GeroScience
https://doi.org/10.1007/s11357-026-02311-x

ORIGINAL ARTICLE

Plasma metabolomic and inflammatory profiles associated with low physical function in heart failure: an integrated cardiac–metabolic–muscular phenotype

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Received: 2 February 2026 / Accepted: 6 May 2026

Abstract Dysregulated metabolomic profiles (dysmetabolism) have been observed in patients with heart failure (HF). In this study, we investigated metabolomic profiling differences in HF with reduced physical-functional capacity, hereafter termed reduced "muscle health" for brevity (HF-RM) compared to preserved muscle health (HF-PM) and healthy adults (NonHF). The HF-RM label denotes a screening-level functional phenotype consistent with EWGSOP2 "probable sarcopenia" and Fried physical-frailty constructs and is not intended to imply primary skeletal myopathy independent of cardiac limitation. Exploratory objectives included compared HFrEF and HFpEF subtypes to detangle muscle and metabolomic phenotype from cardiac markers. Twenty-five patients with HF (67.9 ± 10.0 years) and 29 NonHF (67.8 ± 11.1 years) underwent body composition, muscle strength, and lifestyle habits assessments. Reduced muscle health was defined based on low physical activity and sex-specific cut-offs for handgrip strength (HGS) and/or 30-s chair stand test (30CST). Energy and inflammatory metabolites were assessed via untargeted plasma metabolomic profiling. Statistical analyses were conducted using SPSS and MetaboAnalyst. Agnostic principal component analysis revealed elevated branched-chain amino acids (BCAA) and reduced glutamine, methionine and tryptophan in HF-RM vs. NonHF-PM controls ($p < 0.05$). Compared to HF-PM, HF-RM had lower galacturonic acid-1-phosphate,

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11357-026-02311-x.

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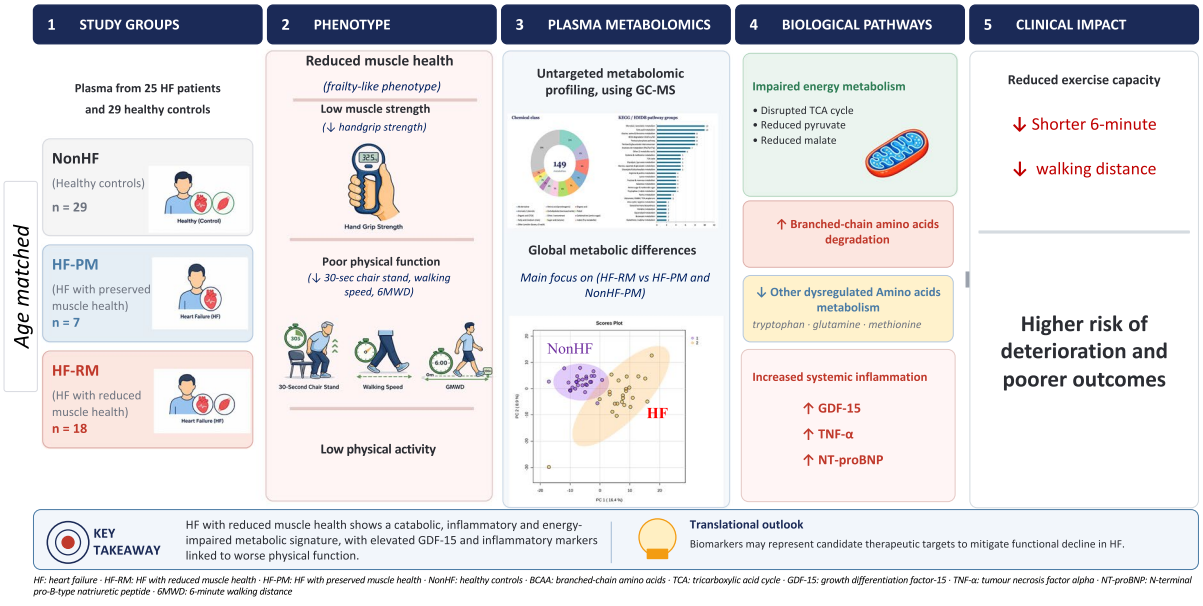
Published online: 25 May 2026

methionine, indole-3-propionic acid, and pyruvic and malic acid ($p < 0.05$). Significant negative correlations were found between N-terminal pro-B-type natriuretic peptide, tumour necrosis factor-alpha, GDF-15, HGS/BMI, appendicular lean soft tissue index (ALSTI)/BMI, and 30CST ($p < 0.05$). Among HF, higher HGS/body mass index (BMI) was linked to longer 6MWD ($\beta = 0.677$, $p < 0.001$) and shorter walking speed ($\beta = -0.532$, $p = 0.006$). HF-RM compared to NonHF-PM had significantly elevated growth differentiation factor-15 (GDF-15) levels (median 1202 vs. 344 pg/ml, $p < 0.01$). HF-RM is marked by impaired energy and fatty acid metabolism, and elevated BCAA catabolism, inflammation, and GDF-15. These biomarkers may represent candidate therapeutic targets to mitigate functional decline in HF, although the present cross-sectional design precludes causal inference. Also, HGS/BMI shows promise as a body-load-normalised functional indicator in HF; given the absence of group differences in absolute strength and the predominantly sarcopenic-obesity phenotype of this cohort, this exploratory finding requires confirmation in larger and HFrEF/HFpEF-balanced studies.

Graphical abstract



HF: heart failure · HF-RM: HF with reduced muscle health · HF-PM: HF with preserved muscle health · NonHF: healthy controls · BCAA: branched-chain amino acids · TCA: tricarboxylic acid cycle · GDF-15: growth differentiation factor-15 · TNF-α: tumour necrosis factor alpha · NT-proBNP: N-terminal pro-B-type natriuretic peptide · 6MWD: 6-minute walking distance

Keywords Heart failure · Muscle weakness · Metabolomics · Energy metabolism · Sarcopenia

Introduction

Heart failure (HF) is a progressive clinical cardiometabolic syndrome, imposing a significant burden on healthcare systems, particularly among ageing populations. Muscle weakness, physical frailty, and sarcopenia are increasingly recognised as highly common non-cardiac comorbidities in HF. Physical frailty, characterised by impaired muscle health (reduced strength and endurance), affects both HF with reduced ejection fraction (HFrEF) and HF with preserved ejection fraction (HFpEF), and significantly reduces quality of life, exercise tolerance, and may increase mortality risk. Low muscle strength is linked to increased severity of HF, as indicated by higher levels of B-type natriuretic peptide (BNP) and its N-terminal fragment (NT-proBNP).

Metabolomics coupled with biochemical blood biomarkers are promising tools to explore the molecular underpinnings of diseased states, including HF.

Previous studies employing metabolomics have shown alterations in energy, amino acid, and lipid metabolism in HF, reflecting the systemic metabolic perturbations driven by cardiac dysfunction. However, knowledge on clinical factors contributing to these metabolic dysregulations is less known. Recently, there has been increasing emphasis on a comprehensive approach that may integrate plasma biomarkers, metabolomic profiling, and clinical data to better understand and disentangle the common mechanisms underlying skeletal muscle weakness in HF.

In this context, clinical biomarkers including transforming growth factor (TGF)-β, and a group of pleiotropic cytokines, particularly activins and growth differentiation factors (GDF), such as GDF-15 and myostatin (also known as GDF-8), have been implicated in various pathological conditions including cardiovascular disease and muscle weakness. In addition, factors such as follistatin-3 antagonists that bind activins/myostatin and prevent them from receptor signalling may possess a key role in energy metabolism. In this study, we provide novel insights into the link between reduced muscle health and various clinical biomarkers, coupled with plasma metabolomics to provide insights into marked metabolic alterations underpinning in HF with reduced (HF-RM) vs. preserved (HF-PM) muscle health.

Methods

Study population

Community-living people with chronic HF were recruited by reviewing the medical records from Aintree Hospital at the Liverpool Hospital Foundation Trust from November 2023 until August 2024. All participants with HF have been diagnosed by cardiologists according to the European Society of Cardiology for HF NICE [10] and ESC 2021 guidelines. This included the NT-proBNP threshold, which was adapted from the National Institute for Health and Care Excellence, and echo ejection fraction estimation using the European Society of Cardiology HF guidelines. Detailed study protocol is available in the Supplementary Material, and current methods highlight the key aspects.

Details of inclusion and exclusion criteria are available. Patients were included if they (i) were age > 50 years old, (ii) were on optimal medical therapy for at least 3 months before study inclusion, and (iii) were able to provide consent and walk a minimum of 10 m, with or without a walking aid. NonHF adults (recruited from Liverpool, UK) were eligible if they were under control for hypertension or hypercholesterolemia without signs or history of chronic conditions and had a body mass index (BMI) between 18 and < 30 kg/m^2.

This study received sponsorship from the University of Liverpool and ethical approval from the London-Queen Square Research Ethics Committee (REC Reference: 23/PR/0050) and has been registered at Clinicaltrials.gov UoL001725. All participants provided written informed consent at enrolment.

Assessment of body composition, muscle strength, and physical function

The descriptive protocol is available in Supplementary Materials. Dual X-ray absorptiometry (GE Lunar Prodigy) was used to assess body composition. Appendicular lean soft tissue (ALST) was calculated by summing lean mass from arms and legs, excluding fat and bone. Muscle strength was measured through handgrip strength (HGS) using a hand dynamometer (JAMAR hydraulic dynamometer), while knee extension strength was assessed by knee extension (Dynamo by VALD). The highest value of maximum three attempts was selected from the participants' dominant arm/leg. Physical function was assessed via 10-m walking speed, 3-m timed-up-and-go testing (TUG), 6-min walking distance (6MWD), and 30-s chair stand test (30CST).

Physical activity assessment

Physical activity was assessed using the IPAQ short form and quantified continuously and categorically. Continuous activity was expressed as metabolic equivalent (MET)–minutes per week, calculated by multiplying minutes per day, days per week, and domain-specific MET values (walking 3.3, moderate 4.0, vigorous 8.0). Categorical classification included three levels. Low indicated no activity or insufficient

activity. Moderate included vigorous activity on ≥3 days for ≥20 min/day, moderate activity or walking on ≥5 days for ≥30 min/day, or any combination on ≥5 days achieving ≥600 MET-minutes/week. High included vigorous activity on ≥3 days totalling ≥1500 MET-minutes/week, or ≥7 days achieving ≥3000 MET-minutes/week.

Muscle phenotype

To allocate participants to reduced compared to preserved muscle health, we adapted Fried's criteria of frailty, regarding low physical activity (IPAQ < 2). Furthermore, low HGS (men < 27 kg and women < 16 kg) and/or low 30CST (< 11 repetitions) were utilised based on the European Working Group on Sarcopenia in Older People (EWGSOP2) criteria. We acknowledge that, in HF, performance-based functional tests (30CST, 6MWD, gait speed) are influenced by cardiac output reserve as well as by intrinsic skeletal muscle properties; the HF-RM label is therefore best interpreted as marking an integrated cardiac–metabolic–muscular functional phenotype rather than a pure myopathic state. Confirmatory sarcopenia diagnosis additionally requires low muscle quantity (ALSTI) per EWGSOP2; we report ALSTI/BMI and HGS/BMI as body-load-normalised indices for completeness, while noting that absolute appendicular lean mass is preserved or elevated in our HF cohort. As a sensitivity analysis, we re-classified HF participants using a stricter criterion (low IPAQ + low HGS, omitting 30CST), with results reported in Supplementary Table S5.

Gas chromatography–mass spectrometry analysis

Blood samples were collected in the morning after an overnight fast. Samples were centrifuged at 2000*g* for 15–20 min at 4 °C, aliquoted into two 2-mL microcentrifuge tubes, and stored at −80 °C. Samples were then transferred to the Liverpool University Biobank Facility and were processed at the University of Liverpool for translational analysis. GC–MS analysis was completed at the Centre for Metabolomics Research, University of Liverpool. Plasma samples were deproteinised in a single batch by adding 300 µL of ice-cold acetonitrile/methanol (1:1) to 100 µL of homogenised plasma spiked with deuterated internal standards, vortexed for 15 s, and centrifuged at 30,130*g* for 15 min. Supernatant was transferred into microcentrifuge tubes (Eppendorf, Stevenage, UK) and lyophilised for 16 h in a vacuum centrifuge (Savant SpeedVac SPD130DLX, Savant vapor trap RVT5105, (Fisher Scientific, Loughborough, UK). Pooled quality control (QC) and process blanks were prepared in tandem. Lyophilised samples, QCs, and blanks were derivatised in a two-step process, randomised and analysed via autosampler injection with GC–MS. An Agilent 7250 GC-ToF–MS equipped with an HP-5 ms (length 30 m, inner diameter 0.25 mm, film thickness 0.25 µm) capillary column (Agilent, Cheadle, UK) was utilised, with an oven temperature gradient of 70 °C, 4 min hold, 20 °C/min to 300 °C, 4 min hold, and 300 °C transfer line temperature. MassHunter Acquisition software was set to automatically recalibrate the mass spectrometer following every four sample injections. Data were processed through spectral deconvolution, alignment using QC data, blank subtraction, and retention index assignment. Peak annotation was performed using an in-house spectral library concatenated with purchased external libraries for untargeted discovery. Signal correction and quality assessment across the batch runs were carried out using pooled QC samples. The full experimental protocol is available in detail in Supplementary Data 1.

Metabolomic analysis

The imputed data were log-transformed for normalisation before PCA agnostic clustering. We performed PCA in MetaboAnalyst 6.0 and examined the loading plots. The top three principal components were identified as the most significant contributors. Hierarchical clustering was performed based on correlation of metabolites and displayed heatmaps using MetaboAnalyst 6.0. Plasma metabolites among the groups were compared using the peak intensity data (after log transformation and normalisation), with independent *t*-test adjusted for the Benjamini–Hochberg method for pairwise comparison and ANOVA for the Fisher's least significant difference (LSD) for three-way comparisons**.** Mummichog pathway activity functional analysis was performed using Mummichog functional analysis in MetaboAnalyst 6.0, which infers pathway-level enrichment directly from high-resolution mass spectrometry features without prior metabolite identification. Significant features were mapped to KEGG

pathways, and pathway significance was assessed using permutation-based testing with mass accuracy–constrained metabolite matching.

Statistical analysis of clinical data

Demographic, clinical, laboratory, and body composition and physical function data were compared across four groups: HF-PM, HF-RM, NonHF-PM, and NonHF-RM. Normality testing was employed through a Shapiro–Wilk test. Distributions across groups were compared with the Kruskal–Wallis test, followed by the Mann–Whitney *U* tests, and independent *t*-tests were used for continuous variables during the assessment of outcomes in the two groups. A chi-squared test was used for categorical variables. Least significant difference (LSD) test was applied for multiple comparisons of biomarkers, and exact *P* values were reported to show statistical significance for each pairwise comparison. Statistical significance was considered $p < 0.05$, using IBM SPSS version 31. Pearson correlations were conducted between clinical biomarkers and physical function metrics, including HGS indexed to BMI (HGS/BMI), ALSTI indexed to BMI (ALSTI/BMI), total fat mass, and 30CST. Biomarkers measured included C-reactive protein (CRP), interleukin-6 (IL-6), NT-proBNP, tumour necrosis factor-alpha (TNF-α), activin A, GDF-15, myostatin, follistatin-3, and activin A to follistatin ratio. Significance was assessed at $p < 0.05$ and 0.01 levels (2-tailed).

Biomarker assays

This study employed enzyme-linked immunosorbent assay (ELISA) to quantify GDF-15, myostatin, activin A, follistatin-3, TNF-α [Thermo Fisher Scientific, Cat. No. KAC1751], IL-6 [Thermo Fisher Scientific, Cat. No. EH2IL6], CRP [Thermo Fisher Scientific, Cat. No. KHA0031], glucose [Thermo Fisher Scientific, Cat. No. EIAGLUC], insulin [Thermo Fisher Scientific, Cat. No. BMS2003], and NT-proBNP [Elabscience, Cat. No. E-EL-H6126] using commercially available human ELISA kits. Plasma samples were thawed on ice and diluted according to manufacturer guidelines. Standards and samples were plated in duplicate on 96-well microplates. Following incubation with biotin conjugate, horseradish peroxidase–conjugated streptavidin, and tetramethylbenzidine substrate, absorbance was measured at 450 nm (IL-6 at 450 and 540 nm). Biomarker concentrations were determined using standard curves. Data availability is shown in Supplementary Materials Appendix Table A3. Insulin resistance was estimated using the homeostasis model assessment of insulin resistance (HOMA-IR), calculated from fasting plasma glucose and fasting serum insulin after an overnight fast: fasting insulin (μU/mL) × fasting glucose (mmol/L)/22.5. Glucose values measured in mg/dL were converted to mmol/L prior to calculation.

Results

Participant characteristics are presented in Tables 1 and 2. In HF-PM and HF-RM, sex distribution did not differ significantly (7 [5 men/2 women] vs. 18 [14 men/4 women], $p = 0.56$). Mean age was similar between groups (HF-PM 68.4 ± 10.7 years; HF-RM 67.8 ± 10.1 years) ($p = 0.89$). BMI was also comparable between the two HF subgroups (HF-PM 31.3 ± 6.3 kg/m^2 vs. HF-RM 30.4 ± 7.0 kg/m^2, $p = 0.628$). HF-RM had the significantly lower ALSTI/BMI among groups ($p = 0.03$), and the highest plasma concentrations of GDF-15, NT-proBNP, TNF-α, and HOMA-IR, and lower concentration for activin A (Fig. 1). All results are presented in the Supplemental Tables 1–10.

Plasma metabolomic functional analysis

A total of 149 unique metabolites and 296 unidentified features were detected, main classes shown in Fig. 2A. A PCA scores plot displayed clear separation of HF and NonHF samples according to the PC1 axis, with a total explained variance (TEV) of 16.4% (Fig. 2B, C). Mummichog pathway enrichment analysis comparing HF with NonHF controls identified several metabolic pathways with the strongest perturbations among the top 10% of significant features (Fig. 2D, Supplementary Table S1). The most enriched pathways were β-alanine metabolism, branched-chain amino-acid degradation, C21-steroid hormone biosynthesis, and vitamin A metabolism, each showing multiple significant hits and the lowest gamma-adjusted probabilities. Additional pathways related to urea cycle/amino-group metabolism

Table 1 Baseline Demographic and clinical characteristics of all participants according to muscle phenotype

Variable	NonHF-PM	NonHF-RM	HF-PM	HF-RM	*p* (overall, K-W)	*p* (HF-RM vs NonHF-PM)	*p* (HF-RM vs HF-PM)	*p* (HF-PM vs NonHF-PM)	*p* (NonHF-RM vs NonHF-PM)
Sample, *n* (men/women)	21 (11/10)	8 (3/5)	7 (5/2)	18 (14/4)	0.174	-	-	-	-
Age (years)	68.4 (12.3)	66.5 (8.0)	68.4 (10.7)	67.8 (10.1)	0.961	0.789	0.832	0.936	0.751
HFpEF/HFrEF	-	-	3/4	4/14	-	-	-	-	-
Systolic BP (mmHg)	133.1 (13.5)	122.5 (7.3)	131.7 (13.7)	120.1 (18.5)	**0.048**	**0.024**	0.146	0.560	**0.039**
Sedentary behaviour (min/d)	309.4 (144.8)	377.1 (102.3)	285.0 (158.5)	416.0 (151.2)	0.102	0.075	0.065	0.448	0.164
Moderate physical activity (min/week)	60.7 (36.5)	40.0 (36.3)	94.3 (127.8)	17.2 (42.4)	**0.003**	**< 0.001**	**0.046**	0.878	0.339
Vigorous physical activity (min/week)	37.2 (25.4)	41.9 (81.9)	42.9 (113.4)	1.1 (4.7)	**< 0.001**	**< 0.001**	0.479	0.059	0.169
Metabolic equivalent of task score	3489.8 (1852.3)	1310.5 (471.0)	4119.5 (2486.3)	718.5 (683.1)	**< 0.001**	**< 0.001**	**0.001**	0.721	**0.004**
Mini Nutritional Assessment (MNA) score	13.3 (1.2)	12.4 (2.1)	13.0 (1.0)	10.6 (2.3)	**< 0.001**	**< 0.001**	**0.009**	0.373	0.428
SARC-F	0.3 (0.6)	1.0 (1.4)	1.7 (1.7)	2.9 (3.5)	**0.031**	**0.009**	0.852	**0.018**	0.180
SarQoL	83.7 (10.0)	75.5 (16.6)	65.8 (12.8)	57.9 (17.6)	**< 0.001**	**< 0.001**	0.193	**0.004**	0.326
Insomnia Severity Index	3.6 (3.2)	5.4 (6.7)	7.0 (4.2)	10.8 (8.2)	**0.020**	**0.005**	0.249	0.072	0.844
Insulin (pg/mL)	496.6 (700.4)	900.3 (744.1)	287.1 (200.4)	818.9 (1126.0)	0.054	0.125	0.087	0.790	**0.034**
Glucose (mg/dL)	84.1 (16.0)	83.1 (11.9)	93.0 (17.3)	111.8 (60.9)	**0.046**	**0.022**	0.418	0.064	1.000

Values are mean (SD) for continuous variables (Kruskal–Wallis omnibus, Mann–Whitney post-hoc) and n (m/w) or n (% of group) for categorical. Bold *p*-values indicate $p < 0.05$. Replaces the original superscript a–f notation with explicit pairwise-comparison columns per Reviewer #1

and sex-steroid biosynthesis were also highlighted, whereas broader amino-acid, bile acid, and fatty acid pathways exhibited weaker or single-metabolite signals. Together, these findings suggest coordinated disruptions in amino-acid handling and steroid/retinoid metabolism in HF.

Univariate volcano analysis ($\log_2$ fold-change vs Mann–Whitney p, Benjamini–Hochberg FDR) was performed on the 149 annotated metabolites (Fig. 3A) (Supplementary Tables S9–S10) and top 20 metabolites compared across four groups (Fig. 3B). The HF-RM vs HF-PM contrast did not survive FDR (smallest $q \approx 0.31$) and is reported as exploratory: at raw $p < 0.05$, HF-RM showed lower isoleucine, valine, glutamic acid, phenylalanine, cystine, and indole-3-propionic acid, with higher pyruvic, malic, 2-oxoglutaric, and 2-hydroxyglutaric acid and glutamine, a pattern consistent with TCA-cycle intermediate accumulation and depleted amino acid precursors, suggesting impaired anaplerotic flux. Per-metabolite group distributions are shown in Fig. 3B. In HF-RM vs NonHF-PM, 49 metabolites passed FDR ($q < 0.05$): upregulated in HF-RM included cystineamine, N-acetyl-aspartic acid, phenylalanine, isoleucine, alanine, and 2-oxoglutaric acid; and downregulated included galactonic acid 1,4-lactone, octadecenoic acid methyl ester, gluconic acid 1,4-lactone, glyoxylic acid, histidine, and asparagine—consistent with disrupted fatty acid/TCA handling and increased BCAA and aromatic amino-acid catabolism (Fig. 3B).

Metabolic alterations HF-RM

In pairwise comparisons between HF-RM and NonHF-PM (Supplementary Data 1), several metabolites were significantly higher in HF-RM ($p < 0.05$), including cystamine ($p < 0.001$), alanine ($p < 0.001$), N-acetyl-L-aspartic acid ($p < 0.001$), methionine ($p < 0.001$), and galactose ($p < 0.001$). Between

Table 2 Body composition, muscle strength and physical function by HF and muscle phenotype

Variable	NonHF-PM	NonHF-RM	HF-PM	HF-RM	*p* (overall, K-W)	*p* (HF-RM vs NonHF-PM)	*p* (HF-RM vs HF-PM)	*p* (HF-PM vs NonHF-PM)	*p* (NonHF-RM vs NonHF-PM)
Body mass index (kg/m^2)	25.1 (2.8)	26.5 (3.5)	31.3 (6.3)	30.4 (7.0)	**0.020**	**0.011**	0.628	**0.020**	0.143
Fat mass (kg)	20.8 (4.6)	26.8 (8.3)	33.2 (10.1)	33.3 (14.3)	**0.002**	**0.001**	0.790	**0.004**	**0.049**
Fat-free mass (kg)	49.3 (10.5)	45.3 (6.8)	55.4 (11.2)	56.7 (9.7)	**0.029**	**0.032**	0.929	0.145	0.469
Appendicular lean mass (kg)	21.2 (5.5)	18.9 (3.8)	24.0 (6.1)	23.8 (4.4)	0.068	0.105	0.929	0.263	0.354
ALSTI (kg/m^2)	7.4 (1.0)	6.9 (1.1)	8.6 (1.6)	8.0 (1.2)	**0.037**	0.118	0.326	**0.041**	0.281
ALSTI/BMI	0.3 (0.0)	0.3 (0.0)	0.3 (0.1)	0.3 (0.0)	**0.040**	**0.015**	0.699	0.196	**0.022**
ALSTI/body weight	0.3 (0.0)	0.3 (0.0)	0.3 (0.1)	0.3 (0.0)	**0.014**	**0.007**	0.701	0.104	**0.008**
Body fat (%)	31.0 (4.9)	37.8 (7.8)	41.7 (12.0)	36.9 (8.1)	**0.007**	**0.013**	0.458	**0.010**	**0.012**
Visceral adipose tissue (cm^3)	1024.1 (696.9)	1153.1 (691.3)	2413.3 (1847.6)	2503.8 (1400.3)	**0.005**	**0.001**	0.641	**0.039**	0.570
Bone mineral density (g/cm^2)	1.2 (0.2)	1.2 (0.1)	1.2 (0.2)	1.2 (0.1)	0.881	0.670	0.812	0.533	0.978
6-min walking distance (m)	501.3 (79.7)	480.3 (33.2)	453.5 (53.9)	386.4 (110.4)	**0.010**	**0.003**	0.270	0.144	0.558
Time-up-and-go (s)	7.4 (1.8)	7.9 (0.8)	8.7 (1.2)	11.6 (4.7)	**< 0.001**	**< 0.001**	0.146	**0.044**	0.251
30-s chair stand (reps)	14.2 (3.1)	11.2 (1.8)	13.9 (0.9)	9.2 (3.1)	**< 0.001**	**< 0.001**	**< 0.001**	0.892	**0.012**
Gait speed (m/s)	1.4 (0.3)	1.3 (0.1)	1.2 (0.2)	1.1 (0.3)	**0.015**	**0.003**	0.364	0.124	0.222
Handgrip strength (kg, absolute)	35.6 (9.4)	30.3 (10.0)	37.0 (12.2)	30.7 (12.0)	0.369	0.254	0.193	0.770	0.196
Handgrip strength/BMI	1.4 (0.3)	1.2 (0.4)	1.2 (0.5)	1.1 (0.5)	0.097	**0.017**	0.574	0.376	0.114
Handgrip strength/body weight	0.5 (0.1)	0.4 (0.1)	0.4 (0.1)	0.4 (0.1)	**0.014**	**0.001**	0.220	0.249	0.237
Knee extension strength (kg)	36.5 (16.1)	30.7 (16.0)	36.4 (14.3)	30.3 (14.1)	0.557	0.284	0.380	0.958	0.324

Values are mean (SD); Kruskal–Wallis omnibus with Mann–Whitney post-hoc. Bold p-values indicate $p < 0.05$. Absolute HGS, ALSTI, ALSTI/BMI, ALSTI/body weight, HGS/BMI and HGS/body weight are reported side-by-side to allow comparison with body size–adjusted indices (Reviewer #1, Major Concern 3)

group differences were also identified in tricarboxylic acid (TCA) cycle intermediates such as malic acid ($p < 0.001$), fumaric acid ($p = 0.008$), and glutaric acid 2-oxo ($p < 0.001$) and BCAAs such as isoleucine ($p = 0.0174$) and valine ($p = 0.003$).

The PCA results did not yield a significant model between HF-RM and HF-PM. The supervised analyses, partial least squares-discriminant analysis (PLS-DA), were applied to select the variables responsible for the separation shown by the models. The first two components showed the highest accuracy which were used as loading plots to generate the variable of importance (VIP > 1.0) contributing to the model. Differing VIPs between HF-RM compared to HF-PM based on raw *p*-values included lower galacturonic acid-1-phosphate ($p = 0.03$), glutamic acid ($p = 0.04$), indole-3-propionic acid ($p < 0.01$), methionine ($p = 0.04$), and phenylalanine ($p = 0.04$). On the contrary, higher levels of 2-hydroxy-glutaric acid were observed vs. HF-PM ($p = 0.02$).

Associations of HGS/BMI with biomarkers and muscle health

Significant correlations were observed: HGS/BMI was negatively associated with NT-proBNP ($r = -0.414$, $p = 0.011$) and GDF-15 ($r = -0.346$, $p = 0.020$); ALSTI/BMI was negatively associated with TNF-α ($r = -0.304$, $p = 0.042$) and GDF-15 ($r = -0.305$, $p = 0.049$); total fat mass was positively associated with TNF-α ($r = +0.414$, $p = 0.004$) and GDF-15 ($r = +0.308$, $p = 0.042$), and negatively with the activin A/follistatin-3 ratio ($r = -0.350$, $p = 0.039$); 30CST was negatively associated with NT-proBNP ($r = -0.433$, $p = 0.008$) and GDF-15 ($r = -0.634$, $p < 0.001$) (Table 3). HGS/BMI demonstrated strong associations with multiple functional outcomes, with consistently larger effects observed in HF. In NonHF, HGS/BMI predicted ALSTI ($\beta = 0.533$, $p = 0.003$) and knee extension strength ($\beta = 0.611$–0.680, all $p < 0.001$) and was inversely associated with 10-m walking speed ($\beta = -0.441$,

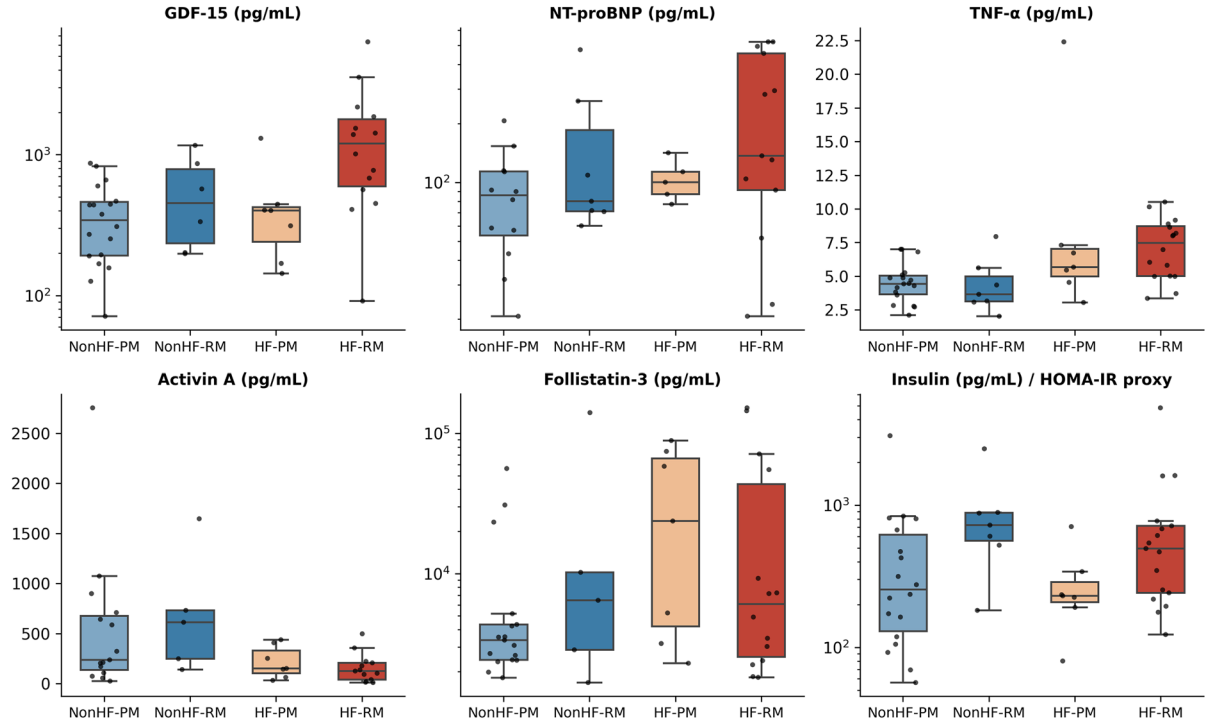


Fig. 1 Group-level circulating biomarker concentrations (not metabolomic data; metabolomic results are presented in Fig. 2) in heart failure (HF) and NonHF participants, stratified by muscle phenotype (PM vs RM). Box plots show median, interquartile range and individual data points on the raw scale (y-axis log-scaled where useful for readability); raw concentrations, group-wise fold-changes vs NonHF-PM and effect sizes (Hedges' g) are reported in Supplementary Table S4. **A**, GDF-15; **B**, NT-proBNP; **C**, TNF; **D**, Activin A; **E**, Follistatin-3, and **F**, HOMA-IR are shown for direct comparison of HF-RM with NonHF-PM, NonHF-RM and HF-PM. Overall group differences were assessed by Kruskal–Wallis with Mann–Whitney post hoc pairwise comparisons. Statistical significance was set at $p < 0.05$

$p = 0.017$) and TUG ($\beta = -0.549$, $p = 0.002$) (Supplementary Table S2). In HF, HGS/BMI was not associated with ALSTI ($\beta = 0.098$, $p = 0.642$) but remained strongly linked to knee extension strength ($\beta = 0.448$–0.720, all $p \leq 0.025$), 10-m walking speed ($\beta = -0.532$, $p = 0.006$), 6MWD ($\beta = 0.677$, $p < 0.001$), TUG ($\beta = -0.547$, $p = 0.005$), and 30CST ($\beta = 0.506$, $p = 0.01$). In contrast, ALSTI/BMI associations were attenuated or were non-significant, except for knee extension strength ($\beta = 0.529$, $p = 0.009$) and TUG ($\beta = -0.445$, $p = 0.033$) (Supplementary Table S3).

Sensitivity and HF subtype analyses

To address the contribution of HF subtype and the choice of classifying criteria to the observed signature, three pre-specified sensitivity analyses were performed (Supplementary Tables S5–S8). First, when HF-RM was redefined using a stricter EWGSOP2-aligned criterion (low IPAQ + low HGS, omitting 30CST), only three patients met the stricter definition; in this small subgroup GDF-15 ($p = 0.006$) and TNF-α ($p = 0.006$) remained significantly higher than in NonHF-PM, indicating that the inflammatory and cardiac-stress signature is not an artefact of the 30CST inclusion (Supplementary Table S5). Second, within the HF cohort, HFrEF ($n = 18$) and HFpEF ($n = 7$) were directly compared for each biomarker; no biomarker reached $p < 0.05$ between subtypes, suggesting that the HF-RM signature is not strongly confounded by HF subtype despite the imbalanced subtype distribution between HF-PM and HF-RM (Supplementary Table S6). Third, in

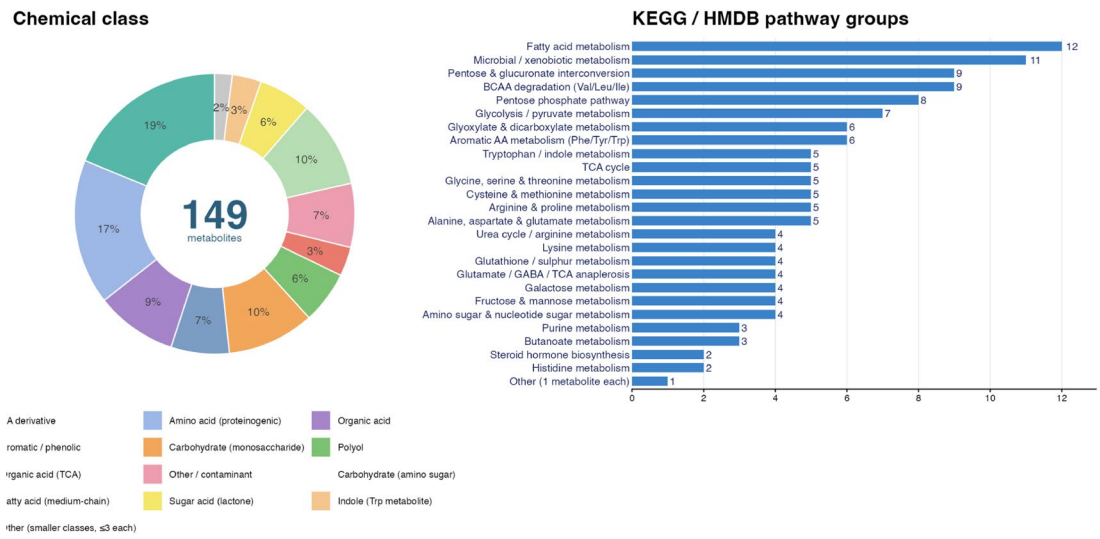
linear models of log-transformed biomarkers within HF, HF-RM remained an independent predictor of GDF-15 ($\beta = 0.557$ on $\log_{10}$ scale, $p = 0.010$) and insulin ($\beta = 0.418$, $p = 0.017$) after adjustment for EF subtype; NT-proBNP was no longer significant after adjustment ($p = 0.58$), reinforcing the interpretation that NT-proBNP elevation reflects cardiac severity rather than the HF-RM phenotype per se (Supplementary Table S7). HGS findings were robust across normalisation choices: HGS/body weight and BMI-adjusted HGS residuals showed the same pattern as HGS/BMI (Supplementary Table S8).

Discussion

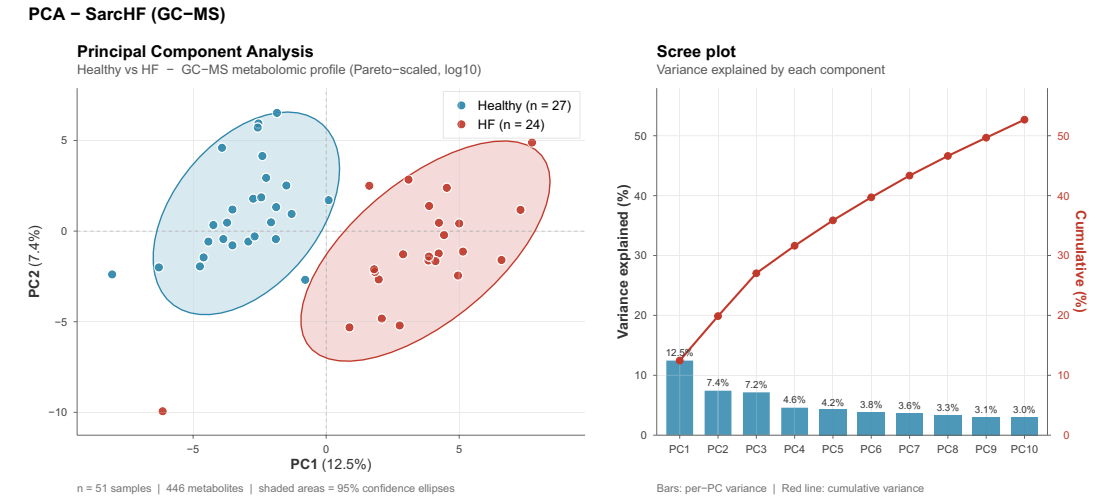
Findings from this study provide novel insights into the biological crosstalk between muscle weakness, HF, and circulating molecular pathways that may drive muscle catabolism in HF. Using an untargeted metabolomic profiling approach, we identified a distinct catabolic metabolic signature that parallels reductions in muscle strength among patients with HF. The concurrent increase in BCAA degradation, altered TCA intermediates, and elevations in TNF-α, NT-proBNP, and GDF-15 may reflect a state of systemic cardiac stress and coordinated alterations in metabolic, inflammatory, and skeletal muscle-derived pathways that could contribute to HF-RM. Moreover, metabolomic analyses revealed disruptions across fatty acid–related metabolites, amino acid metabolism, and glycolytic pathways in both HF-PM and HF-RM groups compared with NonHF controls, indicating impaired energy production and a sustained catabolic phenotype. Two interpretive caveats are important. First, the HF-RM label denotes an integrated cardiac–metabolic–muscular functional phenotype rather than a primary myopathic state. The performance-based tests used in our screening definition (30CST, 6MWD, gait speed) are partially determined by cardiac output reserve, and NT-proBNP was directionally highest in the HF-RM stratum (median 137 vs 86 pg/mL in NonHF-PM, ~ 1.6-fold; Supplementary Table S4), and rose markedly in the stricter sensitivity subgroup defined by low IPAQ + low HGS only (median 522 pg/mL, $n = 1$; Supplementary Table S5), indicating that this subgroup represents a more advanced HF state. Our findings are therefore best read as the metabolic and inflammatory correlates of advanced HF in patients who also display reduced physical-functional capacity. Second, absolute appendicular lean mass and absolute strength are preserved or even elevated in our HF cohort, while body-load-normalised indices (ALSTI/BMI, HGS/BMI) are reduced in HF-RM. This pattern is consistent with a sarcopenic-obesity/cardiometabolic phenotype (mean BMI ~ 30 kg/m^2, body fat 37–42%) rather than classic atrophic sarcopenia, in line with the body size–adjusted indices recommended by the FNIH sarcopenia project and validated for HF by Tinggaard et al. The metabolic perturbations we report should accordingly be interpreted as the signature of sarcopenic-obesity superimposed on HF, not as evidence of overt muscle wasting. Noteworthy that the prespecified sensitivity analysis omitting 30CST (low IPAQ + low HGS only) confirmed that the inflammatory and cardiac-stress signal persisted even within this stricter subgroup, indicating that this signature is not an artefact of including a cardiac-output-dependent functional test in the classifier.

In our cohort, HGS/BMI consistently predicted ALSTI and muscle strength in NonHF, whereas in HF, it predicted muscle strength but not ALSTI, suggesting that HF-related metabolic impairments may weaken the link between muscle quantity and function. These observations indicate that HF could disrupt both structural and functional muscle relationships, making HGS/BMI a more robust functional marker than ALSTI/BMI. Tinggaard et al. previously showed that ALSTI/BMI predicts walking capacity and muscle weakness; our findings suggest this relationship may be bidirectional and that HGS/BMI may be a stronger predictor. Despite our smaller sample, this implies that handgrip strength assessment can offer meaningful clinical value at the individual level, though larger studies are needed to confirm these observations. Importantly, the interpretation of HGS/BMI as a body-load-normalised functional marker is supported by sensitivity analyses using alternative normalisations. When HGS was expressed per kilogram of body weight, a body size adjustment that does not introduce a height redundancy, the negative correlations with NT-proBNP and TNF-α were stronger than those obtained with HGS/BMI, and the positive correlation with the activin A/follistatin-3 ratio reached significance only when body-weight normalisation was applied. Absolute HGS, by contrast,

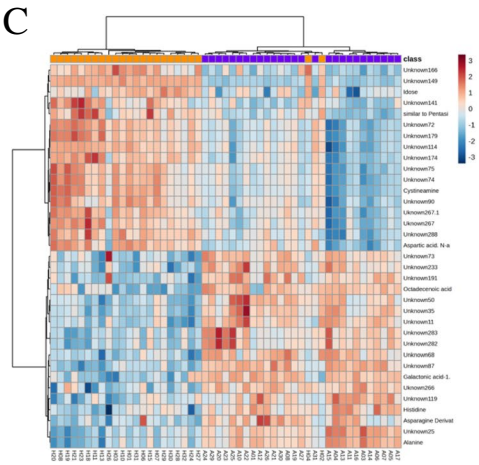
A


Chemical class
149
metabolites
19%
2%
3%
6%
10%
7%
3%
6%
10%
7%
9%
17%
KEGG / HMDB pathway groups
Fatty acid metabolism 12
Microbial / xenobiotic metabolism 11
Pentose & glucuronate interconversion 9
BCAA degradation (Val/Leu/Ile) 9
Pentose phosphate pathway 8
Glycolysis / pyruvate metabolism 7
Glyoxylate & dicarboxylate metabolism 6
Aromatic AA metabolism (Phe/Tyr/Trp) 6
Tryptophan / indole metabolism 5
TCA cycle 5
Glycine, serine & threonine metabolism 5
Cysteine & methionine metabolism 5
Arginine & proline metabolism 5
Alanine, aspartate & glutamate metabolism 5
Urea cycle / arginine metabolism 4
Lysine metabolism 4
Glutathione / sulphur metabolism 4
Glutamate / GABA / TCA anaplerosis 4
Galactose metabolism 4
Fructose & mannose metabolism 4
Amino sugar & nucleotide sugar metabolism 4
Purine metabolism 3
Butanoate metabolism 3
Steroid hormone biosynthesis 2
Histidine metabolism 2
Other (1 metabolite each) 1
0 2 4 6 8 10 12
A derivative
Amino acid (proteinogenic)
Organic acid
romatic / phenolic
Carbohydrate (monosaccharide)
Polyol
rganic acid (TCA)
Other / contaminant
Carbohydrate (amino sugar)
atty acid (medium-chain)
Sugar acid (lactone)
Indole (Trp metabolite)
ther (smaller classes, ≤3 each)

B


PCA – SarcHF (GC–MS)
Principal Component Analysis
Healthy vs HF – GC–MS metabolomic profile (Pareto–scaled, log10)
Healthy (n = 27)
HF (n = 24)
PC2 (7.4%)
PC1 (12.5%)
n = 51 samples | 446 metabolites | shaded areas = 95% confidence ellipses
Scree plot
Variance explained by each component
Variance explained (%)
Cumulative (%)
12.5%
7.4%
7.2%
4.6%
4.2%
3.8%
3.6%
3.3%
3.1%
3.0%
PC1 PC2 PC3 PC4 PC5 PC6 PC7 PC8 PC9 PC10
Bars: per–PC variance | Red line: cumulative variance

C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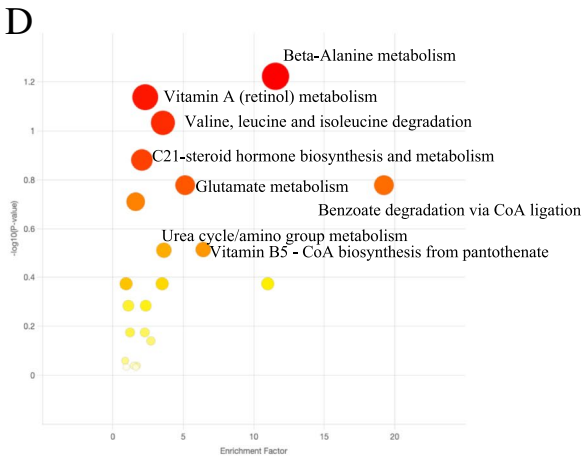
D


Beta-Alanine metabolism
Vitamin A (retinol) metabolism
Valine, leucine and isoleucine degradation
C21-steroid hormone biosynthesis and metabolism
Glutamate metabolism
Benzoate degradation via CoA ligation
Urea cycle/amino group metabolism
Vitamin B5 - CoA biosynthesis from pantothenate
-log10(P-value)
Enrichment Factor

◂**Fig. 2** **A** All counts are based on the 149 annotated GC–MS plasma metabolite s. Each metabolite is assigned to one primary KEGG/HMDB pathway. **B** PCA shows distinct clusters for HF compared to NonHF [*F*-value 44.636; R-squared 0.4767; *p*-value (based on 999 permutations): $P = 0.001$] (metabolomic data for 1 HF and 2 NonHF was not available). **B** Classification of metabolites based on number of downregulated and upregulated in HF compared to NonHF. **C** Hierarchical clustering heatmap of participants (columns) using the plasma metabolome demonstrates similar separation of the HF from NonHF. **D** Mummichog pathway activity profile for HF compared to NonHF controls based on top 10% peaks

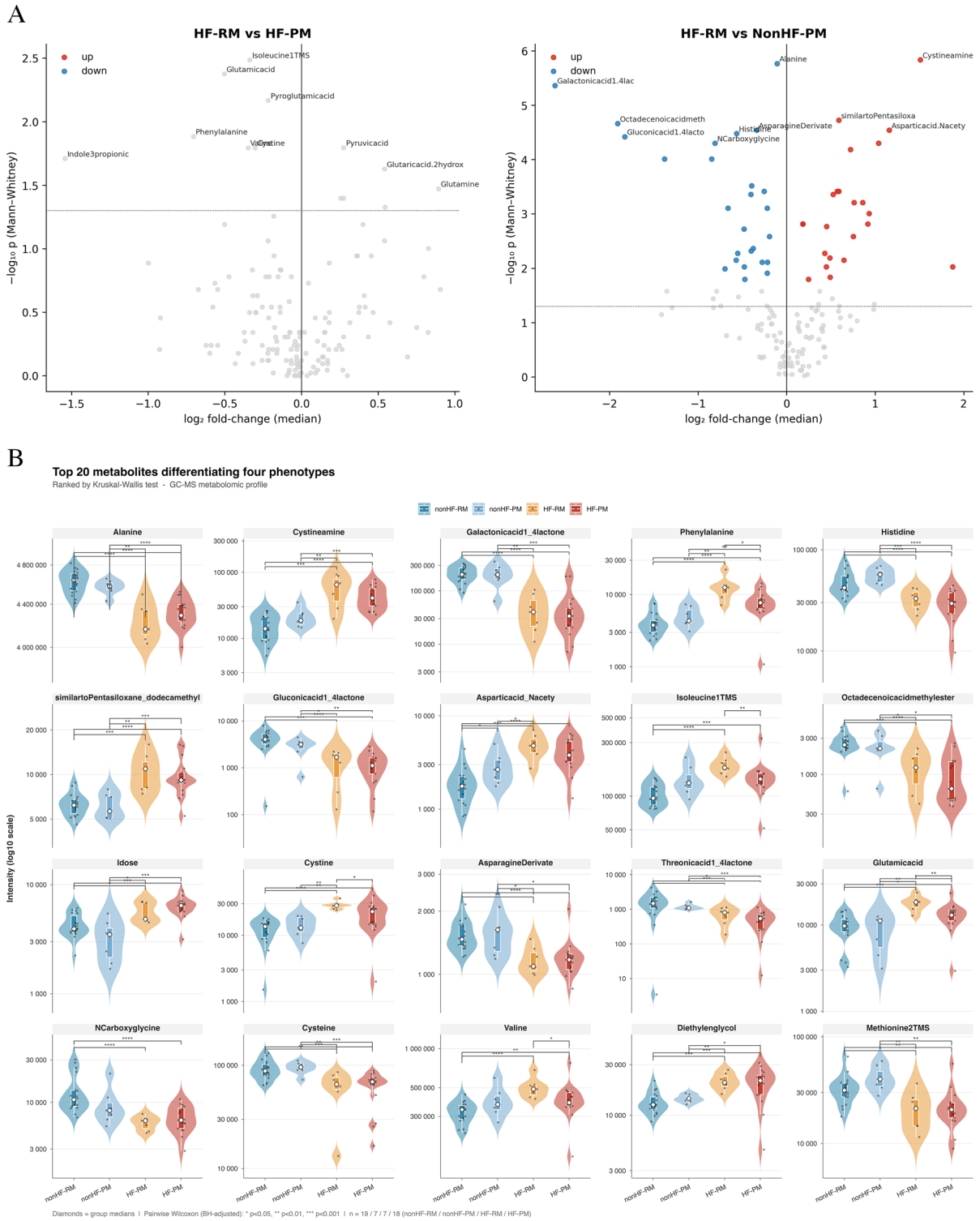
was associated only with NT-proBNP. The body-load-normalised relationship between strength and the cardiac–inflammatory axis is therefore not a mathematical artefact of dividing strength by BMI in an obese cohort: it is robust across normalisation choices and is in fact strongest with the cleanest body size adjustment, supporting the biological interpretation that mass-relative force generation, rather than absolute strength, is what couples to cardiac stress and systemic inflammation in HF.

Collectively, these biomarker–function associations indicate a potential link between elevated inflammatory and cardiac-stress markers and diminished physical function in HF. Among them, the inverse coupling of GDF-15 with 30CST stands out as a candidate indicator of functional decline and sarcopenia risk, in line with observations from the PROTECT cohort of older medical patients. The biological magnitude of these elevations (reported in Results and Supplementary Tables S4–S5) suggests clinically meaningful shifts in the cardiac-stress and inflammatory axis, with the most cardiac-decompensated patients falling within the HF-RM phenotype. These findings align with the hypothesis that chronic inflammation and cardiac stress may share a bidirectional relationship with muscle weakness. However, the cross-sectional nature of the data limits causal inference, warranting longitudinal studies to elucidate these correlations. A specific concern is that the HF-PM and HF-RM sub-strata differed in HF subtype distribution, raising the possibility that the inflammatory–metabolic signature attributed to reduced muscle health might instead reflect HFrEF-specific biology. As shown in the sensitivity analyses (Results; Supplementary Tables S6–S7), HF subtype was not a major driver of the biomarker differences observed between HF-PM and HF-RM, and EF-adjusted linear models indicated that GDF-15 and insulin tracked the HF-RM phenotype independently of EF, whereas NT-proBNP did not. This pattern is consistent with the interpretation that NT-proBNP elevation reflects myocardial wall stress (i.e. HF severity), while the GDF-15 and metabolic/inflammatory signal is at least partially independent of EF. We acknowledge that disentangling muscle-health phenotype from HF subtype definitively will require a larger, balanced HFrEF/HFpEF cohort.

Metabolic alterations in heart failure with reduced muscle health

Our findings regarding the HF-RM phenotype revealed several differentially expressed metabolites, including galacturonic acid-1-phosphate, glutamic acid, indole-3-propionic acid, methionine, phenylalanine, and 2-hydroxy-glutaric acid. These findings, although based on raw p-values, suggest that HF-RM may amplify metabolic perturbations compared to patients with HF-PM. However, elevation of amino acids such as glutamic acid and phenylalanine in HF-RM may yield controversial results regarding their link to frailty [1, 2]. Regarding HF, higher phenylalanine has been linked to increased mortality and HF (re)hospitalisation [3, 4], while glutamic acid may reflect HF severity [5], which may be exacerbated by frailty. Furthermore, the higher levels of galacturonic acid-1-phosphate, a carbohydrate metabolite, may reflect altered glycosaminoglycan metabolism, potentially contributing to impaired tissue remodelling, considering that proteomics have demonstrated it as a top pathway associated with frailty [6]. It is worth noting that the HF-RM cohort exhibited lower 30CST performance, reduced weekly moderate physical activity, and lower MET and MNA scores compared to HF-PM.

These changes were accompanied by higher levels of glucose, TNF-α, and GDF-15, but lower levels of activin A in HF-RM vs. HF-PM. Elevated GDF-15 is a biomarker of inflammation and oxidative stress that has been previously linked to sarcopenia and frailty. The elevated levels of TNF-α in our study are in line with evidence on its link with frailty in cardiovascular diseases [7], while increased GDF-15 may not only


A
HF-RM vs HF-PM
up
down
Isoleucine1TMS
Glutamicacid
Pyroglutamicacid
Phenylalanine
Valine
Pyruvicacid
Indole3propionic
Glutaricacid.2hydrox
Glutamine
−log10 p (Mann–Whitney)
log2 fold-change (median)
HF-RM vs NonHF-PM
up
down
Galactonicacid1.4lac
Alanine
Cystineamine
Octadecenoicacidmeth
Gluconicacid1.4lacto
Histidine
AsparagineDerivate
similartoPentasiloxa
Aspartic acid.Nacety
NCarboxyglycine
−log10 p (Mann–Whitney)
log2 fold-change (median)
B
Top 20 metabolites differentiating four phenotypes
Ranked by Kruskal-Wallis test - GC-MS metabolomic profile
nonHF-RM
nonHF-PM
HF-RM
HF-PM
Alanine
Cystineamine
Galactonicacid1_4lactone
Phenylalanine
Histidine
similartoPentasiloxane_dodecamethyl
Gluconicacid1_4lactone
Asparticacid_Nacety
Isoleucine1TMS
Octadecenoicacidmethylester
Idose
Cystine
AsparagineDerivate
Threonicacid1_4lactone
Glutamicacid
NCarboxyglycine
Cysteine
Valine
Diethylenglycol
Methionine2TMS
Intensity (log10 scale)
Diamonds = group medians | Pairwise Wilcoxon (BH-adjusted): * p<0.05, ** p<0.01, *** p<0.001 | n = 19 / 7 / 7 / 18 (nonHF-RM / nonHF-PM / HF-RM / HF-PM)

◂**Fig. 3** **A** Plasma metabolite volcano plots. Log_2 fold-change of group medians versus $-log_{10}$ p (Mann–Whitney) for all 149 annotated metabolites. Red, increased in HF-RM; blue, decreased; grey, non-significant. Dotted horizontal line, $p = 0.05$. Significance is FDR-corrected (Benjamini–Hochberg, $q < 0.05$) for the left panel; the right panel reports unadjusted p only (no FDR survivors) and is exploratory. Top ten lowest-p metabolites are labelled. Full statistics in Supplementary Tables S9 and S10. **B** Represent violin plots for the top 20 differential metabolites across groups

serve as a marker of HF hospitalisation, mortality [8], inflammation, or malnutrition [9], but also of frailty and/or impaired muscle health severity in HF. In addition, higher activin A has been linked to impaired cardiac remodelling [10], and myocardial damage [11] in humans and murine models, which contradict our results on the detrimental impact muscle weakness may confer in HF. Our data showed that the ratio of activin A to follistatin-3 was lower in both the total HF and HF-RM groups compared to controls, suggesting that follistatin-3 may mask activin A action more effectively in the HF-RM population.

HF-RM exhibited a broad range of metabolic alterations compared to NonHF-PM. For instance, elevated amino acids (e.g. alanine, methionine, isoleucine, glutamic acid, ornithine, and cystine) and carbohydrate metabolites (e.g. galactose, galactonic acid, and glycerolaldopyranosid) suggest increased metabolic stress. Conversely, reduced levels of energy metabolism intermediates (e.g. pyruvic acid, malic acid, and 2-oxo-glutaric acid) may indicate compromised mitochondrial energy production, a feature consistent with the relationship between frailty and impaired energy homeostasis, which was also observed in the total HF vs. NonHF. The lower concentrations of tryptophan, histidine, serine, cysteine, and asparagine further highlight the catabolic state in HF-RM, linking these to potentially accelerated muscle wasting and reduced production of serotonin, kynurenines, and NAD^+ as shown previously [12]. Thus, patients with HF-RM may exhibit extensive metabolic perturbations, including elevated amino acids and carbohydrate metabolites, and reduced energy intermediates compared to NonHF-PM.

Clinical and research implications

The notable muscle weakness in HF-RM was apparent at the similar age with total NonHF and HF-PM counterparts, suggesting that muscle weakness may start at a younger age in this population, which is an established risk factor for worsened prognosis in HF. The distinct metabolic profiles identified in this study could have significant implications for the management of HF. The elevation of GDF-15 and TNF-α in HF-RM highlights the role of inflammation and oxidative stress in this population, suggesting that potential therapies (pharmacological and non-pharmacological) could be explored to mitigate impaired muscle health–related complications. Notably, GDF-15 has been recently emerged as a therapeutic target in cancer-associated cachexia, a condition characterised by severe muscle and weight loss [13]. In addition, the observed metabolic alterations, particularly in BCAAs, glycolytic metabolites, and the tryptophan-indole pathway, suggest potential therapeutic targets to improve energy metabolism and muscle health in this HF-RM phenotype. For example, interventions aimed at enhancing mitochondrial function or optimising amino acid metabolism could help alleviate symptoms of muscle weakness and improve functional outcomes [14, 15]. Specifically, nutritional status, as indicated by lower MNA scores in HF-RM, may be a critical factor, given its link to lower energy and protein intake that may amplify sarcopenia risk or sarcopenia progression. Additionally, the lower 30CST performance and low physical activity levels in the HF-RM group mark further the need for tailored nutritional and exercise programmes to improve physical function. Furthermore, larger sample sizes or more sensitive analytical approaches may be needed in studies to fully elucidate muscle health–specific metabolic signatures. Future studies could employ longitudinal designs to explore how these metabolic changes evolve over time and evaluate their prognostic impact on clinical outcomes such as hospitalisation or mortality.

Study limitations

The relatively small sample size and cross-sectional nature preclude causal interpretations and to investigate the physical frailty bidirectionally to HF, particularly for muscle health and/or HF phenotype-specific (HFrEF and HFpEF) analyses, where statistical significance was based on raw *p*-values rather than adjusted models. Additionally, the consideration of comorbidities and different prescribed medications may have an impact on metabolomic analyses.

Table 3 Pearson correlations between circulating biomarkers and absolute, BMI-normalised and body-weight-normalised muscle and body composition indices

Index	CRP (mg/L)	IL-6 (pg/mL)	NT-proBNP (pg/mL)	TNF-α (pg/mL)	Activin A (pg/mL)	GDF-15 (pg/mL)	GDF-8 (ng/mL)	Follistatin-3 (pg/mL)	Activin A/Follistatin-3
HGS (kg, absolute)	−0.202 ($p=0.183$, $n=45$)	0.168 ($p=0.505$, $n=18$)	−0.365* ($p=0.026$, $n=37$)	−0.127 ($p=0.390$, $n=48$)	−0.024 ($p=0.886$, $n=40$)	−0.247 ($p=0.101$, $n=45$)	−0.036 ($p=0.837$, $n=36$)	0.001 ($p=0.994$, $n=43$)	0.063 ($p=0.716$, $n=36$)
HGS/BMI	−0.172 ($p=0.258$, $n=45$)	−0.004 ($p=0.988$, $n=18$)	−0.414* ($p=0.011$, $n=37$)	−0.282 ($p=0.052$, $n=48$)	0.060 ($p=0.711$, $n=40$)	−0.346* ($p=0.020$, $n=45$)	−0.028 ($p=0.870$, $n=36$)	−0.056 ($p=0.720$, $n=43$)	0.227 ($p=0.183$, $n=36$)
HGS/body weight	−0.206 ($p=0.175$, $n=45$)	0.033 ($p=0.898$, $n=18$)	−0.463** ($p=0.004$, $n=37$)	−0.353* ($p=0.014$, $n=48$)	0.119 ($p=0.465$, $n=40$)	−0.402** ($p=0.006$, $n=45$)	−0.064 ($p=0.709$, $n=36$)	−0.122 ($p=0.434$, $n=43$)	0.341* ($p=0.042$, $n=36$)
ALSTI (kg/m^2, absolute)	−0.158 ($p=0.306$, $n=44$)	0.109 ($p=0.678$, $n=17$)	0.089 ($p=0.599$, $n=37$)	0.199 ($p=0.180$, $n=47$)	−0.127 ($p=0.442$, $n=39$)	0.070 ($p=0.654$, $n=44$)	−0.016 ($p=0.928$, $n=35$)	0.040 ($p=0.800$, $n=42$)	−0.238 ($p=0.169$, $n=35$)
ALSTI/BMI	−0.166 ($p=0.294$, $n=42$)	−0.148 ($p=0.585$, $n=16$)	−0.148 ($p=0.383$, $n=37$)	−0.304* ($p=0.042$, $n=45$)	0.079 ($p=0.644$, $n=37$)	−0.305* ($p=0.049$, $n=42$)	0.060 ($p=0.738$, $n=33$)	−0.120 ($p=0.461$, $n=40$)	0.162 ($p=0.366$, $n=33$)
ALSTI/body weight	−0.169 ($p=0.274$, $n=44$)	−0.169 ($p=0.517$, $n=17$)	−0.163 ($p=0.334$, $n=37$)	−0.291* ($p=0.048$, $n=47$)	0.077 ($p=0.641$, $n=39$)	−0.311* ($p=0.040$, $n=44$)	0.055 ($p=0.752$, $n=35$)	−0.102 ($p=0.521$, $n=42$)	0.159 ($p=0.361$, $n=35$)
Total fat mass (kg)	0.074 ($p=0.633$, $n=44$)	0.192 ($p=0.462$, $n=17$)	0.167 ($p=0.324$, $n=37$)	0.414** ($p=0.004$, $n=47$)	−0.162 ($p=0.326$, $n=39$)	0.308* ($p=0.042$, $n=44$)	0.087 ($p=0.620$, $n=35$)	0.143 ($p=0.365$, $n=42$)	−0.350* ($p=0.039$, $n=35$)
30-s chair stand (reps)	−0.057 ($p=0.709$, $n=45$)	−0.051 ($p=0.842$, $n=18$)	−0.433** ($p=0.008$, $n=37$)	−0.149 ($p=0.311$, $n=48$)	0.293 ($p=0.066$, $n=40$)	−0.634*** ($p=0.000$, $n=45$)	−0.040 ($p=0.818$, $n=36$)	−0.130 ($p=0.407$, $n=43$)	0.433** ($p=0.008$, $n=36$)

Each cell shows Pearson r (*$p<0.05$, **$p<0.01$, ***$p<0.001$) with p-value and pairwise-complete n on the second line. The original table reported only HGS/BMI and ALSTI/BMI; absolute HGS, absolute ALSTI, HGS/body weight and ALSTI/body weight have been added in response to Reviewer #1, Minor Concern 3. ALSTI/body weight is reported because dividing ALSTI (kg/m^2) by BMI (kg/m^2) introduces a redundant body-height term, whereas dividing by body weight gives a clean body size adjustment

MSI level 2 annotations are not definitive metabolite identifications. Future work would be to run authentic standards to confirm the ones not already in CMR library, although hits in Mummichog give extra confidence. Despite these constraints, the robust application of GC–MS in clinical and community-dwelling cohorts provides further mechanistic insights into HF and muscle health. The HF-RM screening definition is based on Fried physical-frailty (low IPAQ) plus EWGSOP2 "probable sarcopenia" criteria (low HGS) and/or low 30CST; because performance-based tests are partly determined by cardiac output reserve in HF, the HF-RM phenotype is best interpreted as integrated cardiac–metabolic–muscular dysfunction rather than as primary skeletal myopathy. Confirmatory sarcopenia (low ALSTI per EWGSOP2) was not used to define groups, and absolute appendicular lean mass was preserved or elevated in our HF cohort, identifying a sarcopenic-obesity rather than atrophic-sarcopenia phenotype. Group sizes were modest ($n=21$, 8, 7, 18) and the HF-PM ($n=7$) and HF-RM ($n=18$) strata were imbalanced for HF subtype (3 HFpEF/4 HFrEF and 4 HFpEF/14 HFrEF, respectively); although our sensitivity analyses suggest that HF subtype is not a major confounder of the GDF-15, TNF-α, and BCAA signal (Supplementary Tables S6–S7), confirmation in a larger, balanced HFrEF/HFpEF cohort is required. Finally, body-load-normalised strength indices (HGS/BMI) are interpretively useful but mathematically sensitive to body composition; we therefore report sensitivity analyses using HGS/body weight and BMI-adjusted residuals (Supplementary Table S8), which show a directionally consistent pattern. These findings highlight the need for integrated approaches to manage HF-RM, incorporating nutritional, exercise, and potentially pharmacological interventions to address metabolic and functional deficits.

Conclusions

This study provides novel insights into the metabolic alterations associated with HF and reduced muscle health, highlighting distinct profiles in fatty acid, amino acid, and carbohydrate metabolism. HF-RM demonstrates impaired energy metabolism, marked by a potentially reduced ability to replenish excess TCA intermediates. Elevated levels of inflammation, GDF-15, and BCAAs and their catabolites may indicate higher systemic catabolism, a particularly prominent feature in HF-RM.

Acknowledgements The authors would like to express their sincere gratitude to all participants of this study. We also gratefully acknowledge the Liverpool University Biobank for the provision of material, Faculty of Health and Life Sciences, University of Liverpool. Especial thanks to Mrs. Katie Bullock for her support in biomarker analysis and Mr. Lewis Harding for support in the recruitment process.

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Funding This study received funding from Dunhill Medical Trust (Dr Masoud Isanejad) and Liverpool Shared Research Facility (Dr Masoud Isanejad), and the Professor John Glover Memorial Award (Konstantinos Prokopidis).

Data Availability All aggregated data are presented in the supplementary materials as tables.

Declarations

Competing interests The authors declare no competing interests.

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