity (Spearman's rho = –0.188, *P*<0.001; and 0.131, *P*=0.006, respectively). HDL antioxidative-capacity and anti-inflammatory capacity were not correlated with each other.

Tables S4 and S5 show determinants of HDL functionality at baseline and at 9 months and changes of HDL functionality in univariable and multivariable regression analysis, respectively. Change in HDL functionality over time was, with respect to all 3 measures, most strongly determined by their respective baseline HDL functionality.

In regression analysis among statin users, rosuvastatin use was associated with a greater reduction of anti-inflammatory capacity over time compared with other statins (standardized beta = –0.155; t value, –2.071; *P*=0.040). Statin dose was not associated with measures of HDL functionality.

HDL Cholesterol Efflux Is Associated With Mortality

In the current study population, 114 (26%) patients died, 115 (26%) were hospitalized for HF, and 183 (41%) either died or were hospitalized for HF during a median follow-up of 21 months. In Cox regression analysis (Table 2), higher baseline HDL cholesterol efflux was significantly associated with lower risk of all-cause mortality, which remained significant after adjustment for the previously described BIOSTAT-CHF risk model, enriched with age and log HDL cholesterol (hazard ratio, 0.81; 95% CI, 0.71–0.92; *P*=0.001). However, HDL cholesterol efflux was not associated with HF hospitalization and the composite end point. Hazard ratios on a continuum of HDL cholesterol efflux are displayed in Figure 2, demonstrating values on the lower end being associated with higher risk of mortality, and high values being associated with lower risk. HDL antioxidative capacity and anti-inflammatory capacity were not associated with clinical outcome, nor were changes of HDL functionality over time.

Correlations Between HDL Functionality and the HDL Proteome

In the HDL proteome cohort consisting of 86 patients with proteome data at baseline, median HDL cholesterol efflux was 6.6% (5.3–7.9), median HDL antioxidative capacity was 44.0% (32.0–51.8) reduction of TBARs, and median anti-inflammatory capacity was 32.0% (–2.0 to 55.8) reduction of vascular cell adhesion molecule-1 expression by HDL at baseline. Figure 3 shows that several of the HDL proteins that were previously found to have the strongest association with outcome in this cohort were significantly correlated with HDL functionality. This applied particularly to apolipoprotein A1, which was the only protein from this set that was associated with all 3 HDL functionality measures (rho=0.409, *P*<0.001 for HDL cholesterol efflux; rho=–0.326, *P*=0.002 for anti-oxidative capacity; and rho=–0.282, *P*=0.009 for anti-inflammatory capacity). Others mainly correlated

Table 2. Cox Regression of HDL Cholesterol Efflux and Outcome

Variable	Univariable		Corrected for Age + Log HDL Cholesterol		Corrected for Previous + BIOSTAT-CHF Risk Model†	
	HR* (95% CI)	*P* Value	HR* (95% CI)	*P* Value	HR* (95% CI)	*P* Value
Mortality	0.88 (0.81–0.95)	0.002	0.84 (0.74 – 0.95)	0.005	0.81 (0.71–0.92)	0.001
HF hospitalization	0.99 (0.92–1.06)	0.690				
Mortality or HF hospitalization	0.95 (0.89–1.00)	0.068				

All-cause mortality: age, log blood urea nitrogen, log NT-proBNP, hemoglobin, and beta-blocker use at baseline.
HF hospitalization: age, HF hospitalization in previous year, peripheral edema, systolic blood pressure, and eGFR.
Combined endpoint: age, HF hospitalization in previous year, systolic blood pressure, log NT-proBNP, hemoglobin, high-density lipoprotein, sodium, and beta-blocker use at baseline.
BIOSTAT-CHF, A Systems Biology Study to Tailored Treatment in Chronic Heart Failure; HDL, high-density lipoprotein; HF, heart failure; HR, hazard ratio; NT-proBNP, N-terminal pro-B-type natriuretic peptide.
*Reported as per unit increase of HDL cholesterol efflux (%).
†Variables in BIOSTAT-CHF risk score.

with HDL cholesterol efflux (alpha-2-HS-glycoprotein, rho=0.269, *P*=0.012; apolipoprotein C3, rho=0.307, *P*=0.004; kallistatin, rho=0.326, *P*=0.002). HDL antioxidative capacity was associated only with apolipoprotein A1, and anti-inflammatory capacity was additionally associated with kallistatin (rho=−0.325, *P*=0.002).

DISCUSSION

This study shows that HDL cholesterol efflux and anti-inflammatory capacity significantly decreased over time in patients with HF, whereas HDL antioxidative capacity increased. HDL cholesterol efflux was independently associated with risk of all-cause mortality after adjustment for age, HDL cholesterol, and the developed risk model, which includes established prognostic factors in HF, whereas anti-inflammatory and antioxidative capacity were not associated with clinical outcome. Finally, we show that certain proteins in the HDL proteome correlated with HDL functionality, with apolipoprotein A1 emerging as the main protein that was associated with all 3 measures of HDL functionality. To the best of our knowledge, this is the first study to comprehensively investigate multiple measures of HDL functionality with serial measurements in a relatively large number of patients with HF, while furthermore linking HDL functionality to proteome composition.

From Studying HDL Cholesterol Concentrations to Studying HDL Quality in Heart Failure

Recently, low HDL cholesterol was found to be among the strongest predictors of death or HF hospitalization in BIOSTAT-CHF,[1] yet recent randomized controlled trials have failed to show that increasing HDL cholesterol concentrations with niacin or cholesteryl ester transfer protein inhibitors reduces cardiovascular events.[7] Beyond quantities of HDL cholesterol, a static measure, focus has therefore largely shifted to studying quality of HDL, a dynamic measure. These complex particles carry many different proteins (with >80 proteins consistently identified) in varying abundances that are involved in many processes.[26,27] HDL is a heterogenic lipoprotein; its composition and functionality can be altered in various diseases, such as coronary artery disease and chronic kidney disease.[8] In experimental studies, HDL has shown to be cardioprotective through its antioxidative, anti-inflammatory, antiapoptotic, and endothelial-protective effects.[6] Only few smaller studies showed that in patients with HF HDL cholesterol efflux, antioxidative capacity, and anti-inflammatory capacity are reduced compared with controls,[12,14] that HDL antioxidative capacity is a predictor of mortality,[15] and that lower arylesterase activity (being used as a proxy integrating antioxidative and anti-inflammatory effects) was associated with adverse cardiac events.[28,29]

In the present study, including a larger number of patients, we showed that higher HDL cholesterol efflux was independently associated with lower risk of mortality in HF. Since HDL cholesterol efflux was not associated with HF hospitalization, this relationship with mortality might be through non–HF-specific related mechanisms. Such reasoning is supported by the fact that HDL cholesterol efflux is also strongly associated with mortality in myocardial infarction and chronic coronary artery disease in the general population.[23,30–32] However, other measures of HDL functionality were not associated with outcome in our study. HDL is involved with all stages of reverse cholesterol transport with cholesterol efflux representing the first and arguably the most important step, possibly making this a central metric of overall HDL function.[27] Mechanisms of HDL in reducing antioxidative stress and inflammation are furthermore complex and

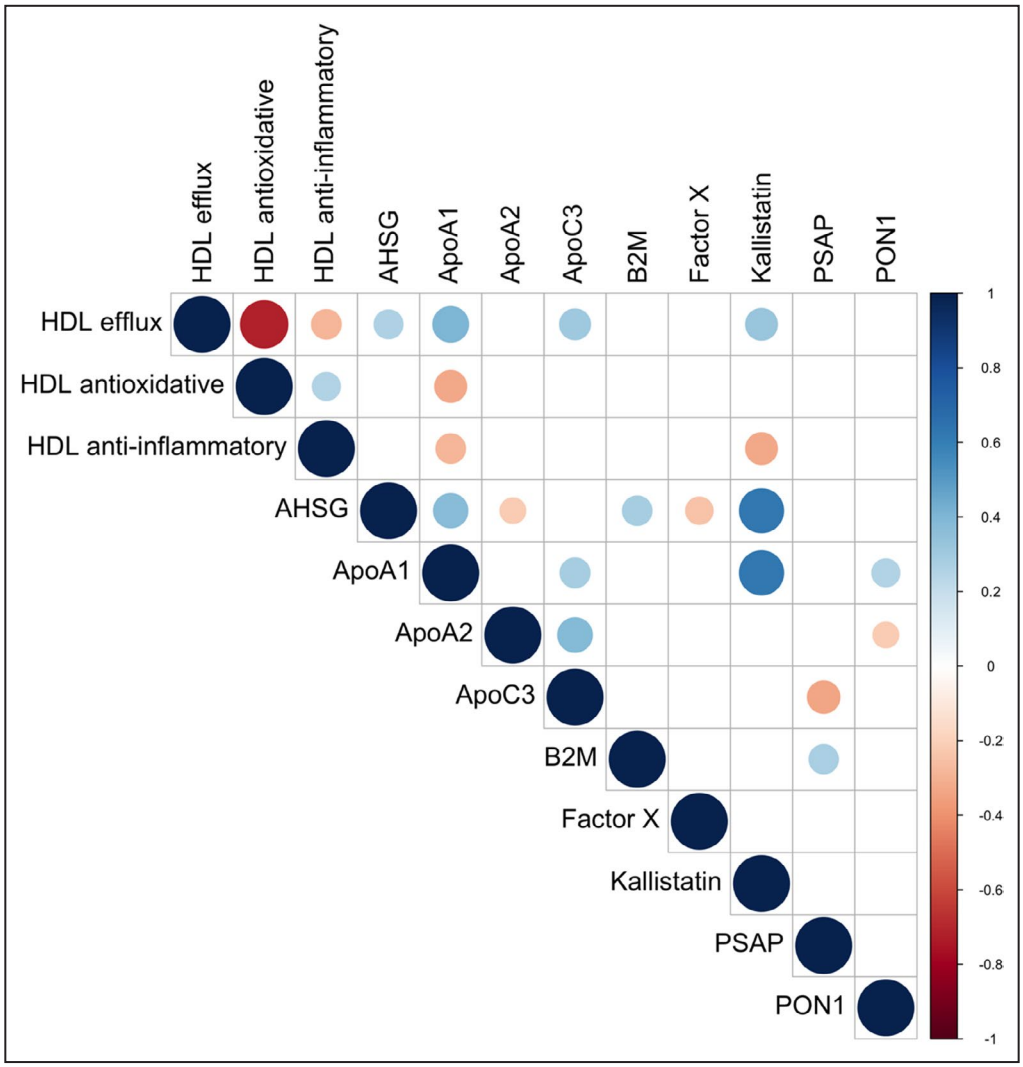

Figure 3. Correlation plot of HDL functionality measures and proteins of the HDL proteome. N (patients) = 86.

Correlation plot where the size and color of the dots represent the magnitude and direction of the association. Dark blue represents a strong positive correlation, whereas dark red represents a strong negative correlation. AHSG, Alpha-2-HS-glycoprotein; ApoA1, apolipoprotein A1; ApoA2, apolipoprotein A2; ApoC3, apolipoprotein C3; B2M, beta-2-microglobulin; Factor X, coagulation factor X; HDL, high-density lipoprotein; PSAP, pulmonary surfactant-associated protein B; PON1, serum paraoxonase/arylesterase 1.

remain incompletely understood. Anti-inflammatory and antioxidative effects have been shown to have a significant impact in inflammatory and autoimmune disorders and are partly directed toward atheroprotection (such as decreasing plaque inflammation)[27]; therefore the magnitude of effects might not be big enough in this mixed HF population. The discrepancy between the present study and previous studies that mostly did show a relationship between those measures and outcome might be found in the use of different methods to assess HDL functionality, the use of paraoxonase-1 arylesterase activity as a surrogate measure for HDL functionality (with inconsistent results[13,28,29]), and different study populations (such as acute/chronic HF, severity of HF, and symptomatic/asymptomatic).

Changes of HDL functionality over time were furthermore not associated with outcome, suggesting that functionality at baseline already determines the prognostic risk with no incremental value from repeated measurements. HDL functionality might develop into a “state of no return” once new-onset or worsening of HF has occurred, even though the general condition and laboratory parameters of these patients improve after treatment. In contrast, antioxidative capacity of HDL increased over time, which is conceivably a counterregulatory effect of HDL in response to systemic oxidative stress. Similar observations have

previously been made, for example, with increased antioxidative responses mounted in smokers.[33] Changes of HDL functionality were very similar when all patients were included at baseline compared with paired differences, confirming that these findings apply regardless of prognosis.

Finally, HDL functionality has been proposed to be affected or important in certain subgroups of interest; clear disturbances have been observed of HDL functionality in patients with chronic kidney disease,[34] and the influence of HDL functionality might be greater in HF with preserved ejection fraction compared with HF with reduced ejection fraction attributable to the effects HDL exerts on cardiovascular comorbidities, such as hypertension and diabetes mellitus.[6] However, we did not observe a difference in HDL functionality between presence and absence of these 2 conditions, keeping in mind the low number of patients with HF with preserved ejection fraction. Furthermore, the influence of statin use and dose on HDL functionality appeared to be little.

The Relationship Between HDL Proteome and Functionality

In an earlier follow-up study with the aim of finding an explanation for the association between low HDL cholesterol and worse outcomes in patients with HF, we showed that HDL proteome composition is markedly different between patients with HF with poor survival compared with those with relatively better survival.[11] In this study, we established 12 proteins that were consistently reported as being part of the HDL proteome and were most strongly associated with outcome, validated using different search techniques.[11] To our knowledge, only 1 study investigated both HDL proteome composition and NO-related activities of HDL as a readout of HDL functionality in a rather limited number of patients with HF but did not provide a direct comparison.[35]

Apolipoprotein A1 seems to be the best reflector of HDL functionality as a whole, with strongest ties to cholesterol efflux. Apolipoprotein A1 is the major structural and functional component of HDL, which is believed to be present in almost all HDL particles, and accounts for ≈70% of total HDL protein content.[36] It is well established that apolipoprotein A1 is involved in promoting efflux (confirmed with a positive association in correlation analysis) and can also act as an antioxidative and anti-inflammatory agent.[27] In line with this, apolipoprotein A1 has also already been shown to be associated with arylesterase activity[29] and adverse outcome in previous HF studies,[4,11] also to a larger extent than LDL cholesterol or HDL cholesterol.[5] However, as mentioned before, antioxidative and anti-inflammatory function are complex processes not solely determined by apolipoprotein A1 but exerted by multiple proteins within the proteome, which might explain the somewhat unexpected finding that apolipoprotein A1 was negatively associated with antioxidative and anti-inflammatory function. An advantage of apolipoprotein A1 is the fact that it is easier to measure than (separate) modalities of HDL functionality, which are thus far still largely reserved for experimental settings, and is therefore more clinically applicable. However, apolipoprotein A1 is also present on chylomicrons and very-low-density lipoproteins, making it not uniquely HDL associated.[36]

Strengths and Limitations

First, we were able to investigate HDL functionality in relation to a large array of different clinical variables and biomarkers in a sizeable subset of this multicenter, multinational, and heterogeneous HF population, which is a strength of our study. The heterogeneous nature of the study population also renders the results more easily applicable to the general HF population. Furthermore, we included 3 measures of HDL functionality with well-established methodology and detailed, high-quality data on HDL proteome composition all in 1 comprehensive study.

However, we were formally not able to show causality because of the retrospective nature of this study. No validation of results in an independent cohort is also a limitation of our study. Some analyses included fewer patients because of missing values. Furthermore, from a technical perspective, we feel that it is important to point out that there is no established gold standard of measuring HDL functionality. This includes the methods for HDL isolation as well as the respective assay conditions. For example, isolating HDL by precipitating apolipoprotein B–containing lipoproteins is feasible for the study of larger cohorts,[12,22,24,30–32] preserves the HDL proteome, and captures pre-beta HDL particles. However, certain plasma components other than HDL remain in these preparations. In contrast, with an ultracentrifugation method, a somewhat purer HDL preparation could be obtained on the expense of losing HDL-associated proteins as well as pre-beta HDL particles because of the centrifugal forces applied and the cutoffs handled. Further, longer centrifugation protocols using higher temperatures can result in additional alterations of HDL particles.[9] Therefore, HDL function assays, although, as in our study, well standardized within the laboratory, are not comparable to the measurement of parameters in routine clinical chemistry laboratories and need to be judged in the context of the HDL isolation method and the assay conditions applied. This complicates comparison between studies from different groups. Finally,

the measurements were performed in a subset of patients, and even though no substantial differences were observed between included and excluded patients, informed censoring cannot be fully ruled out.

Clinical Relevance and Future Perspectives

We have studied HDL functionality to a detailed extent in HF and also directly linked HDL functionality to HDL proteome composition. We found that HDL cholesterol efflux might be a clinically relevant target aspect of HDL functionality for therapeutic intervention. Future studies should confirm whether HDL cholesterol efflux is a causal factor, but evidence so far appears compelling. In future studies, it will also be interesting to address the potential of recombinant HDL, which has been shown to favorably alter HDL metabolism.[7] Additionally, one small study showed that the ability of HDL to stimulate endothelial NO production could be partially restored with exercise training in advanced HF,[35] also representing an interesting way to possibly increase functionality of HDL, along with the many other benefits of exercise training. Finally, in addition to studying HDL content and functionality, focusing on subfractions of HDL particles also appears to be promising, with smaller particles being more favorably associated with outcome and better functionality than less dense particles. This has recently been reported in the largest HF population to date, including both reduced and preserved ejection fraction.[37]

CONCLUSIONS

Better HDL cholesterol efflux at baseline was associated with a lower risk of mortality during follow-up independent of HDL cholesterol. HDL cholesterol efflux and anti-inflammatory capacity declined during follow-up in patients with HF, possibly signifying a "state of no return" for HDL functionality. Combined, these data indicate that measures of HDL function may provide clinical information beyond static HDL cholesterol concentration in patients with HF. Apolipoprotein A1 emerged as an important protein that is associated with all 3 measures of HDL functionality.

ARTICLE INFORMATION

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Supplementary Material

Tables S1–S5
Figures S1–S3

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Supplemental Material

Table S1. Comparison of baseline characteristics between present study cohort and patients not in study subset.

Variable	HDL functionality cohort (N = 446)	Patients without HDL functionality measurements (N = 2,070)	P-value
Clinical variables			
Age (years)	69 ± 12	69 ± 12	0.880
Sex (male), *n* (%)	323 (72)	1523 (74)	0.659
NYHA classification (III/IV), *n* (%)	246 (55)	1276 (62)	**0.015**
Systolic blood pressure (mmHg)	123 ± 21	125 ± 22	**0.020**
Diastolic blood pressure (mmHg)	74 ± 13	75 ± 13	0.062
Heart rate (bpm)	81 ± 21	80 ± 19	0.122
LVEF (%)	30 (24 – 35)	30 (25 – 37)	0.475
HFpEF/LVEF>45%, *n* (%)	31 (7)	131 (6)	0.616
Diabetes mellitus, *n* (%)	125 (28)	694 (34)	**0.028**
Smoking (past or current), *n* (%)	282 (63)	1291 (62)	0.686
Primary heart failure etiology, n (%)			

Ischemic heart disease	194 (43)	932 (45)	0.747
Hypertension	45 (10)	211 (10)	0.877
Cardiomyopathy	104 (23)	527 (25)	0.592
Valvular disease	37 (8)	153 (7)	0.690
Medication, n (%)			
ACE inhibitor/ARB	328 (74)	1492 (72)	0.569
Beta-blocker	373 (84)	1720 (83)	0.836
Loop diuretics	445 (99)	2059 (99)	0.635
MRA	233 (52)	1106 (53)	0.686
Laboratory values			
Hemoglobin (g/dL)	13.3 ± 1.9	13.2 ± 1.9	0.291
Sodium (mmol/L)	140 (137 – 142)	140 (137 – 142)	0.763
Potassium (mmol/L)	4.2 (3.9 – 4.5)	4.2 (3.9 – 4.6)	0.354
ASAT (U/L)	27 (20 – 39)	25 (19 – 34)	**0.025**
ALAT (U/L)	26 (18 – 44)	25 (16 – 37)	**0.043**
HDL cholesterol (mmol/L)	1.1 (0.8 – 1.3)	1.0 (0.8 – 1.3)	0.653
LDL-cholesterol (mmol/L)	2.4 (1.8 – 3.2)	2.4 (1.8 – 3.2)	0.945
Total cholesterol (mmol/L)	4.0 (3.3 – 5.0)	4.1 (3.3 – 5.0)	0.583
Renal function			

Creatinine (µmol/L)	99 (85 – 126)	103 (84 – 132)	0.440
eGFR (mL/min/1.73m^2)	61 ± 23	60 ± 23	0.608
Urea (mmol/L)	11.2 (7.6 – 17.2)	11.4 (7.6 – 18.6)	0.373
Natriuretic peptides			
NT-proBNP (pg/mL)	3915 (2330 – 7391)	4342 (2373 – 8648)	0.256
BNP (pg/mL)	259 (106 – 505)	225 (89 – 465)	0.069
Event rates, n (%)			
Mortality	114 (26)	554 (27)	0.644
- Days to event	646 (487 – 831)	645 (468 – 819)	0.263
HF hospitalization	115 (26)	507 (24)	0.608
- Days to event	564 (275 – 798)	550 (251 – 774)	0.359
Combined endpoint	183 (41)	850 (41)	1
- Days to event	564 (275 – 798)	550 (251 – 774)	0.359

ACE, angiotensin-converting enzyme; ALAT, alanine aminotransferase; ARB, angiotensin receptor blocker; ASAT, aspartate aminotransferase; BNP, brain natriuretic peptide; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; HFpEF, heart failure with preserved ejection fraction; LDL, low-density lipoprotein; LVEF, left ventricular ejection fraction; MRA, mineralocorticoid receptor antagonist; NT-proBNP, N terminal pro brain natriuretic peptide; NYHA, New York Heart Association

Table S2. Baseline characteristics of the HDL proteome cohort including differences between this cohort and the HDL functionality cohort and BIOSTAT-CHF patients not in the proteome cohort

Variable	HDL proteome cohort* (n=86)	HDL functionality cohort (n=446)	P-value†	BIOSTAT-CHF patients not included in HDL proteome cohort (n=2,430)	P-value‡
Clinical variables					
Age (years)	70 ± 9	69 ± 12	0.241	69 ± 12	0.200
Sex (male), *n* (%)	75 (87)	323 (72)	**0.006**	1771 (73)	**0.005**
NYHA classification (III/IV), *n* (%)	30 (35)	246 (55)	**<0.001**	1452 (60)	**<0.001**
Systolic blood pressure (mmHg)	121 ± 23	123 ± 21	0.648	125 ± 22	0.172
Diastolic blood pressure (mmHg)	73 ± 13	74 ± 13	0.541	75 ± 13	0.143

Heart rate (bpm)	78 ± 17	81 ± 21	0.078	80 ± 20	0.200
LVEF (%)	28 (21 – 30)	30 (24 – 35)	**0.004**	30 (25 – 37)	**<0.001**
HFpEF/LVEF>45%, *n* (%)	0 (0)	31 (7)	**0.014**	162 (7)	**0.015**
Diabetes mellitus, *n* (%)	38 (44)	125 (28)	**0.004**	781 (32)	**0.026**
Smoking (past or current), *n* (%)	66 (77)	282 (63)	**0.020**	1507 (62)	**0.010**
Primary heart failure etiology, n (%)					
Ischemic heart disease	86 (100)	194 (43)	**<0.001**	1040 (43)	**<0.001**
Hypertension	0 (0)	45 (10)	**0.036**	255 (10)	**0.020**
Cardiomyopathy	0 (0)	104 (23)	**<0.001**	630 (26)	**<0.001**
Valvular disease	0 (0)	37 (8)	**0.045**	189 (8)	**0.028**
Medication, n (%)					
ACE inhibitor/ARB	59 (69)	328 (74)	0.418	1761 (72)	0.506
Beta-blocker	71 (83)	373 (84)	0.931	2022 (83)	0.990
Loop diuretics	86 (100)	445 (99)	1	2418 (100)	1
MRA	51 (59)	233 (52)	0.279	1288 (53)	0.298
Laboratory values					
Hemoglobin (g/dL)	13.1 ± 1.6	13.3 ± 1.9	**0.013**	13.2 ± 1.9	**0.019**

Sodium (mmol/L)	139 (136 – 141)	140 (137 – 142)	0.083	140 (137 – 142)	0.073
Potassium (mmol/L)	4.2 (4.0 – 4.7)	4.2 (3.9 – 4.5)	0.585	4.2 (3.9 – 4.6)	0.774
ASAT (U/L)	24 (17 – 34)	27 (20 – 39)	**0.035**	25 (19 – 35)	0.134
ALAT (U/L)	22 (15 – 33)	26 (18 – 44)	**0.024**	25 (17 – 38)	0.083
HDL cholesterol (mmol/L)	1.0 (0.7 – 1.3)	1.1 (0.8 – 1.3)	0.121	1.1 (0.9 – 1.3)	0.129
LDL-cholesterol (mmol/L)	2.1 (1.7 – 2.8)	2.4 (1.8 – 3.2)	0.085	2.5 (1.8 – 3.2)	0.060
Total cholesterol (mmol/L)	3.5 (3.1 – 4.4)	4.0 (3.3 – 5.0)	**0.020**	4.1 (3.4 – 5.0)	**0.006**
Renal function					
Creatinine (µmol/L)	115 (100 – 150)	99 (85 – 126)	**<0.001**	102 (83 – 129)	**<0.001**
eGFR (mL/min/1.73m^2)	52 ± 19	61 ± 23	**<0.001**	61 ± 23	**<0.001**
Urea (mmol/L)	19.6 (11.7 – 28.4)	11.2 (7.6 – 17.2)	**<0.001**	11.2 (7.5 – 17.9)	**<0.001**
Natriuretic peptides					

NT-proBNP (pg/mL)	6920 (3447 – 11119)	3915 (2330 – 7391)	**0.022**	4226 (2358 – 8325)	**0.033**
BNP (pg/mL)	423 (207 – 694)	259 (106 – 505)	**<0.001**	224 (92 – 462)	**<0.001**

* Patients of the HDL proteome cohort were selected from BIOSTAT-CHF based on the characteristics that they could be matched with a 1:1 death/survivor ratio of cardiovascular cause within a follow-up period of 12 months. Patients were furthermore matched for important prognostic criteria. Therefore, there are inherent differences between this cohort and the overall BIOSTAT-CHF cohort and the HDL functionality cohort. Detailed methodology on the selection procedure has been published previously.[11]

†Comparison between HDL proteome cohort and HDL functionality cohort

‡Comparison between HDL proteome cohort and BIOSTAT-CHF patients not included in HDL proteome cohort

ACE, angiotensin-converting enzyme; ALAT, alanine aminotransferase; ARB, angiotensin receptor blocker; ASAT, aspartate aminotransferase; BNP, brain natriuretic peptide; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; HFpEF, heart failure with preserved ejection fraction; LDL, low-density lipoprotein; LVEF, left ventricular ejection fraction; MRA, mineralocorticoid receptor antagonist; NT-proBNP, N terminal pro brain natriuretic peptide; NYHA, New York Heart Association

Table S3. Baseline characteristics of the HDL functionality cohort at baseline*

Clinical characteristics	
Age (years)	69 ± 12
Sex (male), *n* (%)	323 (72)
NYHA classification (III/IV), *n* (%)	246 (55)
Systolic blood pressure (mmHg)	123 ± 21
Diastolic blood pressure (mmHg)	74 ± 13
Heart rate (bpm)	81 ± 21
LVEF (%)	30 (24 – 35)
HFpEF/LVEF>45%, *n* (%)	31 (7)
Diabetes mellitus, *n* (%)	125 (28)
Smoking (past or current), *n* (%)	282 (63)
Primary heart failure etiology, n (%)	
Ischemic heart disease	194 (43)
Hypertension	45 (10)
Cardiomyopathy	104 (23)
Valvular disease	37 (8)
Medication, n (%)	
ACE inhibitor/ARB	328 (74)
- ≥50% target dose†	177 (40)
- 100% target dose†	59 (13)
Beta-blocker	373 (84)
- ≥50% target dose†	127 (28)

- 100% target dose†	21 (5)
Loop diuretics	445 (99)
MRA	233 (52)
Statins (total)	229 (51)
- Simvastatin	98 (43)
- Atorvastatin	91 (40)
- Rosuvastatin	21 (9)
- Pravastatin	17 (7)
- Fluvastatin	2 (0)
Laboratory values	
Hemoglobin (g/dL)	13.3 ± 1.9
Sodium (mmol/L)	140 (137 – 142)
Potassium (mmol/L)	4.2 (3.9 – 4.5)
ASAT (U/L)	27 (20 – 39)
ALAT (U/L)	26 (18 – 44)
HDL cholesterol (mmol/L)	1.1 (0.8 – 1.3)
LDL cholesterol (mmol/L)	2.4 (1.8 – 3.2)
Total cholesterol (mmol/L)	4.0 (3.3 – 5.0)
Renal function	
Creatinine (µmol/L)	99 (85 – 126)
eGFR (mL/min/1.73m^2)	61 ± 23
Urea (mmol/L)	11.2 (7.6 – 17.2)
Plasma NGAL (ng/mL)	55 (37 – 89)

Natriuretic peptides	
NT-proBNP (pg/mL)	3915 (2330 – 7391)
BNP (pg/mL)	259 (106 – 505)
ANP (ng/mL)	21 (13 – 31)
Inflammatory markers	
CRP (µg/mL)	13.0 (5.5 – 27.9)
MPO (ng/mL)	28 (23 – 35)
TNFR1A (ng/mL)	1.1 (0.6 – 1.7)

*N=446

†Based on target guideline-recommended treatment doses[37]

ACE, angiotensin-converting enzyme; ANP, atrial natriuretic peptide; ALAT, alanine aminotransferase; ARB, angiotensin receptor blocker; ASAT, aspartate aminotransferase; BNP, brain natriuretic peptide; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; HFpEF, heart failure with preserved ejection fraction; LDL, low-density lipoprotein; LVEF, left ventricular ejection fraction; MPO, myeloperoxidase; MRA, mineralocorticoid receptor antagonist; NGAL, neutrophil gelatinase-associated lipocalin; NT-proBNP, N terminal pro brain natriuretic peptide; NYHA, New York Heart Association; TNFR1A, tumor necrosis factor receptor 1 alfa

Table S4. Univariable regression analysis for determinants of HDL functionality.

	Baseline			9 months			Δ HDL functionality		
Variable	Standardized beta	*T*	P-value	Standardized beta	*T*	P-value	Standardized beta	*T*	P-value
*HDL cholesterol efflux**									
Age	-0.109	-2.305	**0.022**	-0.091	-1.788	0.075	-0.008	-0.153	0.879
Female sex	0.025	0.528	0.598	0.055	1.069	0.286	0.001	0.024	0.981
NYHA class II	-0.432	-2.290	**0.023**	0.038	0.545	0.586	0.568	2.766	**0.006**
NYHA class III	-0.481	-2.522	**0.012**	-0.028	-0.400	0.689	0.540	2.636	**0.009**
NYHA class IV	-0.352	-2.738	**0.006**	0.063	1.190	0.235	0.290	2.168	**0.031**
Systolic blood pressure	0.019	0.397	0.692	0.058	1.137	0.256	0.032	0.626	0.532
Diastolic blood pressure	0.086	1.808	0.071	0.101	1.972	**0.049**	-0.026	-0.511	0.610
Heart rate	-0.089	-1.881	0.061	-0.009	-0.173	0.863	0.034	0.667	0.505

LVEF†	0.054	1.066	0.287	0.049	0.706	0.481	-0.016	-0.286	0.775
Presence of diabetes	0.029	0.605	0.546	-0.061	-1.198	0.232	-0.056	-1.102	0.271
Never smoked	0.016	0.340	0.734	-0.056	-1.090	0.276	-0.063	-1.222	0.222
Ischemic heart disease as primary HF etiology	0.012	0.243	0.808	-0.026	-0.498	0.619	0.003	0.054	0.957
Hypertension as primary HF etiology	0.043	0.908	0.364	-0.117	-2.286	**0.023**	-0.111	-2.159	**0.032**
Cardiomyopathy as primary HF etiology	-0.030	-0.628	0.531	0.148	2.892	**0.004**	0.113	2.201	**0.028**

Valvular disease as primary HF etiology	0.008	0.166	0.868	0.008	0.146	0.884	-0.040	-0.778	0.437
ACE-inhibitor/ARB use	-0.041	-0.866	0.387	0.030	0.582	0.561	0.071	1.399	0.163
Beta blocker use	0.086	1.824	0.069	0.041	0.793	0.428	-0.003	-0.067	0.946
Loop diuretic use	0.022	0.460	0.646	-0.074	-1.456	0.146	-0.005	-0.088	0.930
MRA use	0.053	1.110	0.268	-0.016	-0.320	0.749	-0.013	-0.262	0.793
Statin use	0.056	1.176	0.240	0.092	1.808	0.071	0.006	0.125	0.901
Hemoglobin	0.144	3.024	**0.003**	0.074	1.195	0.233	-0.003	-0.050	0.960
Sodium†	0.099	2.069	**0.039**	0.027	0.462	0.644	-0.007	-0.137	0.891
Potassium†	0.065	1.368	0.176	-0.098	-1.694	**0.091**	0.014	0.269	0.788
ASAT†	-0.076	-1.314	0.190	0.061	0.771	0.442	0.061	0.977	0.330
ALAT†	-0.065	-1.203	0.230	0.087	1.213	0.227	0.063	1.079	0.282
HDL cholesterol†	0.182	2.678	**0.008**	-0.048	-0.563	0.574	-0.067	-0.890	0.375

LDL cholesterol†	0.092	1.302	0.194	0.115	1.284	0.202	0.052	0.664	0.508
Total cholesterol†	0.151	2.459	**0.015**	0.180	2.281	**0.024**	-0.014	-0.213	0.831
Serum creatinine†	-0.074	-1.573	0.116	-0.070	-1.228	0.220	-0.004	-0.072	0.943
eGFR	0.092	1.949	0.052	0.092	1.613	0.108	-0.007	-0.136	0.892
Urea†	-0.086	-1.717	0.087	-0.092	-1.483	0.139	0.060	1.088	0.277
Plasma NGAL†	-0.066	-1.374	0.170	-0.159	-3.060	**0.002**	-0.057	-1.097	0.273
NT-proBNP†	-0.148	-3.044	**0.002**	-0.099	-1.112	0.268	-0.032	-0.613	0.540
BNP†	-0.099	-2.072	**0.039**	-0.133	-2.563	**0.011**	-0.027	-0.511	0.609
ANP†	-0.081	-1.687	0.092	-0.054	-1.034	0.302	0.009	0.174	0.862
CRP†	-0.193	-4.077	**<0.001**	-0.038	-0.717	0.474	0.083	1.611	0.108
MPO†	-0.064	-1.329	0.185	-0.058	-1.109	0.268	-0.058	-1.125	0.261
TNFR1A†	-0.132	-2.767	**0.006**	-0.118	-2.254	**0.025**	0.002	0.040	0.968
Baseline HDL cholesterol efflux				-0.040	-0.777	0.438	-0.743	-21.670	**<0.001**

HDL anti-oxidative capacity									
Age	-0.029	-0.621	0.535	0.045	0.871	0.385	0.056	1.102	0.271
Female sex	0.046	0.964	0.335	0.006	0.124	0.901	-0.021	-0.417	0.677
NYHA class II	0.163	0.859	0.391	0.005	0.071	0.944	-0.317	-1.532	0.126
NYHA class III	0.203	1.060	0.290	0.004	0.054	0.957	-0.331	-1.603	0.110
NYHA class IV	0.070	0.545	0.586	-0.019	-0.360	0.719	-0.179	-1.333	0.183
Systolic blood pressure	-0.061	-1.276	0.203	-0.036	-0.695	0.488	0.021	0.414	0.679
Diastolic blood pressure	-0.020	-0.431	0.667	-0.020	-0.388	0.698	-0.007	-0.141	0.888
Heart rate	0.052	1.086	0.278	-0.070	-1.367	0.172	-0.052	-1.013	0.312
LVEF†	-0.025	-0.485	0.628	-0.065	-0.936	0.350	0.078	1.430	0.154
Presence of diabetes	-0.028	-0.599	0.550	0.005	0.095	0.924	0.026	0.514	0.607
Never smoked	-0.061	-1.289	0.198	-0.004	-0.075	0.940	0.060	1.162	0.246

Ischemic heart disease as primary HF etiology	-0.042	-0.883	0.378	0.045	0.867	0.386	0.061	1.189	0.235
Hypertension as primary HF etiology	0.003	0.055	0.956	-0.042	-0.815	0.416	-0.017	-0.330	0.741
Cardiomyopathy as primary HF etiology	0.015	0.321	0.748	-0.083	-1.613	0.108	-0.062	-1.211	0.227
Valvular disease as primary HF etiology	-0.028	-0.595	0.552	0.025	0.487	0.626	0.025	0.479	0.632
ACE-inhibitor/ARB use	0.027	0.579	0.563	-0.093	-1.822	0.069	-0.115	-2.270	0.024

Beta blocker use	-0.035	-0.742	0.459	-0.123	-2.422	**0.016**	-0.049	-0.957	0.339
Loop diuretic use	-0.076	-1.616	0.107	0.077	1.503	0.134	0.064	1.242	0.215
MRA use	0.015	0.313	0.754	-0.011	-0.224	0.823	-0.016	-0.311	0.756
Statin use	-0.051	-1.068	0.268	0.126	2.477	**0.014**	0.071	1.397	0.163
Hemoglobin	-0.085	-1.764	0.078	0.123	1.976	**0.049**	0.021	0.411	0.681
Sodium†	-0.053	-1.112	0.267	0.034	0.579	0.563	0.060	1.154	0.249
Potassium†	-0.015	-0.323	0.747	-0.100	-1.730	0.085	-0.057	-1.101	0.272
ASAT†	0.040	0.696	0.487	0.136	1.712	0.088	-0.015	-0.237	0.813
ALAT†	0.048	0.883	0.378	0.080	1.106	0.270	-0.042	-0.710	0.479
HDL cholesterol†	0.003	0.050	0.960	-0.014	-0.167	0.867	-0.065	-0.870	0.386
LDL cholesterol†	-0.096	-1.362	0.175	-0.015	-0.168	0.867	0.044	0.560	0.576
Total cholesterol†	-0.097	-1.569	0.118	-0.031	-0.389	0.698	-0.013	-0.199	0.843
Serum creatinine†	0.039	0.827	0.409	0.050	0.874	0.383	0.017	0.334	0.738
eGFR	-0.039	-0.816	0.415	-0.048	-0.839	0.402	-0.024	-0.459	0.646

Urea†	-0.048	-0.955	0.340	-0.031	-0.507	0.612	0.052	0.942	0.347
Plasma NGAL†	0.024	0.496	0.620	0.120	2.295	**0.022**	0.034	0.654	0.513
NT-proBNP†	0.066	1.346	0.179	-0.095	-1.064	0.289	-0.036	-0.687	0.492
BNP†	0.016	0.337	0.736	0.096	1.829	0.068	0.022	0.419	0.676
ANP†	0.005	0.109	0.913	0.104	1.985	**0.048**	0.036	0.700	0.484
CRP†	0.010	0.199	0.843	0.008	0.151	0.880	-0.014	-0.271	0.786
MPO†	-0.008	-0.171	0.864	0.004	0.069	0.945	0.046	0.895	0.371
TNFR1A†	0.053	1.104	0.270	0.097	1.860	0.064	0.019	0.367	0.714
Baseline anti-oxidative capacity				-0.073	-1.423	0.156	-0.756	-22.510	**<0.001**
HDL anti-inflammatory capacity									
Age	0.004	0.082	0.935	-0.066	-1.300	0.194	-0.054	-1.061	0.290
Female sex	0.036	0.762	0.447	-0.033	-0.652	0.515	-0.057	-1.105	0.270
NYHA class II	-0.044	-0.233	0.816	0.079	1.126	0.261	0.066	0.318	0.750
NYHA class III	-0.064	-0.333	0.739	0.100	1.446	0.149	0.146	0.707	0.480

NYHA class IV	-0.061	-0.473	0.636	-0.047	-0.894	0.372	0.038	0.284	0.777
Systolic blood pressure	0.032	0.676	0.500	0.155	3.053	**0.002**	0.041	0.797	0.426
Diastolic blood pressure	0.030	0.632	0.527	0.184	3.626	**<0.001**	-0.061	-1.190	0.235
Heart rate	0.009	0.183	0.855	-0.155	-3.057	**0.002**	-0.063	-1.228	0.220
LVEF†	-0.001	-0.018	0.986	-0.072	-1.039	0.300	-0.019	-0.342	0.732
Presence of diabetes	-0.072	-1.517	0.130	0.061	1.189	0.235	0.100	1.965	0.050
Never smoked	-0.037	-0.774	0.439	-0.070	-1.359	0.175	-0.042	-0.829	0.408
Ischemic heart disease as primary HF etiology	0.010	0.208	0.835	0.048	0.934	0.351	0.046	0.898	0.370

Hypertension as primary HF etiology	-0.009	-0.183	0.855	-0.104	-2.032	0.043	-0.091	-1.772	0.077
Cardiomyopathy as primary HF etiology	-0.052	-1.083	0.279	0.052	1.009	0.314	0.066	1.290	0.198
Valvular disease as primary HF etiology	-0.020	-0.412	0.681	-0.000	-0.008	0.994	0.023	0.436	0.663
ACE-inhibitor/ARB use	-0.044	-0.926	0.355	-0.031	-0.598	0.550	0.060	1.170	0.243
Beta blocker use	-0.005	-0.106	0.916	-0.027	-0.531	0.596	0.016	0.317	0.751
Loop diuretic use	0.003	0.059	0.953	-0.052	-1.019	0.309	0.001	0.014	0.989
MRA use	0.069	1.456	0.146	-0.018	-0.346	0.730	-0.023	-0.448	0.654
Statin use	-0.039	-0.823	0.411	0.038	0.744	0.458	0.092	1.805	0.072

Hemoglobin	0.021	0.444	0.657	0.048	0.768	0.443	-0.014	-0.271	0.786
Sodium†	-0.004	-0.076	0.940	-0.012	-0.204	0.839	-0.028	-0.532	0.595
Potassium†	0.039	0.821	0.412	-0.001	-0.010	0.992	0.061	1.185	0.237
ASAT†	0.030	0.523	0.601	0.040	0.501	0.617	0.013	0.210	0.834
ALAT†	0.009	0.167	0.868	0.102	1.416	0.159	0.028	0.474	0.636
HDL cholesterol†	-0.071	-1.038	0.300	-0.093	-1.089	0.278	-0.070	-0.932	0.353
LDL cholesterol†	-0.018	-0.253	0.800	-0.105	-1.170	0.244	-0.027	-0.341	0.734
Total cholesterol†	-0.038	-0.616	0.538	-0.115	-1.452	0.149	-0.051	-0.766	0.445
Serum creatinine†	-0.032	-0.668	0.505	0.054	0.954	0.341	0.005	0.097	0.923
eGFR	0.025	0.534	0.593	-0.028	-0.482	0.630	0.019	0.371	0.711
Urea†	-0.009	-0.185	0.853	0.006	0.090	0.928	0.044	0.811	0.418
Plasma NGAL†	0.055	1.134	0.258	0.068	1.298	0.195	-0.016	-0.304	0.761
NT-proBNP†	-0.005	-0.096	0.924	0.045	0.499	0.619	-0.005	-0.009	0.992
BNP†	0.001	0.022	0.982	0.031	0.593	0.554	0.051	0.987	0.324

ANP†	-0.022	-0.457	0.648	-0.042	-0.795	0.427	-0.005	-0.090	0.929
CRP†	0.066	1.377	0.169	0.004	0.084	0.933	-0.067	-1.287	0.199
MPO†	-0.001	-0.027	0.979	0.031	0.594	0.553	0.008	0.147	0.883
TNFR1A†	-0.002	-0.043	0.966	-0.019	-0.355	0.723	-0.006	-0.124	0.902
Baseline anti-inflammatory capacity				0.019	0.375	0.708	-0.524	-12.010	**<0.001**

Cells in grey indicate that none of the available variables were determinants.

*Log-transformed for baseline and 9 months.

†Log transformed

ACE, angiotensin-converting enzyme; ANP, atrial natriuretic peptide; ASAT, aspartate aminotransferase; BNP, brain natriuretic peptide; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; HF, heart failure; NGAL, neutrophil gelatinase-associated lipocalin; NT-proBNP, N terminal pro brain natriuretic peptide; NYHA, New York Heart Association; TNFR1A, tumor necrosis factor receptor 1 alfa

Table S5. Multivariable linear regression analysis for determinants of HDL functionality

	Baseline			9 months			Δ HDL functionality		
Variable	Standardized beta	*T*	P-value	Standardized beta	*T*	P-value	Standardized beta	*T*	P-value
*HDL cholesterol efflux**									
Age	-0.211	-3.147	**0.002**						
HDL cholesterol†	0.207	3.089	**0.003**						
Total cholesterol†				0.178	2.286	**0.024**			
Hypertension as prim. HF etiology				-0.197	-2.539	**0.012**	-0.080	-2.338	**0.020**
Baseline cholesterol efflux†							-0.746	-21.802	**<0.001**
NYHA class II							0.212	3.729	**<0.001**
NYHA class III							0.145	2.557	**0.011**

HDL anti-oxidative capacity‡									
Beta blocker use				-0.141	-2.278	**0.024**			
Hemoglobin				0.137	2.210	**0.028**			
Baseline anti-oxidative capacity							-0.751	-22.448	**<0.001**
ACE-inhibitor use							-0.071	-2.122	**0.035**
HDL anti-inflammatory capacity§									
Heart rate†				-0.143	-2.805	**0.005**			
Systolic blood pressure				0.129	2.083	**0.038**			
Diastolic blood pressure				-0.126	-2.047	**0.041**			
Baseline anti-inflammatory capacity							-0.524	-11.956	**<0.001**

Hypertension as prim. HF etiology							-0.089	-2.032	0.043

Cells in grey indicate that none of the available variables were determinants.

*Log-transformed for baseline and 9 months. Adjusted R^2 models: baseline efflux, 0.068; 9 months, 0.059; delta: 0.574.

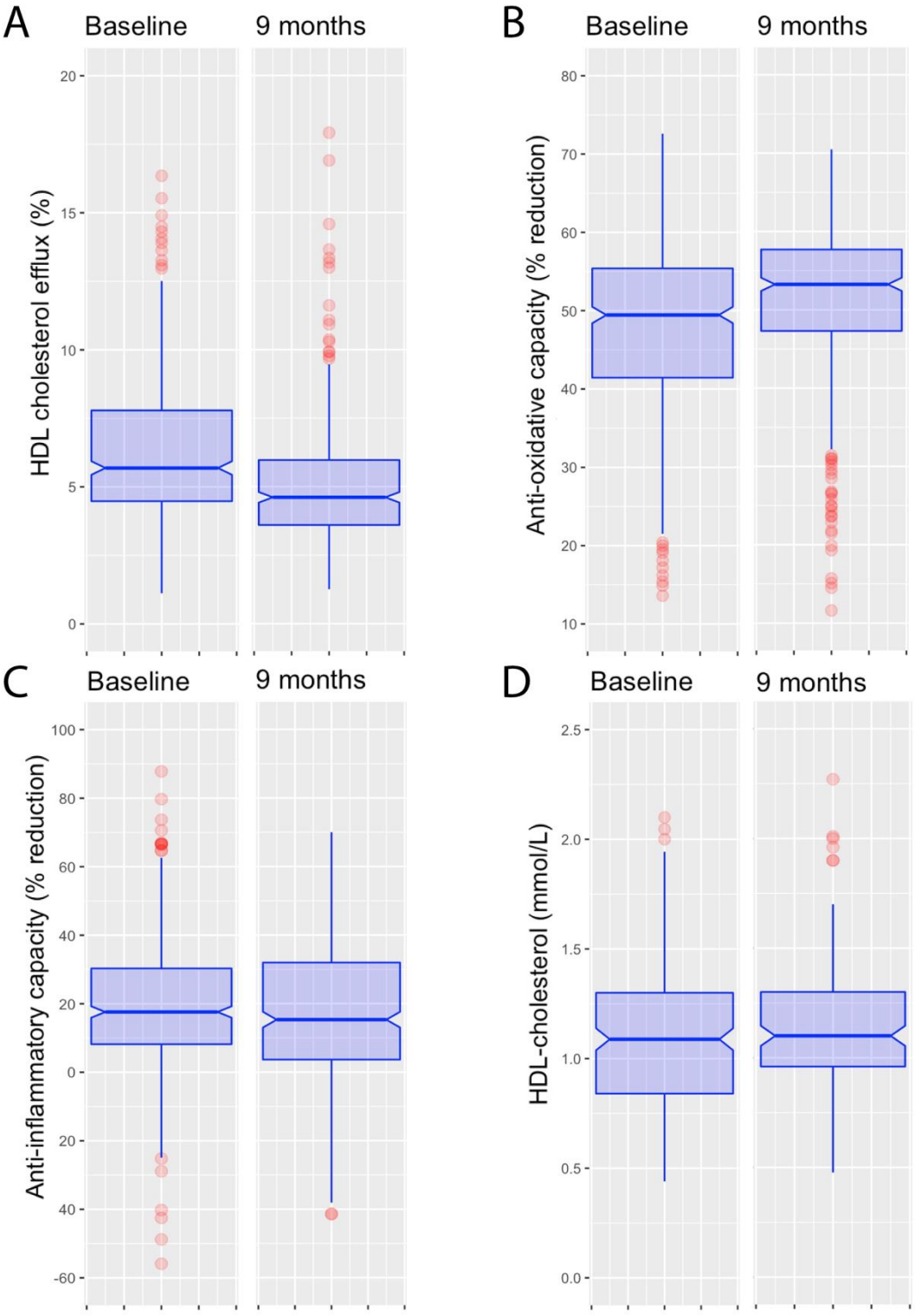
†Log transformed

‡Adjusted R^2 models: anti-oxidative capacity 9 months: 0.027; delta: 0.574.

§Adjusted R^2 models: anti-inflammatory capacity 9 months: 0.029; delta: 0.279.

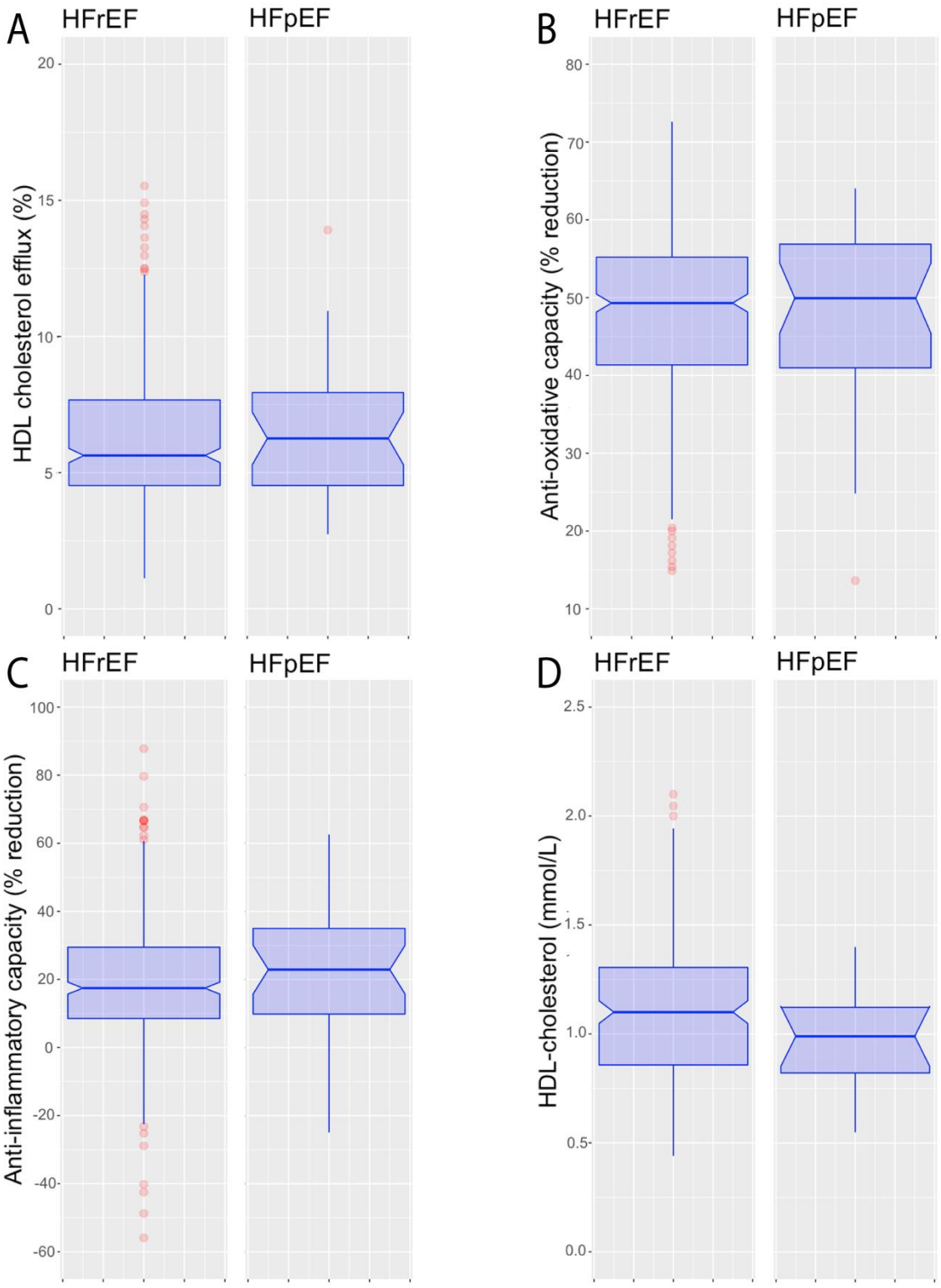
ACE, angiotensin-converting enzyme; HDL, high-density lipoprotein; HF, heart failure; NYHA, New York Heart Association

Figure S1. Differences in HDL functionality (relative to control) between baseline and 9 months (unpaired).



N (patients) = 446 at baseline, *N* (patients) = 383 at 9 months. P-values resulting from the Wilcoxon Rank Sum test for paired samples. A) HDL cholesterol efflux, P<0.001 B) Anti-oxidative capacity, P<0.001 C) Anti-inflammatory capacity, P=0.046 D) HDL cholesterol concentration, P=0.040.

Figure S2. Differences in HDL functionality at baseline between heart failure with preserved ejection fraction and heart failure with reduced ejection fraction.



A) HDL cholesterol efflux. Number of patients: 359 HFrEF, 31 HFpEF, P = 0.773 B) Anti-oxidative capacity. Number of patients: 359 HFrEF, 31 HFpEF, P = 0.683 C) Anti-inflammatory capacity. Number of patients: 359 HFrEF, 31 HFpEF, P = 0.431 D) HDL cholesterol concentration. Number of patients: 183 HFrEF, 12 HFpEF, P = 0.215

Figure S3. Correlation plots showing associations among HDL functionality measures.