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RESEARCH ARTICLE

Distinct pathophysiological pathways in women and men with heart failure

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Aims Clinical differences between women and men have been described in heart failure (HF). However, less is known about the underlying pathophysiological mechanisms. In this study, we compared multiple circulating biomarkers to gain better insights into differential HF pathophysiology between women and men.

Methods and results In 537 women and 1485 men with HF, we compared differential expression of a panel of 363 biomarkers. Then, we performed a pathway over-representation analysis to identify differential biological pathways in women and men. Findings were validated in an independent HF cohort (575 women, 1123 men). In both cohorts, women were older and had higher left ventricular ejection fraction (LVEF). In the index and validation cohorts respectively, we found 14/363 and 12/363 biomarkers that were relatively up-regulated in women, while 21/363 and 14/363 were up-regulated in men. In both cohorts, the strongest up-regulated biomarkers in women were leptin and fatty acid binding protein-4, compared to matrix metalloproteinase-3 in men. Similar findings were replicated in a subset of patients from both cohorts matched by age and LVEF. Pathway over-representation analysis revealed increased activity of pathways associated with lipid metabolism in women, and neuro-inflammatory response in men (all $p < 0.0001$).

Conclusion In two independent cohorts of HF patients, biomarkers associated with lipid metabolic pathways were observed in women, while biomarkers associated with neuro-inflammatory response were more active in men. Differences in inflammatory and metabolic pathways may contribute to sex differences in clinical phenotype observed in HF, and provide useful insights towards development of tailored HF therapies.

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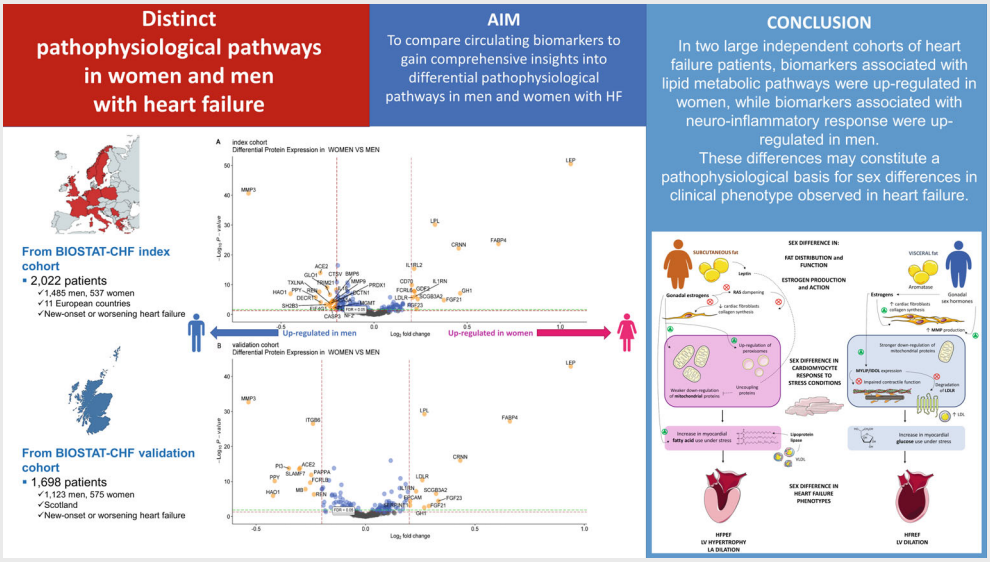
Graphical Abstract



Distinct pathophysiological pathways in women and men with heart failure. An analysis on biomarkers from the Systems Biology Study to Tailored Treatment in Chronic Heart Failure (BIOSTAT-CHF).

Keywords Heart failure • Sex • Women • Pathway analysis • Inflammation • Lipids

Introduction

Heart failure (HF) is a debilitating syndrome with a lifetime risk of 1 in 5, equal in women and men.[1,2] However, most evidence that shaped clinical guidelines in HF was based on studies in which women were under-represented.[3–5]

The recent focus on sex differences led to the acknowledgement of sex-specific features in HF.[6,7] HF with reduced ejection fraction (HFrEF) is more common in men, while HF with preserved ejection fraction (HFpEF) more frequently affects women,[8,9] men tend to develop HF at a younger age, and the risk factors for HF are different in women and men.[6,8,10] Moreover, we recently observed that women with HFrEF might need lower doses of angiotensin-converting enzyme inhibitors (ACEi) or angiotensin receptor blockers (ARBs) and beta-blockers than men to achieve outcome benefit.[11] In addition, women with HFpEF appeared to gain more benefit from mineralocorticoid receptor antagonists (MRAs) and angiotensin receptor–neprilysin inhibitors (ARNI) then men.[12,13]

Despite this new clinical evidence, the therapeutic approach to HF is still the same in women and men, and the mechanistic understanding of the underlying sex differences in HF pathophysiology is limited.[14–17]

Connecting disease-related phenotypes with underlying molecular mechanisms is crucial for better understanding of complex diseases like HF. A systems biology approach allows to study how different phenotypes are generated, with techniques spanning across the analysis of phenotype, biomarker and genetic layers, integrating them to gain insights on the key components and interactions of biological systems within the disease.[18,19] Such integrated knowledge constitutes the basis for an improvement in disease therapy, allowing tailored therapeutic approaches and personalized treatment regimens.

The aim of our study was to compare differentially expressed circulating biomarkers and associated pathophysiological pathways between women and men with HF, to gain better insights into sex-specific HF pathophysiology.

Methods

Patient population

A Systems Biology Study to Tailored Treatment in Chronic Heart Failure (BIOSTAT-CHF) was a European project, including two prospective, observational, multinational cohorts. The design and primary results of BIOSTAT-CHF have been published elsewhere.[20] Briefly, the index cohort included a total of 2516 patients from 11 centres in Europe, with signs and symptoms of worsening HF and either a left ventricular ejection fraction (LVEF) ≤40%, or plasma concentrations of N-terminal pro-B-type natriuretic peptide (NT-proBNP) >2000 ng/L. Included patients were either treatment naïve or receiving <50% of the

target dose of ACEi/ARBs and beta-blockers at the time of inclusion. Patients were enrolled from December 2010 to December 2012. Median follow-up was 21 months.

A second independent cohort from six centres in Scotland, the validation cohort, included 1738 patients with HF and a previous documented HF admission requiring diuretic treatment, not previously treated or receiving ≤50% of target doses of ACEi/ARBs and/or beta-blockers. Patients were recruited from October 2010 until April 2014. Median follow-up was 21 months. This study complies with the Declaration of Helsinki, and medical ethics committees of participating centres approved the study. All patients provided written informed consent. Details on the study plan are provided in online supplementary *Appendix S1*.

Biomarkers

The Olink Proteomics® Multiplex Cardiovascular (CVD)-II, CVD-III, Immune Response and Oncology-II panels of 363 unique proteins from different pathophysiological domains were used to investigate the biomarker profiles in both BIOSTAT-CHF index and validation cohorts (details in online supplementary *Appendix S1*).[21] Complete biomarker data were available in 2022/2516 and 1698/1738 patients from the index and validation cohorts, respectively.

Statistical analyses

Normally distributed continuous variables were displayed as mean ± standard deviation, non-normally distributed variables as median and interquartile range (IQR), categorical variables as numbers with percentages. Group comparisons were tested using Student's *t*-tests, Mann–Whitney U tests, or Chi-square tests where appropriate.

Differences in expression of the 363 biomarkers in women versus men with HF were tested using Linear Models for Microarray data analysis (Limma) software (version 3.34.9), using a fold change cut-off of 1.15 (equal to a log2 fold change cut-off of 0.2), and a false discovery rate <0.05 according to the Benjamini-Hochberg method.[22] Differential expression analysis was also adjusted for age and diabetes for their biological and metabolic relevance.

The biomarkers that were up-regulated in women or in men were further studied by using pathway over-representation analysis, a method that can identify associated biological pathways based on circulating biomarker profiles in specific subgroups.[23–25] Pathway over-representation analysis was performed with ClueGO using knowledge from established pathways in publicly available databases (i.e. Gene Ontology [GO], Reactome, and the Kyoto Encyclopedia of Genes and Genomes [KEGG]) using the hypergeometric test and the default Bonferroni step down method for multiple testing corrections (family-wise error rate).[26,27] All tests were performed two-sided, and a *p*-value of <0.05 was considered statistically significant.

Because baseline age and LVEF represent major differences between women and men, not only in BIOSTAT-CHF but also in the HF population, a sensitivity analysis was performed on 576 subjects from the index cohort, and 496 subjects from the validation cohort, matched by age and LVEF using the MatchIt package in R.[6,8] Differential expression analysis of biomarkers was performed, and adjusted for age and the presence of diabetes (more details in online supplementary *Appendix S1*).

Sex differences in the association of the differentially expressed biomarkers with outcome (all-cause death) were investigated using logistic regression, univariable and adjusted for selected covariates (age, LVEF and biomarker concentration). Forrest plots were obtained.

Statistical analyses were performed using R, A Language and Environment for Statistical Computing, version 3.5.1 (R Foundation for Statistical Computing, Vienna, Austria), IBM SPSS Statistics for Windows, version 23.0.0.3 (IBM Corp., Armonk, NY, USA), and Cytoscape (version 3.8.0) with plugin ClueGO (version 2.5.7) and CluePedia (version 1.5.7).[26,27]

Results

Index cohort

Clinical characteristics

Baseline characteristics of the index cohort, stratified by sex, are displayed in *Table 1*. Of 2022 patients considered for the analysis of patients enrolled in the index cohort, 537 (27%) were women, and 1485 (73%) men. Women were older (71 vs. 67 years old, $p < 0.001$), and had similar body mass index (BMI, 27.4 vs. 28 kg/m^2, $p = 0.05$) as compared to men. Fewer women had ischaemic HF (42% vs. 58%, $p < 0.001$), atrial fibrillation (40% vs. 47%, $p = 0.006$), and a HF hospitalization in the past year (27% vs. 32%, $p = 0.018$). Signs and symptoms of HF at presentation were fairly similar in women and men except for hepatomegaly, which was less prevalent in women, and orthopnea, which was more common in women (*Table 1*).

Echocardiographic parameters

A baseline echocardiogram was performed in 88% of patients in the index cohort (online supplementary *Table S5*). At presentation, men had larger left ventricular (LV) dimensions (LV end-diastolic diameter [LVEDD] 62 vs. 56 mm, $p < 0.001$) and lower LVEF (30% vs. 35%, $p < 0.001$). On the other hand, women presented with higher prevalence of LV hypertrophy, as defined using guidelines' sex specific definition (79% vs. 68%, $p < 0.001$) and larger indexed left atrial dimensions (26 vs. 24 mm/m^2, $p < 0.001$) (*Table 2*).[14]

Biomarker concentrations

In the index cohort, 14 biomarkers were up-regulated in women as compared to men, and 21 biomarkers were up-regulated in men as compared to women (*Figure 1*, online supplementary *Table S7*). The most prominently up-regulated biomarkers in women as compared with men were leptin (fold change 2.08, $p = 1.18 \times 10^{-48}$), and fatty acid binding protein 4 (FABP4, fold change 1.59, $p = 1.87 \times 10^{-22}$). The most strongly up-regulated biomarker in men as compared with women was matrix metalloproteinase-3 (MMP3, fold change 1.59, $p = 4.23 \times 10^{-39}$). A volcano plot showing all differentially expressed biomarkers in men and women is shown in *Figure 2A*. Adjusted volcano plots are displayed in online supplementary *Figure S1*.

Sensitivity analysis

Baseline characteristics of the matched subset from the index cohort are displayed in online supplementary *Table S2*.

Table 1 Baseline characteristics, stratified by sex, of BIOSTAT-CHF index cohort

	Women	Men	*p*-value
No. of subjects	537	1485	
Demographics			
Age, years	71 ± 12	67 ± 12	<0.001
Race			0.698
Caucasian	531 (98.9)	1468 (98.9)	
Asian	2 (0.4)	8 (0.5)	
Black	2 (0.4)	2 (0.1)	
Other	2 (0.4)	7 (0.5)	
BMI, kg/m^2	27.4 ± 5.9	28 ± 5.3	0.050
Weight, kg	72.3 ± 16.9	85.4 ± 17.9	<0.001
Height, cm	162 ± 7	174 ± 8	<0.001
Clinical profile			
NYHA class I/II/III/IV	7/180/261/70 (1.4/34.7/50.4/13.5)	35/536/700/172 (2.4/37.1/48.5/11.9)	0.294
LVEF, %	34.6 ± 11.8	30 ± 10	<0.001
Pulmonary rales			0.140
No	224 (43.1)	694 (48.0)	
Single base	66 (12.7)	176 (12.2)	
Bi-basilar	230 (44.2)	575 (39.8)	
Oedema			0.212
Not present	181 (40.2)	517 (42.1)	
Ankle	135 (30.0)	350 (28.5)	
Below knee	92 (20.4)	280 (22.8)	
Above knee	42 (9.3)	82 (6.7)	
Jugular venous pressure	121 (35.5)	328 (32.3)	0.302
Hepatomegaly	50 (9.3)	229 (15.5)	0.001
Orthopnoea	212 (39.6)	484 (32.6)	0.005
Systolic blood pressure, mmHg	128 ± 25	124 ± 21	<0.001
Diastolic blood pressure, mmHg	74 ± 15	75 ± 13	0.030
Heart rate, bpm	82 ± 21	79 ± 19	0.003
Type of visit			0.243
Scheduled outpatient clinic	119 (22.2)	378 (25.5)	
Unscheduled outpatient clinic	22 (4.1)	69 (4.6)	
Inpatient hospitalization	396 (73.7)	1038 (69.9)	
Heart failure history			
Months since first diagnosis	1.9 [0.1, 35]	3.7 [0.2, 42.6]	0.233
Past heart failure hospitalization	143 (26.6)	479 (32.3)	0.018
Heart failure aetiology			
Ischaemic	226 (42.1)	856 (57.6)	<0.001
Hypertensive	314 (58.5)	766 (51.6)	0.014
Cardiomyopathy	197 (36.7)	600 (40.4)	0.288
Valvular	218 (40.6)	553 (37.2)	0.205
Other	101 (18.8)	205 (13.8)	0.018
Medical history			
Hypertension	358 (66.7)	888 (59.8)	0.006
Atrial fibrillation	216 (40.2)	702 (47.3)	0.006
Myocardial infarction	145 (27.0)	608 (40.9)	<0.001
PCI	75 (14.0)	346 (23.3)	<0.001
CABG	56 (10.4)	292 (19.7)	<0.001
Device			<0.001
None	461 (86.0)	1084 (73.2)	
Pacemaker	40 (7.5)	106 (7.2)	
ICD	14 (2.6)	138 (9.3)	
Biventricular pacer (CRT)	6 (1.1)	32 (2.2)	
Biventricular pacer (CRT) and ICD	15 (2.8)	121 (8.2)	

Table 1 (Continued)

	Women	Men	*p*-value
Diabetes mellitus	162 (30.2)	483 (32.5)	0.342
COPD	71 (13.2)	275 (18.5)	0.006
Peripheral artery disease	50 (9.3)	173 (11.6)	0.161
Stroke	52 (9.7)	140 (9.4)	0.930
Medication			
ACE inhibitors or ARBs	367 (68.3)	1089 (73.3)	0.031
Target dose	69 (12.8)	192 (12.9)	1.000
Percent of optimal dose	25 [0, 50]	25 [0, 50]	0.223
Beta-blockers	424 (79.0)	1256 (84.6)	0.004
Target dose	42 (7.8)	75 (5.1)	0.025
Percent of optimal dose	25 [0, 50]	25 [0.1, 50]	0.371
MRAs	238 (44.3)	825 (55.6)	<0.001
Percent of optimal dose	25 [25, 25]	25 [25, 50]	0.019
Loop diuretics	536 (99.8)	1476 (99.4)	0.407
Digoxin	76 (14.2)	299 (20.1)	0.003
Laboratory			
Haemoglobin, g/dl	12.7 [11.5, 13.67]	13.6 [12.1, 14.8]	<0.001
Creatinine, mg/dl	1.01 [0.81, 1.28]	1.20 [1.00, 1.55]	<0.001
Urea, mmol/L	9.50 [6.90, 15.20]	11.50 [7.60, 18.57]	<0.001
eGFR (MDRD), ml/min/1.73 m^2	72.5 [55.7, 95.3]	60.1 [45.2, 74.8]	<0.001
Sodium, mmol/L	140 [137, 142]	140 [137, 142]	0.114
Potassium, mmol/L	4.10 [3.80, 4.50]	4.30 [4.00, 4.60]	<0.001
NT-proBNP, ng/L	4387 [2542, 8282]	3822 [2252, 7614]	0.044

Values are mean ± standard deviation, *n* (%), or median [interquartile range].
ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; BMI, body mass index; BP, blood pressure; CABG, coronary artery bypass graft; COPD, chronic obstructive pulmonary disease; CRT, cardiac resynchronization therapy; eGFR, estimated glomerular filtration rate (Modification of Diet in Renal Disease formula); ICD, implantable cardioverter defibrillator; LVEF, left ventricular ejection fraction; MRA, mineralocorticoid receptor antagonist; NT-proBNP, N-terminal pro-B-type natriuretic peptide; NYHA, New York Heart Association; PCI, percutaneous coronary intervention.

Table 2 Echocardiographic characteristics at baseline, stratified by sex, of BIOSTAT-CHF index cohort

	Women	Men	*p*-value
No. of subjects	537	1485	
LVEDD, cm	5.6 [5.1, 6.2]	6.2 [5.7, 6.8]	<0.001
LVESD, cm	4.5 [3.8, 5.2]	5.1 [4.5, 5.9]	<0.001
IVSd, cm	1.0 [0.9, 1.2]	1.0 [0.9, 1.2]	0.014
LVPWd, cm	1.0 [0.9, 1.1]	1.0 [0.9, 1.2]	0.052
LVEF, %	34.6 ± 11.8	30 ± 10	<0.001
HFrEF, *n* (%)	341 (72.1)	1114 (83.7)	<0.001
HFpEF, *n* (%)	60 (12.7)	60 (4.5)	<0.001
E/A	1.20 [0.75, 2.00]	1.40 [0.80, 2.38]	0.012
E/A > 2.5	27 (15.2)	98 (22.2)	0.063
LAD, cm	4.47 (0.80)	4.83 (0.78)	<0.001
LAD index, cm/m^2	2.56 (0.50)	2.44 (0.43)	<0.001
LV mass, g	219.90 (73.37)	276.14 (86.91)	<0.001
LV mass index, g/m^2	125.09 (42.01)	138.83 (44.25)	<0.001
LV hypertrophy, *n* (%)	289 (78.5)	678 (68.3)	<0.001

HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; IVSd, interventricular septum (diastole); LAD, left atrial diameter; LV, left ventricular; LVEDD, left ventricular end-diastolic diameter; LVEF, left ventricular ejection fraction; LVESD, left ventricular end-systolic diameter; LVPWd, left ventricular posterior wall (diastole).
Note: HFpEF is defined as LVEF ≥50%. HFrEF is defined as LVEF <40%, according to the most recent European Society of Cardiology (ESC) heart failure guidelines. LV mass is calculated using the American Society of Echocardiography (ASE) formula. LAD index and LV mass index are calculated dividing LAD and LV mass (respectively) for body surface area (m^2) as calculated using the DuBois formula, according to the most recent ASE/European Association for Cardiovascular Imaging guidelines. LV hypertrophy is defined as LV mass index ≥95 g/m^2 in women and ≥115 g/m^2 in men according to the most recent ESC heart failure guidelines.

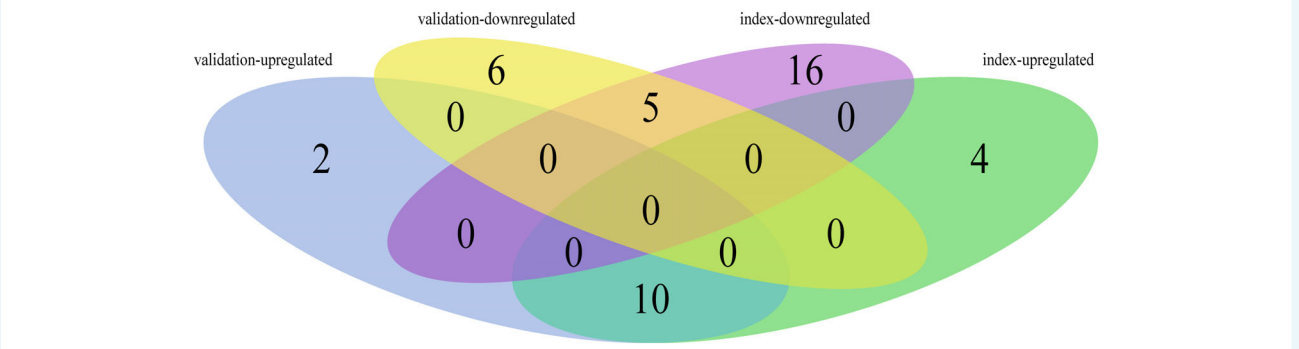


Figure 1 Venn diagram of number of significantly differentially expressed proteins in women versus men in the index cohort and validation cohort. CRNN, FABP4, FGF21, FGF23, GH1, IL1RN, LDLR, LEP, LPL, SCGB3A2 were the biomarkers up-regulated in women as compared to men in both cohorts; ACE2, HAO1, MMP3, PPY and REN were the biomarkers up-regulated in men as compared to women in both cohorts (biomarkers are mentioned using the official symbols of their cognate genes as provided by the HUGO Gene Nomenclature Committee, reported in online supplementary *Table S6*). Consistently, no proteins were up-regulated in one cohort but down-regulated in the other, nor vice versa.

In the matched subset from the index cohort, 11 biomarkers were relatively up-regulated in women, and 24 biomarkers in men (online supplementary *Figures S2* and *S3*).

The most prominently up-regulated biomarkers in women as compared with men were leptin (fold change 2.14, $p = 3.49 \times 10^{-20}$), and FABP4 (fold change 1.42, $p = 1.92 \times 10^{-5}$). The most strongly up-regulated biomarker in men as compared with women was MMP3 (fold change 1.85, $p = 3.45 \times 10^{-25}$).

Pathway over-representation analyses of differentially expressed biomarkers

Pathway over-representation analysis of the 14 up-regulated biomarkers in women in the index cohort revealed four pathways that were specifically enhanced in women with HF: (1) triglyceride catabolic process ($p = 3.56\text{x}10^{-6}$), (2) glycerolipid catabolic process ($p = 3.23 \times 10^{-7}$), (3) plasma lipoprotein assembly, remodelling, and clearance ($p = 1.73 \times 10^{-5}$), and (4) cellular response to low-density lipoprotein (LDL) particle stimulus ($p = 4.48 \times 10^{-7}$) (*Figure 3A*, online supplementary *Table S9*). Pathway over-representation analysis of the 21 up-regulated biomarkers in men in the index cohort revealed two over-expressed pathways in men: (1) neuro-inflammatory response ($p = 7.33 \times 10^{-5}$), and (2) regulation of neuro-inflammatory response ($p = 7.93 \times 10^{-6}$) (*Figure 3C*, online supplementary *Table S11*).

Association with outcome

Regression analysis showed a significant association of most of the differentially expressed biomarkers with all-cause mortality, before and after adjustment for selected covariates (online supplementary *Figure S5*). With the only exception of interleukin-1 receptor-like-2 and peroxiredoxin-1 (adjusted p for interaction = 0.024 and $p = 0.034$, respectively) that were significantly associated with adverse prognosis only in men, all differentially expressed biomarkers carried the same association with all-cause mortality in women and in men.

Validation cohort

Clinical characteristics

The baseline characteristics of patients included in the validation cohort are presented in online supplementary *Table S1*. As a whole, patients enrolled in the index cohort were younger, more often men, had a lower LVEF, and higher plasma NT-proBNP as compared with patients included in the validation cohort.[20]

Similar sex differences trends were observed as in the index cohort: women were slightly older (74 vs. 73 years old, $p = 0.006$), had a similar BMI (29 vs. 29 kg/m^2, $p = 0.103$), though with lower waist/hip ratio (0.93 vs. 1.00, $p < 0.001$), and presented less often with a history of coronary artery disease or atrial fibrillation (online supplementary *Table S1*). Women had a shorter history of HF (12 vs. 19 months, $p = 0.002$), a trend also observed in the index cohort, though they more frequently reported a history of HF hospitalization in the past year (32% vs. 24%, $p = 0.002$). Finally, baseline signs and symptoms of HF were similar in women and men, except peripheral oedema which was more prevalent in women (67% vs. 59%, $p = 0.002$).

Echocardiographic parameters

A baseline echocardiogram was performed in 91% of patients in the validation cohort (online supplementary *Table S5*). Sex differences in echocardiographic features (online supplementary *Table S4*) were similar to those observed in the index cohort. At presentation, men had higher LVEDD (57 vs. 51 mm, $p < 0.001$) and lower LVEF (40% vs. 44%, $p < 0.001$). Women showed larger baseline indexed left atrial dimensions (25 vs. 23 mm/m^2, $p < 0.001$), and slightly higher prevalence LV hypertrophy (71% vs. 68%, $p = 0.566$).

Biomarker concentrations

In the validation cohort, 12 biomarkers were up-regulated in women, 10 of which overlapped with the 14 biomarkers that were found to be up-regulated in women in the index cohort (*Figures 1*

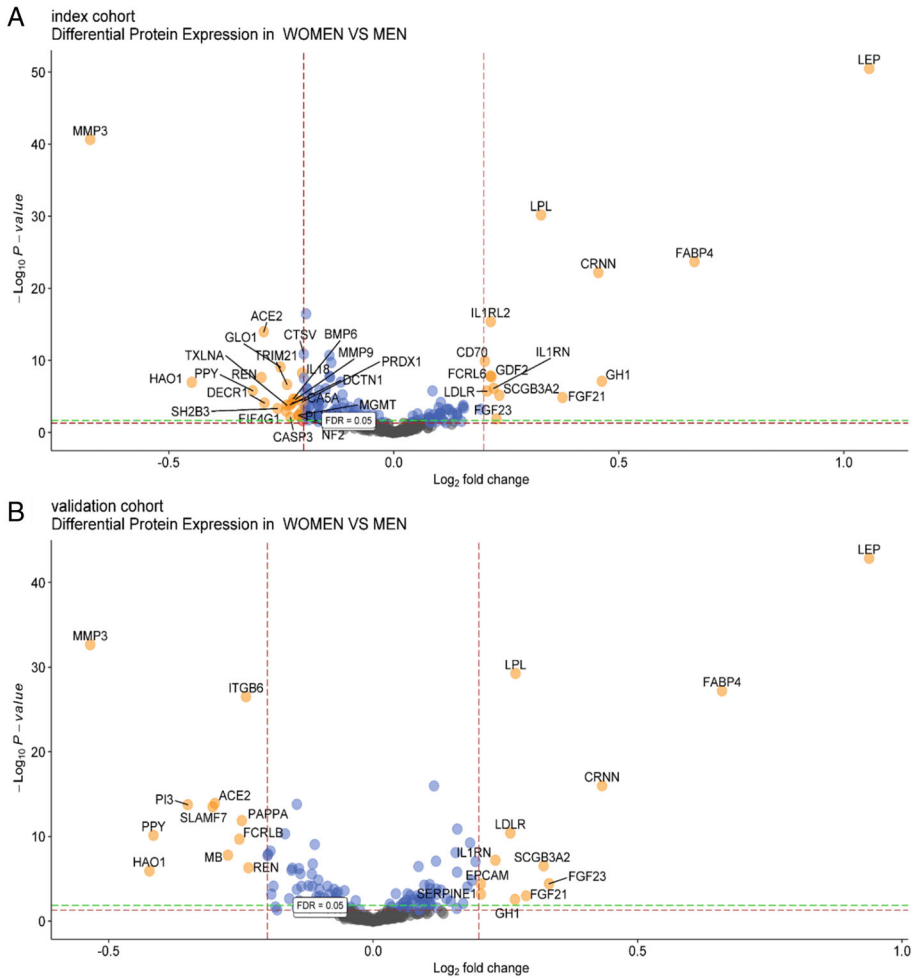


Figure 2 Volcano plots of differential protein expression in women versus men. In the *y*-axis is displayed *p*-value, on the x-axis effect size (positive, right-hand side = up-regulated in women as compared to men; negative, left-hand side = up-regulated in men as compared to women). Labelled are the significant differentially expressed proteins. (*A*) BIOSTAT-CHF index cohort. (*B*) BIOSTAT-CHF validation cohort. Proteins are mentioned using the official symbols of their cognate genes as provided by the HUGO Gene Nomenclature Committee (HGNC), reported in online supplementary *Table S6*.

and *2B*, online supplementary *Table S8* and *Figures S1*). Eleven biomarkers were up-regulated in men as compared to women, 5 of which overlapped with the 21 biomarkers from the index cohort. The strongest up-regulated biomarkers in women were the same as in the index cohort, leptin (fold change 1.92, $p = 4.93 \times 10^{-41}$), and FABP4 (fold change 1.57, $p = 5.59 \times 10^{-26}$). As in the index cohort, the most prominently up-regulated biomarker in men as compared with women was MMP3 (fold change 1.45, $p = 3.91 \times 10^{-31}$).

Sensitivity analysis

Baseline characteristics of the matched subset from the validation cohort are displayed in online supplementary *Table S3*.

In the matched subset from the validation cohort, 8 biomarkers were relatively up-regulated in women, and 14 biomarkers in men (online supplementary *Figures S2* and *S4*).

The most prominently up-regulated biomarkers in women as compared with men were leptin (fold change 1.80, $p = 1.53 \times 10^{-9}$), and FABP4 (fold change 1.48, $p = 6.01 \times 10^{-6}$). The most strongly up-regulated biomarker in men as compared with women was MMP3 (fold change 1.46, $p = 5.62 \times 10^{-11}$).

Pathway over-representation analyses of differentially expressed biomarkers

Pathway over-representation analysis revealed four activated pathways in women with HF from the validation cohort, with complete overlap with those from the index cohort: (1) triglyceride catabolic process ($p = 2.16 \times 10^{-6}$), (2) glycerolipid catabolic process ($p = 1.61 \times 10^{-7}$), (3) plasma lipoprotein assembly ($p = 1.05 \times 10^{-5}$), remodelling, and clearance, and (4) cellular response to LDL particle stimulus ($p = 2.71 \times 10^{-7}$) (*Figure 3B*, online supplementary *Table S10*). The up-regulated biomarkers in men in

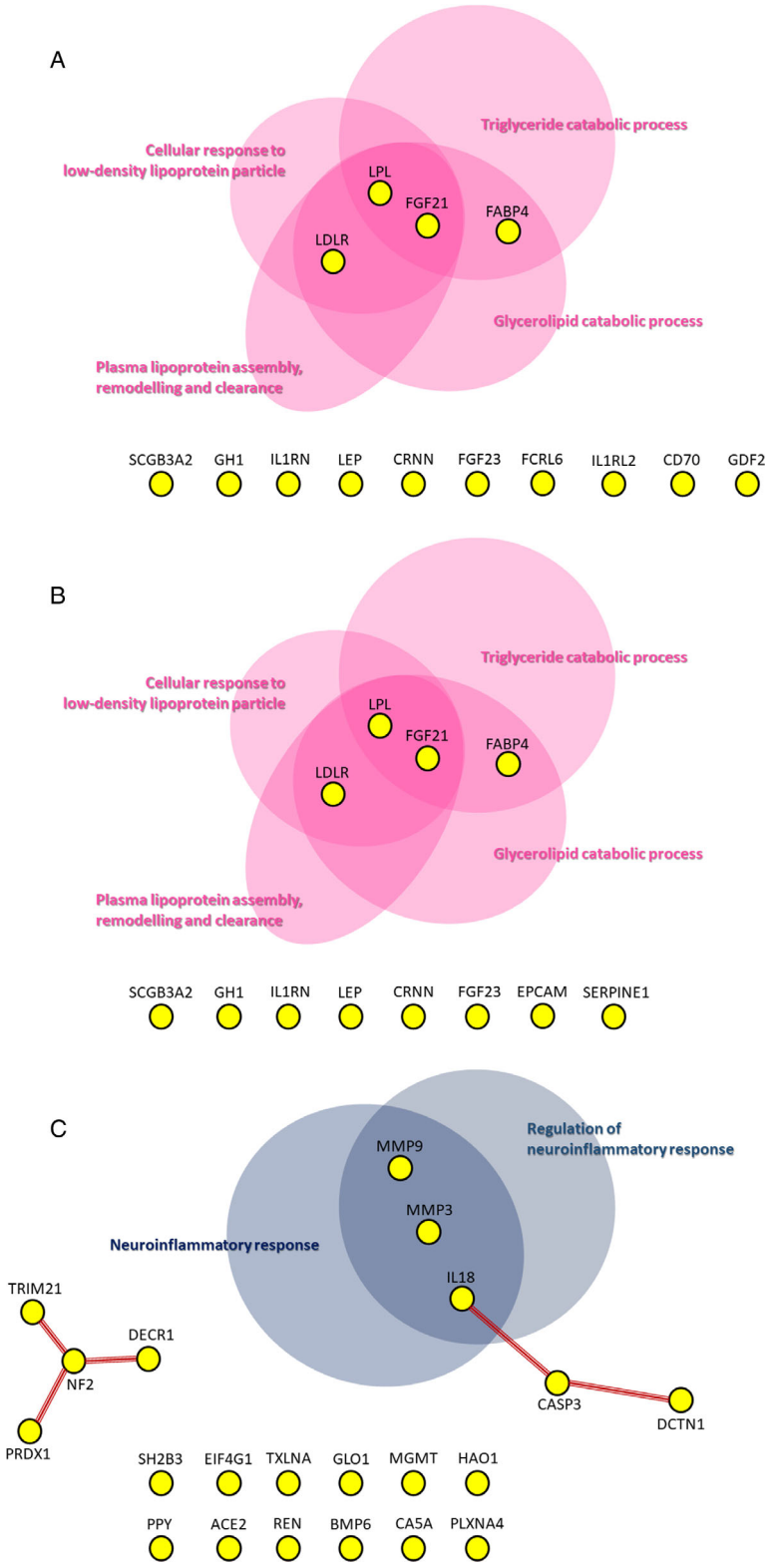


Figure 3 Pathophysiological pathways related to proteins that were up-related in women relative to men (*A*, index cohort; *B*, validation cohort) and men relative to women (*C*, index cohort; N/B equivalent part D for validation cohort is not provided because no pathway was significantly over-represented.) in heart failure. Proteins are depicted as yellow nodes, linking pathways are displayed as clusters labelled with the corresponding pathway name, red lines represent physical interactions between proteins. Proteins are mentioned using the official symbols of their cognate genes as provided by the HUGO Gene Nomenclature Committee (HGNC), reported in online supplementary *Table S6*.

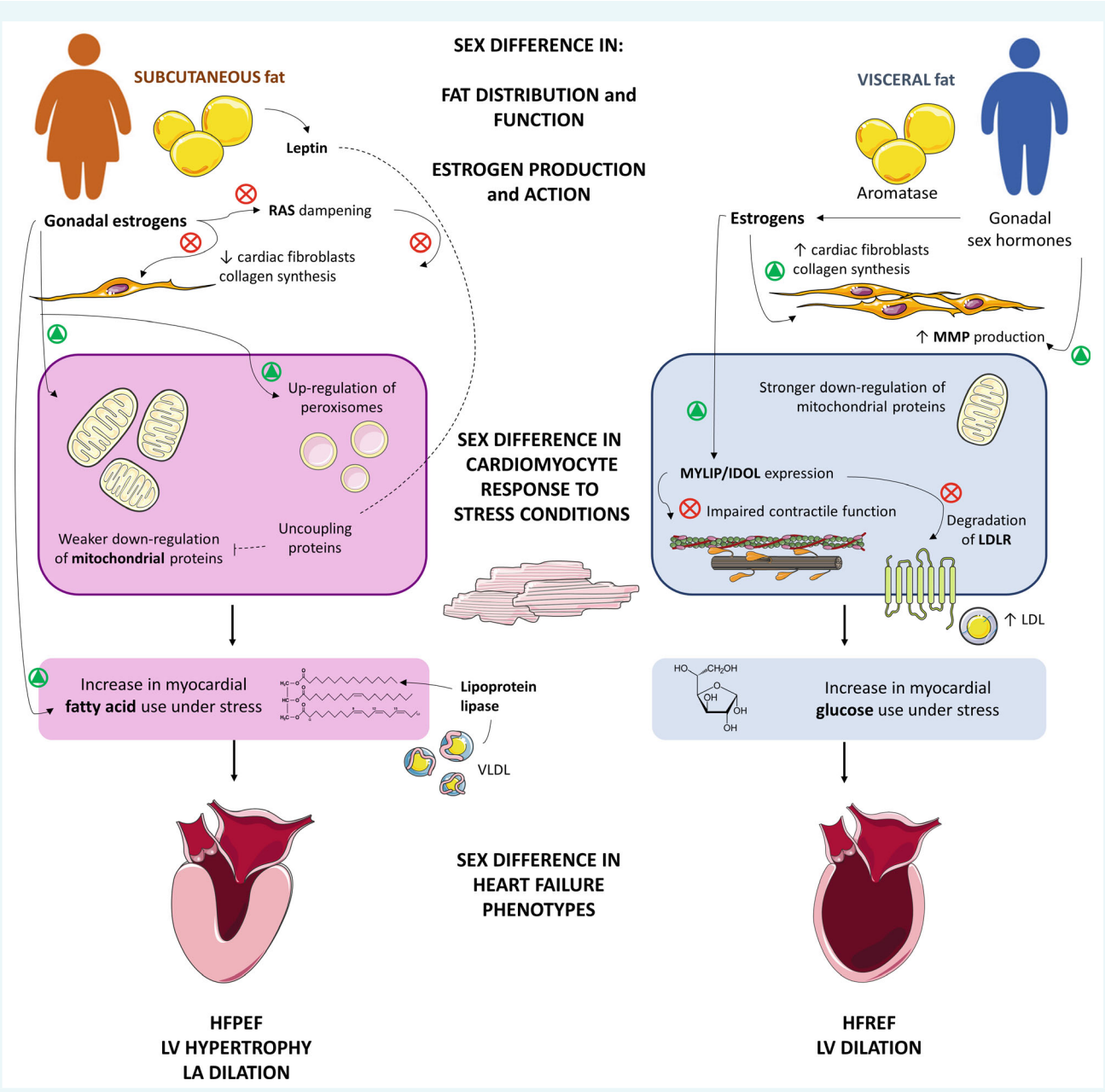


Figure 4 Sex differences in heart failure pathophysiology: not only a disease of an organ, but a disease of an organism. HFPEF, heart failure with preserved ejection fraction; HFREF, heart failure with reduced ejection fraction; LA, left atrial; LDL, low-density lipoprotein; LDLR, low-density lipoprotein receptor; MMP, matrix metalloproteinase; LV, left ventricular; MYLIP/IDOL, myosin regulatory light chain interacting protein/inducible degrader of low-density lipoprotein receptor; RAS, renin–angiotensin system; VLDL, very low-density lipoprotein.

the validation cohort were too few to yield results from pathway over-representation analysis.

Association with outcome

Most of the differentially expressed biomarkers were significantly associated with all-cause mortality, before and after adjustment for selected covariates (online supplementary *Figure S5*). Growth hormone-1, FABP4 and myoglobin (adjusted p for interaction = 0.044, p = 0.004 and p = 0.002, respectively) showed a stronger association with adverse prognosis in men as compared to women. All other biomarkers carried a similar association with outcome in women and men.

Discussion

There are substantial differences between women and men with HF, though not much is known about the pathophysiology underlying these differences. Using a large amount of biomarkers

from different biological domains in two independent cohorts of patients with HF, we observed significant up-regulation of biomarkers associated with pathways involved in fatty acid metabolism and LDL clearance in women, and neuro-inflammatory response in men (*Graphical Abstract*). These findings are in line with existing evidence from clinical and basic science studies (*Figure 4*).

Sex differences in heart failure

Lipid storage and fatty acid metabolism

Numerous studies documented that women have higher body fat mass, preferential subcutaneous fat distribution and fat accumulation in the hip region, while men tend to have higher visceral adipose tissue in the abdominal region.[7,15,28] These differences are also observed in our cohorts, with women having lower waist/hip ratio, despite similar BMI as compared to men.

Besides storage, adipose tissue exerts endocrine functions, secreting leptin, more expressed in women, or metabolizing sex hormones, with the aromatase activity being responsible for most oestrogen production in men.[15,29] Recent evidence suggests that sex-specific molecular mechanisms can influence glucose and lipid metabolism, as well as cardiomyocyte energy metabolism and cardiac function.[15] The major contributors of sex dimorphism in energy homeostasis appear to be the effects of oestrogens and androgens.[30,31] Oestrogens are either produced by gonadal tissue and peaking in fertile age (especially in women), or metabolized from testosterone by adipose tissue and increasing with age (especially in men).[29] Oestrogen receptors differ in location, abundance and effects between sexes.[15,29,32]

Past studies showed that fatty acids provide over 70% of the energy for cardiac function, with lipoprotein lipase catalyzing the hydrolysis of circulating triglycerides into fatty acids for cardiac metabolism.[33] Evidence from animal models and humans suggests a metabolic shift in cardiomyocytes under stress conditions, from fatty acids towards the more oxygen-efficient glucose.[29] However, due to oestrogen-mediated up-regulation of mitochondrial and peroxisome-dependent lipid utilization genes, this translation appears to be less pronounced in women, still relying on mitochondrial function and fatty acid oxidation during stress, exercise, pressure overload, or HF.[29,34,35] This oestrogen-mediated mitochondrial adaptation has been shown to contribute to the development of myocardial hypertrophy in female hearts.[31]

Key elements related to these findings can be found in our analysis, where fatty acid catabolic pathways, lipoprotein lipase and leptin are over-represented in women as compared to men. Notably, in our analysis, also FABP4 was up-regulated in women. FABP4 is an intracellular lipid chaperone involved in fatty acid uptake, transport, and metabolism. Besides incident HF, its circulating levels have been associated with LV hypertrophy, LV diastolic dysfunction, and myocardial intracellular lipid content in human studies, the latter effect partially reversed by FABP4 inhibition in mice.[36–39] All these features are concordant with the echocardiographic phenotype observed in women from our study. Moreover, fibroblast growth factor-21, over-expressed in women in our cohorts, has been shown to prevent induction of pro-oxidative pathways in the heart, while a member of the same family, fibroblast growth factor-23, also over-expressed in women from our study, was related to LV hypertrophy in animal models and humans.[40]

Low-density lipoprotein and lipoprotein metabolism

Low-density lipoprotein receptor (LDLR) is a major determinant of circulating levels of LDL.[41,42] The inducible degrader of LDLR (IDOL) mediates ubiquitylation and removal of membrane LDLR pool, therefore contributing to increased atherogenesis and ischaemic HF.[15,43] Interestingly, IDOL which is also known as the myosin regulatory light chain-interacting protein (MYLIP/IDOL), is induced by oestrogen exposure only in male cardiomyocytes, and exerts a second independent action, leading to dysregulation of myosin regulatory light chain function and to impaired contractile male cardiomyocytes only.[44] Consistently, in our analysis we observed that LDLR is over-expressed in women with HF, and tripartite motif-containing protein 21 (TRIM21), one of MYLIP/IDOL orthologs, is over-expressed in men.

Inflammation, renin–angiotensin system, tissue remodelling and cell death

Considerable evidence suggests greater induction of renin–angiotensin system (RAS)-related genes in men than in women, the latter showing decreased renin levels, ACE activity and angiotensin type 1 (AT1) receptor density as an effect of oestrogens.[45] Oestrogens also activate pathways antagonizing the effects of RAS, including natriuretic peptides.[46] Less is known about androgens, but testosterone seems to increase renin levels and ACE activity.[45] Consistently with these observations, we also detected a renin up-regulation in men, as compared with women.

Furthermore, sex differences in RAS also entail sex differences in tissue remodelling. In fact, angiotensin II, via AT1 receptors, mediates interleukin-18 (IL-18) and matrix metalloproteinase-9 (MMP9) expression, thus promoting inflammation and extracellular matrix degradation, ultimately leading to adverse tissue remodelling.[47,48] Sex differences in extracellular matrix deposition and destruction have also been described, with MMP9 being induced by testosterone and estrogens mediating collagen deposition only in male fibroblasts.[15,49] The inflammatory response related to extracellular matrix destruction (IL-18, MMP3, MMP9) is over-expressed in men in our study, confirming sex differences in the activity of this pathway in HF.

Interestingly, both IL-18 and caspase-3 are up-regulated in men in our analysis. These proteins have been shown to physically interact within the cell, and take part to pyroptosis, a programmed cell death that, unlike apoptosis, is accompanied by an inflammatory response, and plays a role in cardiovascular disease and HF.[50,51]

Potential treatment implications

The findings of this study appear in line with current knowledge about sex differences in treatment response in HF. The higher expression of renin in men may explain why they need higher doses of ACEi/ARBs to achieve outcome benefit in HFrEF, as compared to women.[11] Furthermore, leptin has been shown to increase aldosterone secretion, a response particularly marked in women,

thus providing a potential explanation for the observed benefit of MRAs in women with HFpEF.[12,52,53]

Until recently, most preclinical research in drug development was done using male animals and cells with unidentified sex. However, given the differences in the pathophysiology and outcomes of cardiovascular diseases observed in male and female animal models, a drug or gene modification may be effective in a male animal model and neutral or deleterious in females, or vice versa.[29] Our observation of different pathophysiological pathways in men and women with HF underscores the need for including an adequate number of subjects from both sexes in preclinical and clinical studies in HF, to ensure proper detection of sex difference in treatment response and side effects, and ultimately allow tailored optimization of drug regimens in women and in men. Interestingly, in our analysis on outcome we found that the differentially expressed biomarkers in women and men carried a modest and similar independent association with all-cause mortality. Taken together, our findings may point out relevant biological processes, e.g. related to energetic metabolism or inflammation and extracellular matrix deposition/degradation, that differently interact with HF pathophysiology in men and women. Tackling sex-specific HF mechanisms warrants further investigation with dedicated experiments and studies, to find new therapeutic targets towards a personalized approach to HF therapy.

Inference about the specificity of the sex differences in biomarkers and pathways to HF was beyond the aim of this paper. We studied differential biomarker expression in men and women with HF, without any control group of healthy subjects for comparison. However, analysis of another study comparing circulating biomarkers in men and women with other cardiovascular disease, showed some similarities, though not complete overlap, with our differentially expressed biomarkers.[54] Additionally, we observed considerable overlap of the differentially expressed biomarkers and associated pathways between index and validation cohort, despite the differences in baseline characteristics. Moreover, our sensitivity analysis yielded a similar set of differentially expressed biomarkers in women and men matched by age and LVEF, after adjustment for age and diabetes, though the overall magnitude of the differential expression was lower after matching and adjustment. Finally, even though HF aetiology was different between men and women, past research comparing biomarker patterns in different HF aetiologies, revealed different biomarkers and pathways as compared to our analysis on sex.[25] Overall, these findings suggests a common *core* of differential biomarker expression appearing attributable to sex, with further observed differences potentially explained by the different pathophysiological contexts.[55]

Limitations

First, BIOSTAT-CHF enrolled mainly Caucasian patients, thus limiting the generalizability of results to other ethnicities. Second, echocardiography at baseline was encouraged but not study-mandated, thus entailing a moderate amount of missing data of some parameters. Third, when compared to the actual prevalence of women in the HF population, women resulted to be under-represented in both cohorts; however, this is a common issue in HF studies, and the proportion of women enrolled in BIOSTAT-CHF was similar to most HF studies. Fourth, sex hormones were not included in the panels of assessed biomarkers, thus limiting the direct inference on their potential action on other biological pathways. Furthermore, no detailed information about concomitant hormone replacement therapy was available. Fifth, though containing 363 unique proteins, the set of biomarkers analysed encompassed a limited range of biological process, thus introducing a selection bias. Finally, we performed an analysis on circulating biomarkers to gain insights on sex differences in pathogenic mechanisms in HF. From this analysis we cannot draw conclusions about undergoing pathogenic processes within the heart itself. However, because of the demonstrated complex interplay between the heart and other organs (e.g. kidney, gut, brain, skeletal muscle) in HF, we think of HF as a disease of an organism rather than of an organ. In this perspective, circulating biomarkers are very appropriate to study sex differences in this complex disease.

Conclusion

In two large independent cohorts of HF patients, biomarkers associated with lipid metabolic pathways were up-regulated in women, while biomarkers associated with neuro-inflammatory response were up-regulated in men. Unravelling the interplay between these sex-related biological differences with HF pathophysiology may help to better understand sex differences in clinical phenotype observed in HF.

Supplementary Information

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

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References

1. Roger VL. Epidemiology of heart failure. *Circ Res*. 2013;**113**:646–59.
2. Lloyd-Jones DM, Larson MG, Leip EP, Beiser A, D'Agostino RB, Kannel WB, et al.; Framingham Heart Study. Lifetime risk for developing congestive heart failure: the Framingham Heart Study. *Circulation* 2002;**106**:3068–72.
3. Tsang W, Alter DA, Wijeysundera HC, Zhang T, Ko DT. The impact of cardiovascular disease prevalence on women's enrollment in landmark randomized cardiovascular trials: a systematic review. *J Gen Intern Med*. 2012;**27**:93–8.
4. Jin X, Chandramouli C, Allocco B, Gong E, Lam CSP, Yan LL. Women's participation in cardiovascular clinical trials from 2010 to 2017. *Circulation*. 2020;**141**:540–8.
5. Whitelaw S, Sullivan K, Eliya Y, Alruwayeh M, Thabane L, Yancy CW, et al. Trial characteristics associated with under-enrolment of females in randomized controlled trials of heart failure with reduced ejection fraction: a systematic review. *Eur J Heart Fail* 2021;**23**:15–24.
6. Lam CSP, Arnott C, Beale AL, Chandramouli C, Hilfiker-Kleiner D, Kaye DM, et al. Sex differences in heart failure. *Eur Heart J* 2019;**40**:3859-68c.
7. Packer M, Lam CSP, Lund LH, Maurer MS, Borlaug BA. Characterization of the inflammatory-metabolic phenotype of heart failure with a preserved ejection fraction: a hypothesis to explain influence of sex on the evolution and potential treatment of the disease. *Eur J Heart Fail*. 2020;**22**:1551–67.
8. Meyer S, Brouwers FP, Voors AA, Hillege HL, de Boer RA, Gansevoort RT, et al. Sex differences in new-onset heart failure. *Clin Res Cardiol* 2015;**104**:342–50.
9. Wilkoff BL, Birnie D, Gold MR, Hersi AS, Jacobs S, Gerritse B, et al. Differences in clinical characteristics and reported quality of life of men and women undergoing cardiac resynchronization therapy. *ESC Heart Fail* 2020;**7**:2972–82.
10. Lam CS, Donal E, Kraigher-Krainer E, Vasan RS. Epidemiology and clinical course of heart failure with preserved ejection fraction. *Eur J Heart Fail*. 2011;**13**:18–28.
11. Santema BT, Ouwerkerk W, Tromp J, Sama IE, Ravera A, Regitz-Zagrosek V, et al. Identifying optimal doses of heart failure medications in men compared with women: a prospective, observational, cohort study. *Lancet* 2019;**394**:1254–63.
12. Solomon SD, Claggett B, Lewis EF, Desai A, Anand I, Sweitzer NK, et al.; TOPCAT Investigators. Influence of ejection fraction on outcomes and efficacy of spironolactone in patients with heart failure with preserved ejection fraction. *Eur Heart J* 2016;**37**:455–62.
13. Solomon SD, McMurray JJV, Anand IS, Ge J, Lam CSP, Maggioni AP, et al.; PARAGON-HF Investigators and Committees. Angiotensin-neprilysin inhibition in heart failure with preserved ejection fraction. *N Engl J Med* 2019;**381**:1609–20.
14. Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JG, Coats AJ, et al. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: the Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC). Developed with the special contibution of the Heart Failure Association (HFA) of the ESC. *Eur J Heart Fail*. 2016;**18**:891–975.
15. Gerdts E, Regitz-Zagrosek V. Sex differences in cardiometabolic disorders. *Nat Med*. 2019;**25**:1657–66.
16. Suthahar N, Meems LMG, Ho JE, de Boer RA. Sex-related differences in contemporary biomarkers for heart failure: a review. *Eur J Heart Fail*. 2020;**22**:775–88.
17. DeFilippis EM, Van Spall HGC. Is it time for sex-specific guidelines for cardiovascular disease? *J Am Coll Cardiol*. 2021;**78**:189–92.
18. Kirschner MW. The meaning of systems biology. *Cell*. 2005;**121**:503–4.
19. Ideker T, Galitski T, Hood L. A new approach to decoding life: systems biology. *Annu Rev Genomics Hum Genet*. 2001;**2**:343–72.
20. Voors AA, Anker SD, Cleland JG, Dickstein K, Filippatos G, van der Harst P, et al. A systems BIOlogy study to TAilored treatment in chronic heart failure: rationale, design, and baseline characteristics of BIOSTAT-CHF. *Eur J Heart Fail* 2016;**18**:716–26.
21. Assarsson E, Lundberg M, Holmquist G, Bjorkesten J, Thorsen SB, Ekman D, et al. Homogenous 96-plex PEA immunoassay exhibiting high sensitivity, specificity, and excellent scalability. *PLoS One* 2014;**9**:e95192.
22. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, et al. Limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res* 2015;**43**:e47.
23. Tromp J, Voors AA, Sharma A, Ferreira JP, Ouwerkerk W, Hillege HL, et al. Distinct pathological pathways in patients with heart failure and diabetes. *JACC Heart Fail* 2020;**8**:234–42.
24. Tromp J, Westenbrink BD, Ouwerkerk W, van Veldhuisen DJ, Samani NJ, Ponikowski P, et al. Identifying pathophysiological mechanisms in heart failure with reduced versus preserved ejection fraction. *J Am Coll Cardiol* 2018;**72**:1081–90.
25. Sama IE, Woolley RJ, Nauta JF, Romaine SPR, Tromp J, Ter Maaten JM, et al. A network analysis to identify pathophysiological pathways distinguishing ischaemic from non-ischaemic heart failure. *Eur J Heart Fail* 2020;**22**:821–33.
26. Bindea G, Galon J, Mlecnik B. CluePedia Cytoscape plugin: pathway insights using integrated experimental and in silico data. *Bioinformatics*. 2013;**29**:661–3.
27. Bindea G, Mlecnik B, Hackl H, Charoentong P, Tosolini M, Kirilovsky A, et al. ClueGO: a Cytoscape plug-in to decipher functionally grouped gene ontology and pathway annotation networks. *Bioinformatics* 2009;**25**:1091–3.
28. Liu J, Fox CS, Hickson DA, May WD, Hairston KG, Carr JJ, et al. Impact of abdominal visceral and subcutaneous adipose tissue on cardiometabolic risk factors: the Jackson heart study. *J Clin Endocrinol Metab* 2010;**95**:5419–26.
29. Ventura-Clapier R, Dworatzek E, Seeland U, Kararigas G, Arnal JF, Brunelleschi S, et al. Sex in basic research: concepts in the cardiovascular field. *Cardiovasc Res* 2017;**113**:711–24.
30. Mauvais-Jarvis F. Sex differences in metabolic homeostasis, diabetes, and obesity. *Biol Sex Differ*. 2015;**6**:14.
31. Dworatzek E, Mahmoodzadeh S, Schubert C, Westphal C, Leber J, Kusch A, et al. Sex differences in exercise-induced physiological myocardial hypertrophy are modulated by oestrogen receptor beta. *Cardiovasc Res* 2014;**102**:418–28.
32. Fliegner D, Schubert C, Penkalla A, Witt H, Kararigas G, Dworatzek E, et al. Female sex and estrogen receptor-beta attenuate cardiac remodeling and apoptosis in pressure overload. *Am J Physiol Regul Integr Comp Physiol* 2010;**298**:R1597–606.
33. Augustus AS, Kako Y, Yagyu H, Goldberg IJ. Routes of FA delivery to cardiac muscle: modulation of lipoprotein lipolysis alters uptake of TG-derived FA. *Am J Physiol Endocrinol Metab*. 2003;**284**:E331–9.
34. Kararigas G, Dworatzek E, Petrov G, Summer H, Schulze TM, Baczko I, et al. Sex-dependent regulation of fibrosis and inflammation in human left ventricular remodelling under pressure overload. *Eur J Heart Fail* 2014;**16**:1160–7.
35. Kadkhodayan A, Lin CH, Coggan AR, Kisrieva-Ware Z, Schechtman KB, Novak E, et al. Sex affects myocardial blood flow and fatty acid substrate metabolism in humans with nonischemic heart failure. *J Nucl Cardiol* 2017;**24**:1226–35.
36. Djousse L, Bartz TM, Ix JH, Kochar J, Kizer JR, Gottdiener JS, et al. Fatty acid-binding protein 4 and incident heart failure: the Cardiovascular Health Study. *Eur J Heart Fail* 2013;**15**:394–9.
37. Engeli S, Utz W, Haufe S, Lamounier-Zepter V, Pofahl M, Traber J, et al. Fatty acid binding protein 4 predicts left ventricular mass and longitudinal function in overweight and obese women. *Heart* 2013;**99**:944–8.
38. Fuseya T, Furuhashi M, Yuda S, Muranaka A, Kawamukai M, Mita T, et al. Elevation of circulating fatty acid-binding protein 4 is independently associated with left ventricular diastolic dysfunction in a general population. *Cardiovasc Diabetol* 2014;**13**:126.
39. Rodriguez-Calvo R, Girona J, Rodriguez M, Samino S, Barroso E, de Gonzalo-Calvo D, et al. Fatty acid binding protein 4 (FABP4) as a potential biomarker reflecting myocardial lipid storage in type 2 diabetes. *Metabolism* 2019;**96**:12–21.
40. Itoh N, Ohta H, Nakayama Y, Konishi M. Roles of FGF signals in heart development, health, and disease. *Front Cell Dev Biol*. 2016;**4**:110.
41. Tolleshaug H, Goldstein JL, Schneider WJ, Brown MS. Posttranslational processing of the LDL receptor and its genetic disruption in familial hypercholesterolemia. *Cell*. 1982;**30**:715–24.

42. Brown MS, Goldstein JL. A receptor-mediated pathway for cholesterol homeostasis. *Science*. 1986;**232**:34–47.
43. Sorrentino V, Nelson JK, Maspero E, Marques ARA, Scheer L, Polo S, et al. The LXR-IDOL axis defines a clathrin-, caveolae-, and dynamin-independent endocytic route for LDLR internalization and lysosomal degradation. *J Lipid Res* 2013;**54**:2174–84.
44. Kararigas G, Bito V, Tinel H, Becher E, Baczko I, Knosalla C, et al. Transcriptome characterization of estrogen-treated human myocardium identifies myosin regulatory light chain interacting protein as a sex-specific element influencing contractile function. *J Am Coll Cardiol* 2012;**59**:410–7.
45. Komukai K, Mochizuki S, Yoshimura M. Gender and the renin-angiotensin-aldosterone system. *Fundam Clin Pharmacol*. 2010;**24**:687–98.
46. Nicolaou PA. Sex differences in heart failure medications targeting the renin-angiotensin-aldosterone system. *Eur J Pharmacol*. 2021;**897**:173961.
47. Valente AJ, Yoshida T, Murthy SN, Sakamuri SS, Katsuyama M, Clark RA, et al. Angiotensin II enhances AT1-Nox1 binding and stimulates arterial smooth muscle cell migration and proliferation through AT1, Nox1, and interleukin-18. *Am J Physiol Heart Circ Physiol* 2012;**303**:H282–96.
48. Anzai A, Anzai T, Nagai S, Maekawa Y, Naito K, Kaneko H, et al. Regulatory role of dendritic cells in postinfarction healing and left ventricular remodeling. *Circulation* 2012;**125**:1234–45.
49. Natoli AK, Medley TL, Ahimastos AA, Drew BG, Thearle DJ, Dilley RJ, et al. Sex steroids modulate human aortic smooth muscle cell matrix protein deposition and matrix metalloproteinase expression. *Hypertension* 2005;**46**:1129–34.
50. Wang Q, Wu J, Zeng Y, Chen K, Wang C, Yang S, et al. Pyroptosis: a pro-inflammatory type of cell death in cardiovascular disease. *Clin Chim Acta* 2020;**510**:62–72.
51. Zhou W, Chen C, Chen Z, Liu L, Jiang J, Wu Z, et al. NLRP3: a novel mediator in cardiovascular disease. *J Immunol Res* 2018;**2018**:5702103.
52. Flanagan DE, Vaile JC, Petley GW, Phillips DI, Godsland IF, Owens P, et al. Gender differences in the relationship between leptin, insulin resistance and the autonomic nervous system. *Regul Pept* 2007;**140**:37–42.
53. Packer M. Leptin-aldosterone-neprilysin axis: identification of its distinctive role in the pathogenesis of the three phenotypes of heart failure in people with obesity. *Circulation*. 2018;**137**:1614–31.
54. Eggers KM, Lindhagen L, Baron T, Erlinge D, Hjort M, Jernberg T, et al. Sex-differences in circulating biomarkers during acute myocardial infarction: an analysis from the SWEDEHEART registry. *PLoS One* 2021;**16**:e0249830.
55. De With RR, Artola Arita V, Nguyen BO, Linz D, Ten Cate H, Spronk H, et al. Different circulating biomarkers in women and men with paroxysmal atrial fibrillation: results from the AF-RISK and RACE V studies. *Europace* 2021;**24**:193–201.