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DOI:
10.1016/j.jacbts.2023.11.006

JACC: BASIC TO TRANSLATIONAL SCIENCE VOL. ■, NO. ■, 2024

ORIGINAL RESEARCH

Cardiac Substrate Utilization and Relationship to Invasive Exercise Hemodynamic Parameters in HFpEF

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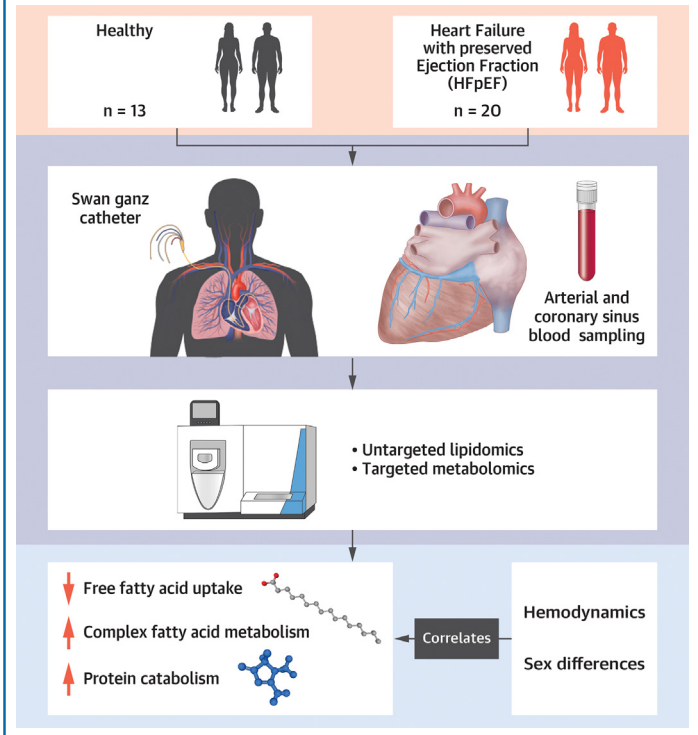
VISUAL ABSTRACT



O'Sullivan JF, et al. J Am Coll Cardiol Basic Trans Science. 2024;■(■):■–■.

HIGHLIGHTS

- **HFpEF is a complex clinical syndrome with incompletely understood pathophysiology. The recent demonstration of a beneficial impact of sodium glucose co-transporter 2 inhibitor therapy in HFpEF has drawn attention to the potential role of deranged cardiac metabolism, although this remains poorly understood.**
- **Arterial and coronary sinus blood samples were collected in nonfasted HFpEF patients and healthy control subjects, in conjunction with detailed physiologic phenotyping. Lipidomic and metabolomic analyses were performed to develop a comprehensive profile of myocardial metabolism.**
- **The data identify the metabolic profile of the HFpEF heart in the nonfasted state as one of less free fatty acid use, with a concomitant transition to more complex lipid generation, and protein catabolism. This effect was more pronounced in female HFpEF hearts, which also displayed a narrower repertoire of lipid class use compared to male hearts. Finally, there was an interdependence of lipid use with cardiac hemodynamics, particularly pulmonary pressures and cardiac index.**
- **Taken together, these data illustrate a complex pattern of metabolic remodeling in the HFpEF heart. Further studies are required to evaluate the impact of recently identified metabolic therapies on myocardial metabolism and their physiologic consequences.**

ISSN 2452-302X https://doi.org/10.1016/j.jacbts.2023.11.006

ABBREVIATIONS AND ACRONYMS

ART = radial artery

BCAA = branched-chain amino acid

BMI = body mass index

Cer = ceramide

CmE = campesterol ester

CS = coronary sinus

FA = free fatty acid

FC = fold change

FDR = false discovery rate

Hex1Cer = hexosylceramide

HF = heart failure

HFpEF = heart failure with preserved ejection fraction

HFrEF = heart failure with reduced ejection fraction

H_2FPEF = standardized HFpEF score

LPC = lysophosphatidylcholine

LPE = lysophosphatidylethanolamine

MAP = mean arterial pressure

MLAC = medium- and long-chain acylcarnitines

OAHFA = (*O*-acyl)-omega-hydroxy fatty acids

PC = phosphatidylcholine

PCWP = pulmonary capillary wedge pressure

PI = phosphatidylinositol

PS = phosphatidylserine

rest_PAS = resting pulmonary systolic pressure

TCA = tricarboxylic acid

TG = triacylglycerol

WE = wax monoester

SUMMARY

We conducted transcardiac blood sampling in healthy subjects and subjects with heart failure with preserved ejection fraction (HFpEF) to compare cardiac metabolite and lipid substrate use. We demonstrate that fatty acids are less used by HFpEF hearts and that lipid extraction is influenced by hemodynamic factors including pulmonary pressures and cardiac index. The release of many products of protein catabolism is apparent in HFpEF compared to healthy myocardium. In subgroup analyses, differences in energy substrate use between female and male hearts were identified. (J Am Coll Cardiol Basic Trans Science 2024;■:■–■) © 2024 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

The recent demonstration of favorable effects of sodium glucose cotransporter inhibitors in both heart failure with reduced (HFrEF) and preserved (HFpEF) ejection fraction has highlighted the potential role of disturbed myocardial metabolism in HF progression.[1-3] To date, limited studies have quantified cardiac nutrient use by measuring transmyocardial concentration gradients in the failing and nonfailing heart[4] and in right ventricular myocardial tissue samples.[5] These studies reveal some commonality of changes in myocardial fatty acid (FA) metabolism in both HFpEF and HFrEF, despite significant differences in the prevalence of diabetes between groups, which is known to influence this metabolic pathway. This work also suggested a decreased capacity of human HFpEF hearts to use alternative fuels (glucose, ketones, and branched-chain amino acids [BCAAs]), consistent with a state of metabolic inflexibility. There was a consistent perturbation of metabolites in HFpEF hearts despite variability in obesity and other comorbidities, suggesting a common metabolic profile in HFpEF.[5]

Despite the progress made in understanding myocardial fuel use and energy generation in HFpEF, the interpretation of recent findings is limited by several important caveats. Studies conducted on myocardial samples from HFpEF patients were obtained from the right ventricular septum,[5] introducing the potentially confounding influences of differential embryologic origins of the right and left ventricles,[6-10] hemodynamic workloads,[11-14] and therefore their fuel use.[15,16] In addition, although tissue levels provide an important index of steady state metabolite levels, they do not necessary reflect the cardiac capacity for uptake/use as reflected by the arteriovenous concentration gradient.[4] Finally, interpretation of myocardial use must also be performed in the context of the physiologic and nutritional environment.[4] As such, there remains a fundamental lack of understanding of the relationship between perturbed hemodynamics and impaired fuel use in HFpEF.

In the current study, we interrogated myocardial fuel use using simultaneous arterial and coronary sinus (CS) sampling in HFpEF and healthy control

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The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the Author Center.

Manuscript received October 10, 2023; revised manuscript received November 2, 2023, accepted November 2, 2023.

subjects. We applied quantitative assessment of metabolites (including central carbon pathways and energetic intermediates) and lipids. HFpEF and control subjects were comprehensively phenotyped physiologically, and disturbances of cardiac substrate use were evaluated in detail in the context of clinically relevant physiologic derangements. The primary objective of our study was to comprehensively characterize metabolic substrate turnover in the myocardium of HFpEF patients and healthy control subjects by measuring concentration differences between CS and radial artery (ART) blood.

METHODS

STUDY POPULATION. The study cohort included 20 patients with HFpEF and 13 healthy control subjects. HFpEF patients referred to D.M.K., J.M., and S.N. for investigation of exertional dyspnea in which HFpEF was suspected were consecutively invited to participate in the study. Healthy control subjects were recruited via advertising in the general community. Exclusion criteria included significant coronary artery disease that had not been revascularized; moderate or greater aortic or mitral valve disease; infiltrative, restrictive, or hypertrophic myocardial disease; pericardial constriction; or significant right ventricular disease (which could confound an investigation focused on a left ventricular diastolic dysfunction-predominant phenotype). Patients with significant pulmonary disease including chronic obstructive pulmonary disease were also excluded. A diagnosis of HFpEF was established by a pulmonary capillary wedge pressure (PCWP) of ≥15 mm Hg at rest or ≥25 mm Hg during symptom-limited exercise[17,18] and complemented by a standardized HFpEF score (H_2FPEF) of ≥6.[19] Healthy volunteers were recruited from the general community and had no known history of significant comorbidities including cardiovascular, pulmonary, or other systemic diseases. The study was approved by the Alfred Hospital Research and Ethics Committee, and all participants provided written informed consent.

HEMODYNAMICS AND TRANSCARDIAC BLOOD SAMPLING. Catheterization studies were conducted in the nonfasted, nonsedated state, and background medications were continued as previously described by us.[20] We performed arterial and CS sampling at rest before exercise testing. In the HFpEF cohort, consecutive patients with suspected HFpEF who agreed to have blood samples collected underwent sampling before final hemodynamic confirmation. The samples from patients with subsequently confirmed HFpEF form the basis of this study. The exercise testing protocol is described in our previous work.[20] In brief, A 3-F arterial line was placed in a radial or brachial artery for blood pressure measurement and blood sampling. An introducer sheath was placed in the right internal jugular or a brachial vein for placement of central venous catheters. The CS was cannulated under fluoroscopy, with the tip placed at least 2 cm within the CS as confirmed by radiographic contrast injection. Subsequent to sampling, a thermodilution catheter was advanced under fluoroscopy for the measurement of right atrial pressure, pulmonary artery pressure, and PCWP. The wedge position was confirmed by fluoroscopy and pressure wave form, and the mean PCWP was measured at end expiration. Cardiac output was measured using thermodilution with measurements taken in triplicate.

Subjects then performed symptom-limited exercise in the supine position on a cycle ergometer mounted to the catheter table at incremental workloads of 0.3 W/kg, 0.6 W/kg, 1 W/kg, and 1.5 W/kg for 3 minutes each as tolerated. Hemodynamics were recorded at each workload, with cardiac output also measured at peak workload.

UNTARGETED LIPIDOMICS. The convention we use in the text for clarity is the fold change (FC) between efflux/CS and influx/ART, with an FC of <1 meaning that metabolites including lipids were lower in CS vs ART and extracted by the heart and a FC of >1 meaning release from the heart. A total of 30 μL human plasma was extracted using the methanol/methyl tert-butyl ether/water solvent system.[21] A full description is provided in the Supplemental Methods.

TARGETED METABOLOMICS ANALYSIS. Targeted liquid chromatography-triple quadripole-mass spectrometry analysis was used to detect a set of water-soluble metabolites in both positive and negative ionization modes using a Shimadzu Nexera LC-30AD liquid chromatography system (Sciex) coupled to a QTRAP 6500+ tandem mass spectrometer (Sciex) operating in multiple-reaction monitoring mode. A full description is provided in Supplemental Methods.

STATISTICAL ANALYSIS. All statistics analyses were performed in R (4.2.2).[22] Metabolomics, lipidomics, and FA data were $\log_2$-transformed and normalized by removing unwanted variation methods.[23,24] Differential abundance analyses for all data types were performed using the lmFit and eBayes functions in the limma R package.[25] A linear model was fitted for each metabolite, lipid, or FA to compare abundance levels between groups with the regression coefficient (β) presented. All comparative analyses were adjusted for $\log_2$-tranformed age. A subject (patient) block

factor was specified to account for individual effects via the duplicateCorrelation[26] function in limma. Significance is defined by a P value less than or equal to 0.05 by the 2-sided Student's t-test after adjustment for multiple comparisons. For the metabolomics pathways, pathways were annotated by the Kyoto Encyclopedia of Genes and Genomes,[27] and competitive gene set tests were performed using cameraPR[28] from limma. The dysregulated lipid classes and pathway were investigated using camera.[28] The lipids were classified based on LipidSearch criteria, and pathways were retrieved from the Small Molecule Pathway Database.[29,30] The analysis of lipid species reaction was performed in BioPAN,[31,32] a software curated by LIPID MAPS, which analyzes possible suppressed or activated chemical reactions given 2 cohorts of interests. False discovery rate (FDR) using the Benjamini-Hochberg method was used to calculate adjusted P values to account for multiple comparisons. R code was used in differential expression analysis.

RESULTS

CLINICAL AND HEMODYNAMIC FEATURES. Samples were obtained from the ART and CS (**Figure 1A**). The clinical and hemodynamic profiles of the control subjects and HFpEF patients are detailed in **Table 1**. Compared with healthy control volunteers, HFpEF patients were older and heavier ($P < 0.001$ and $P = 0.004$, respectively), and women were proportionately more common among HFpEF patients, although age, sex, and body mass index (BMI) were adjusted for in all statistical analyses.

MYOCARDIAL SUBSTRATE EXTRACTION AND RELEASE IN HEALTHY CONTROL SUBJECTS. In healthy control subjects, a complex group consisting of 29 metabolites was identified as being significantly (FDR: <0.05) extracted by the myocardium (**Figure 1B**, **Table 2**, Supplemental Tables 1 to 3) after correction for multiple comparisons. We further classified extracted molecules according to their functions. Creatine, a principal component of the myocardial energy metabolism system, was the most highly extracted metabolite in control hearts (FC: 0.59; $P = 1.08$ e-5). *Trans*-hydroxyproline, which plays an important role in collagen stability, was the next most highly consumed metabolite in control hearts (FC: 0.61; $P = 1.83$ e-5). Glutamate was the third most highly extracted metabolite by the normal myocardium (FC: 0.64; $P = 4.98$ e-5), consistent with recent reports of highly efficient cardiac glutamate extraction (up to 67%) from the circulation.[4]

The methylated form of a ketone body, acetoacetate, methylacetoacetate was the next most highly consumed (FC: 0.83; $P = 0.0014$) metabolite in control hearts. This was followed by xanthurenate, gluconolactone, and uridine; 2-aminoadipate, a lysine product, was also significantly extracted, as was tricarboxylic acid (TCA) cycle intermediate succinate, which accumulates during and regulates cardiac ischemia-reperfusion injury.[33] Consistent with prior reports,[4] the purine metabolite hypoxanthine was also significantly extracted (FC: 0.66; $P = 0.009$). The adenosine metabolite inosine (FC: 0.67; $P = 0.009$) was also significantly extracted during myocardial passage, as was thyroxine (FC: 0.8; $P = 0.01$), which undergoes transformation to its active form triiodothyronine in the heart. The saccharides fructose (FC: 0.77; $P = 0.03$) and glucose (FC: 0.8; $P = 0.036$) were also significantly consumed by the heart, and glucose is known to be extracted and used more under stress conditions.[34] Lactate, another important energetic substrate under stress conditions, was also extracted by control hearts (FC: 0.84; $P = 0.049$).

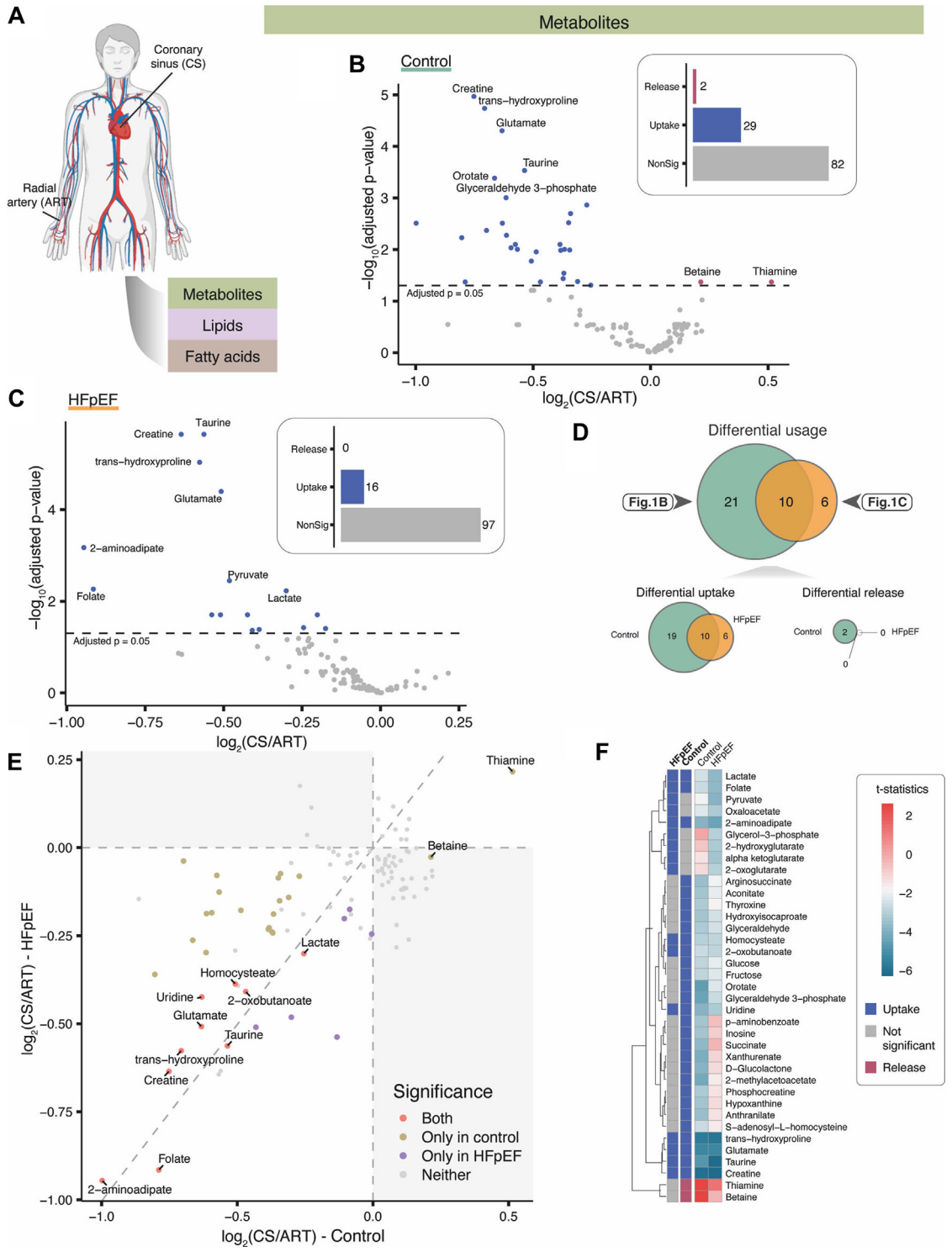
In contrast to the complex array of compounds extracted by the normal heart, only 2 metabolites were significantly released by control hearts: betaine (FC: 1.16; $P = 0.04$) and thiamine (FC: 1.4; $P = 0.04$) (**Figure 1C**, **Table 2**, Supplemental Datasets 1 to 3).

Although there was substantial overlap in the metabolites extracted by control and HFpEF hearts, there was a change in rank order (**Figures 1B and 1C**). As in control hearts, creatine was the most extensively extracted metabolite in HFpEF patients (FC: 0.64; $P = 2.28$ e-6) (**Figure 1C**, **Table 3**, Supplemental Datasets 1 to 3). Taurine, a sulfur amino acid associated with cardiomyopathy, was significantly extracted in control hearts (FC: 0.69; $P = 0.0003$) and more highly by HFpEF hearts (FC: 0.68; $P = 2.28$ e-6). Lactate was also more highly extracted by HFpEF hearts, as were aminoadipate, folate, and pyruvate.

Other metabolites were significantly extracted by HFpEF but not by control hearts. TCA cycle intermediates alpha ketoglutarate and oxaloacetate were significantly extracted by HFpEF but not by control hearts, as was the oncometabolite 2-hydroxyglutarate (FC: 0.69; $P = 0.019$), which is structurally similar to alpha ketoglutarate and known to accumulate in hypoxia.[35]

Metabolites extracted by both control and HFpEF hearts included creatine, taurine, *trans*-hydroxyproline, 2-aminoadipate, glutamate, folate, lactate, uridine, homocysteate, and 2-oxobutanoate (**Figure 1D**). Overall, there were fewer metabolites released/

FIGURE 1 **Healthy Control and HFpEF Patient Substrate Use**



(A) Schematic or blood sampling that was used for metabolomics and lipidomics assessment. (B) Healthy volunteer and HFpEF patient demographics. (C) Volcano plot of extracted and released metabolites in healthy control subjects. (D) Volcano plot of extracted and released metabolites in HFpEF. (E) Log fold change of CS/ART in control vs HFpEF hearts. (F) Summary of differential use, uptake, and release in control and HFpEF hearts. (G) Heatmap and dendrogram of extracted and released metabolites in control and HFpEF hearts. ART = radial artery; CS = coronary sinus; HFpEF = heart failure with preserved ejection fraction; NonSig = nonsignificant.

TABLE 1 Clinical Profiles

	Control Subjects (n = 13)	HFPEF Patients (n = 20)	*P* Value
Demographics			
Age, y	53 ± 8	70 ± 9	<0.001
Male/female	9/4	7/13	0.086
BMI, kg/m^2	26 ± 4	34 ± 9	0.004
Hypertension	–	15 (75)	
Atrial fibrillation	–	12 (60)	
Diabetes	–	6 (30)	
Coronary disease	–	4 (20)	
ACE/ARB	–	16 (80)	
Beta-blocker	–	9 (45)	
H_2FPEF score	0 (0-2)	6 (4-7)	<0.001
Echocardiography			
LVEF, %	63 ± 5	63 ± 5	0.98
LV mass index	70 ± 11	93 ± 26	0.004
LA volume index	29 ± 8	45 ± 14	0.002
Hemodynamics			
Heart rate, beats/min	61 ± 13	68± 14	0.21
SBP, mm Hg	135 ± 15	143 ± 21	0.21
Pulmonary artery, mean, mm Hg	14 ± 3	22 ± 8	<0.001
PCWP, mm Hg	9 ± 3	13 ± 5	0.016
Cardiac index, L/min/m^2	2.7 ± 0.5	2.5 ± 0.5	0.17
exercise PCWP, mm Hg	15 ± 4	31 ± 6	<0.001
exercise CI, L/min/m^2	7.3 ± 1.1	4.5 ± 1.7	<0.001

Values are mean ± SD, or n, n (%), median (Q1-Q3).

ACE = angiotensin-converting enzyme; ARB = angiotensin receptor blocker; BMI = body mass index; HFpEF = heart failure with preserved ejection fraction; LA = left atrium; LV = left ventricle; LVEF = left ventricular ejection fraction; PCWP = pulmonary capillary wedge pressure; SBP = systolic blood pressure.

extracted by HFpEF (0 of 16) compared to control (2 of 29) hearts (Figure 1F). Although there was significant overlap, there was a divergence of extraction of certain metabolites toward healthy hearts (inosine, succinate, xanthurenate, methylacetoacetate, phosphocreatine, hypoxanthine, and anthranilate) and toward HFpEF hearts (glycerol-3-phosphate, hydroxyglutarate, alpha ketoglutarate, and oxoglutarate) (Figure 1F).

TABLE 2 Top 10 Differentially Used Metabolites in Control Subjects (n = 13)

Metabolite	Fold Change	Average $\log_2$ Intensity	T-Statistic	Adjusted *P* Value
Creatine	0.59	20.72	-6.00	1.08×10^{-5}
Trans-hydroxyproline	0.61	19.90	-5.69	1.83×10^{-5}
Glutamate	0.65	21.25	-5.33	4.98×10^{-5}
Taurine	0.69	22.12	-4.78	2.95×10^{-4}
Orotate	0.63	19.98	-4.63	4.14×10^{-4}
Glyceraldehyde 3-phosphate	0.65	14.01	-4.33	9.91×10^{-4}
2-Methylacetoacetate	0.83	18.52	-4.19	1.37×10^{-3}
Xanthurenate	0.79	17.15	-4.04	2.01×10^{-3}
D-Gluconolactone	0.79	18.46	-3.89	3.03×10^{-3}
Uridine	0.65	18.71	-3.84	3.08×10^{-3}

TABLE 3 Top 10 Differentially Used Metabolites in HFpEF Patients (n = 20)

Metabolite	Fold Change	Average $\log_2$ Intensity	T-Statistic	Adjusted *P* Value
Creatine	0.64	20.72	-6.29	2.28×10^{-6}
Taurine	0.68	22.12	-6.22	2.28×10^{-6}
Trans-hydroxyproline	0.67	19.90	-5.76	9.28×10^{-6}
Glutamate	0.70	21.25	-5.31	4.02×10^{-5}
2-Aminoadipate	0.52	19.58	-4.49	6.73×10^{-4}
Pyruvate	0.72	21.03	-3.96	3.56×10^{-3}
Folate	0.53	15.04	-3.79	5.43×10^{-3}
Lactate	0.81	25.58	-3.72	5.91×10^{-3}
Alpha ketoglutarate	0.87	21.24	-3.26	1.98×10^{-2}
Oxaloacetate	0.70	17.23	-3.25	1.98×10^{-2}

HFpEF = heart failure with preserved ejection fraction.

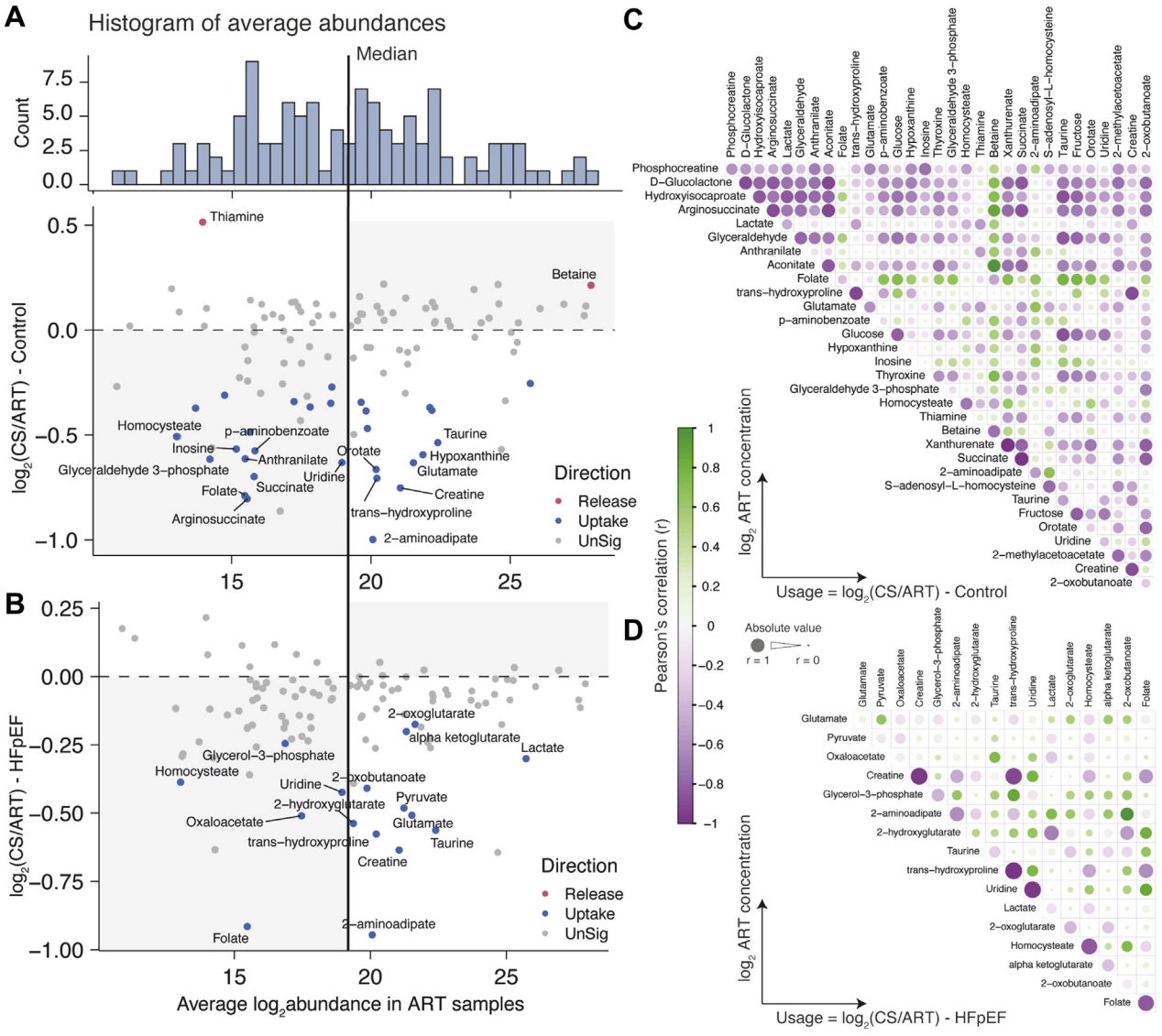
RELATIONSHIP OF METABOLITE EXTRACTION/RELEASE TO CIRCULATING CONCENTRATIONS. We next examined the overall range of abundances of fuel substrates (Figure 2A) and compared uptake/release dependent on circulating concentrations in control (Figure 2A) and HFpEF hearts (Figure 2B). As can be seen in Figure 2A, significantly extracted metabolites in control hearts were both high and low abundance, with the most extracted metabolite, 2-aminoadipate, having only a slightly higher than median concentration. Likewise, the 2 significantly released metabolites—thiamine and betaine—were not released in a concentration-dependent manner.

Likewise, in HFpEF hearts, uptake was independent of the prevailing circulating concentration (Figure 2B). Of the significantly extracted metabolites, lactate was the most abundant, and it was only relatively modestly extracted; conversely, the most extracted metabolites (2-aminoadipate, folate) had comparatively medium (2-aminoadipate) to low (folate) abundance.

In control subjects (Figure 2C), correlation of arterial concentrations of each metabolite with its uptake/release revealed that the majority of metabolites had inverse correlations of use with their own arterial concentration; that is, they were commonly extracted more in the face of lower circulating concentrations or extracted less when arterial concentrations were relatively higher. For example, arginosuccinate, aconitate, *trans*-hydroxyproline, xanthurenate, succinate, and creatine exhibited this pattern. Conversely, thiamine was released despite high arterial concentrations entering the heart. Certain metabolites had similar extraction/circulation relationships in control hearts, for example, phosphocreatine, gluconolactone, hydroxyisocaproate, and argininosuccinate.

In HFpEF hearts (Figure 2D), there were overall less common patterns of use across the metabolites.

FIGURE 2 **Arterial Concentration vs Use of Metabolites in Healthy and HFpEF Hearts**



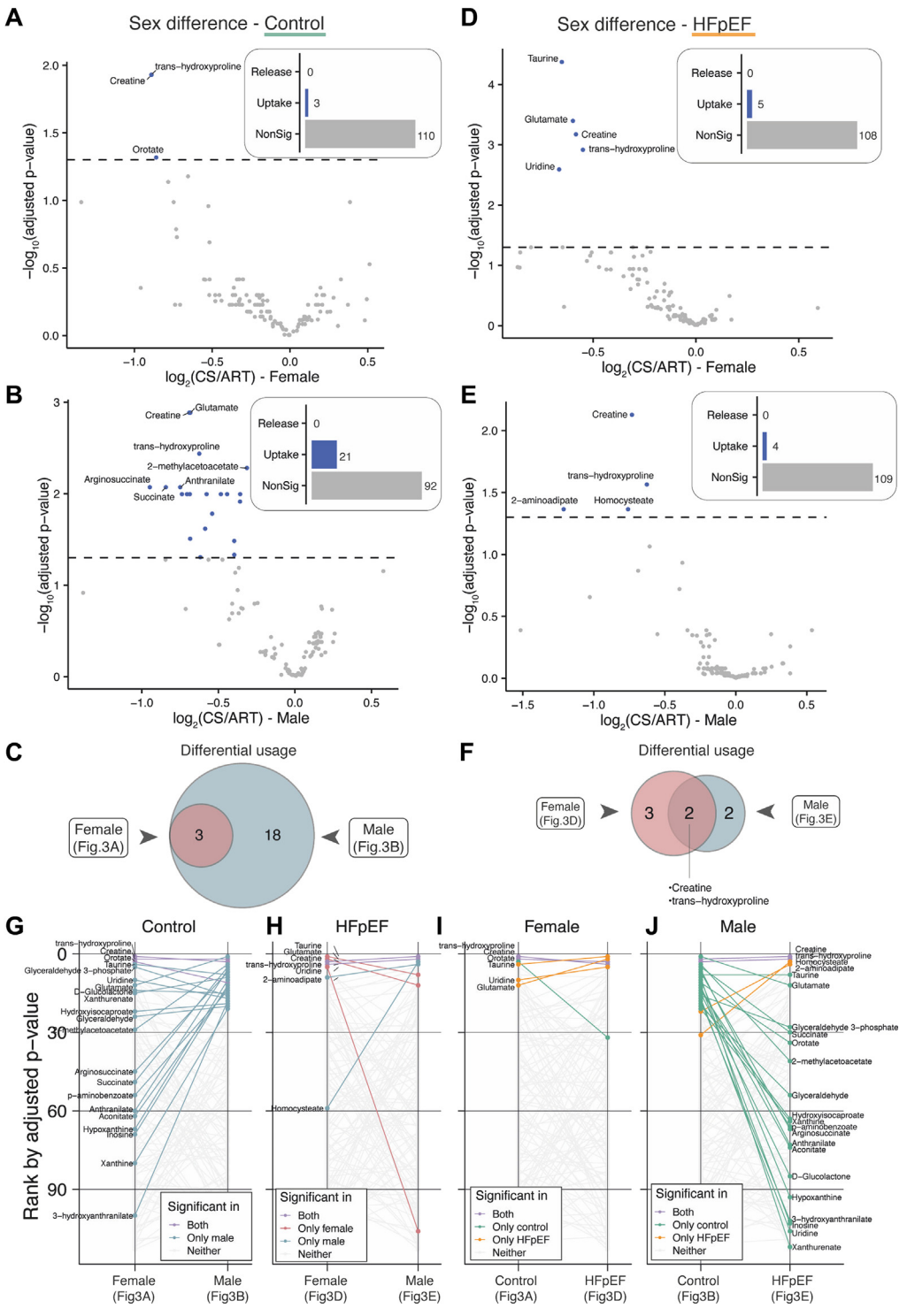
(A) Control hearts: plot of abundance (x-axis) vs $\log_2$ CS/ART, that is, extraction/release. (B) HFpEF hearts: plot of abundance (x-axis) vs $\log_2$ CS/ART, that is, extraction/release. (C) Correlation matrix of arterial concentration vs use in control. (D) Correlation matrix of arterial concentration in HFpEF. UnSig = not significant; other abbreviations as in Figure 1.

Although uridine's use was negatively correlated with its arterial concentration, it was positively correlated with folate concentrations.

SEX-DEPENDENT METABOLITE USE IN CONTROL AND HFpEF. Female (Figure 3A) and male (Figure 3B) control hearts did not release any metabolites at our significance thresholds. Female control hearts extracted 3 metabolites (Figure 3A), whereas male control hearts extracted 21 metabolites (Figure 3B). All 3 metabolites extracted by female control hearts were also extracted by male control hearts (creatine, orotate, and *trans*-hydroxyproline), with the remaining 18 unique to male control hearts (Figure 3C).

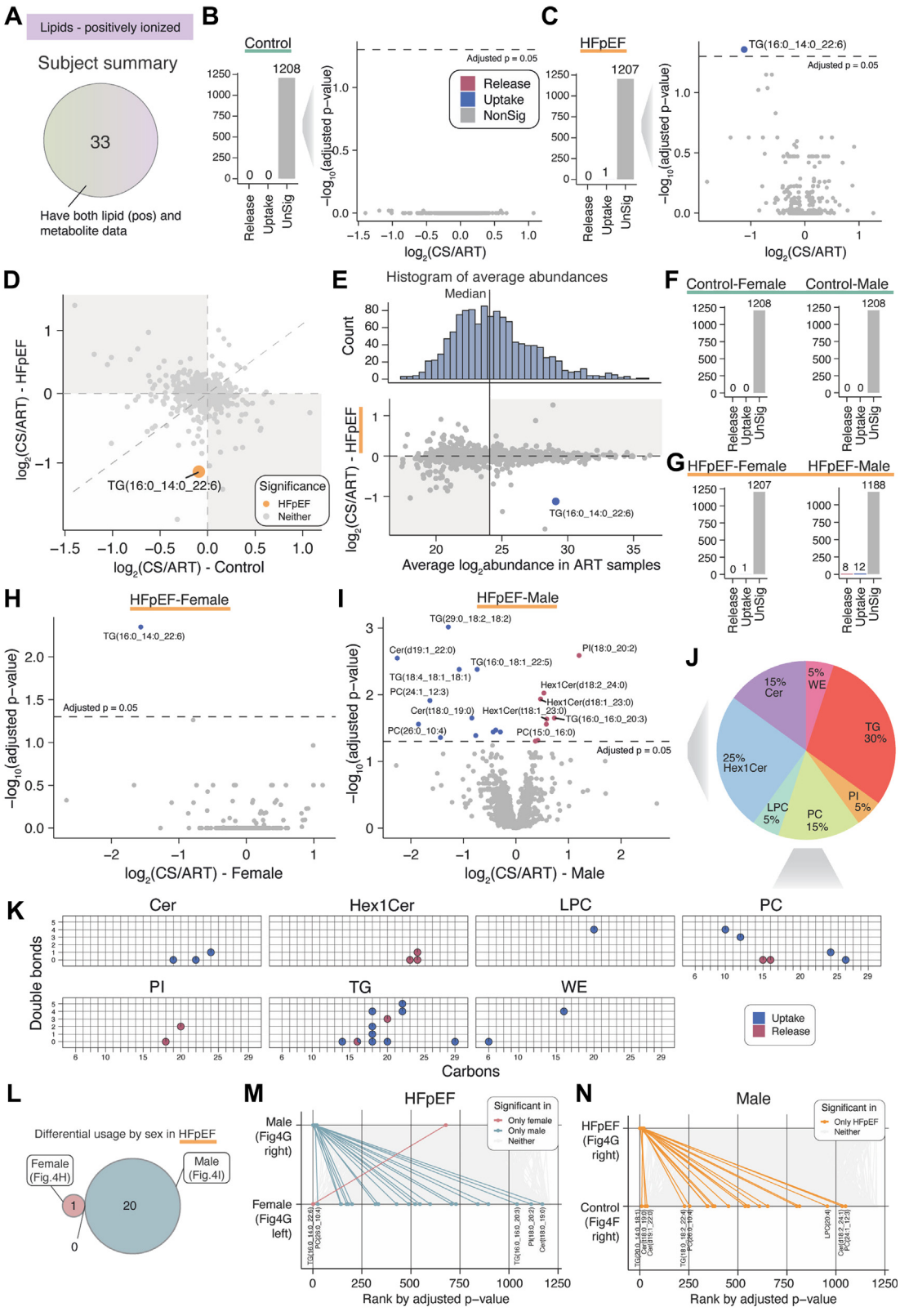
Similarly, both female (Figure 3D) and male (Figure 3E) HFpEF hearts did not release any metabolites at our significance thresholds. However, both female and male HFpEF hearts extracted similar numbers of metabolites. Two extracted metabolites

FIGURE 3 Sex Interaction in Control and HFpEF Metabolite Use



(A) Metabolite uptake and release volcano plot in female control subjects. (B) Metabolite uptake and release volcano plot in male control subjects. (C) Schematic of differential use of male and female control subjects. (D) Metabolite uptake and release volcano plot in female HFpEF. (E) Metabolite uptake and release volcano plot in male HFpEF. (F) Schematic of differential use of male and female control subjects. (G to J) Rank order comparisons of metabolite use in (G) control (female vs male), (H) HFpEF (female vs male), (I) female (control vs HFpEF), and (J) male (control vs HFpEF). Abbreviations as in Figure 1.

FIGURE 4 **Positively Ionized Lipids**



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were common to male and female HFpEF hearts (creatine and *trans*-hydroxyproline), with 3 unique to female and 2 unique to male HFpEF hearts (Figure 3F).

The extracted metabolites in female control hearts were common to male control hearts (*trans*-hydroxyproline, creatine, and orotate) (Figure 3G). Eighteen extracted metabolites were unique to male control hearts and included glycolytic (glyceraldehyde-3-phosphate, glyceraldehyde), TCA cycle (succinate and its modified form, arginosuccinate), nucleosides (hypoxanthine, xanthine, inosine), a tryptophan intermediate (hydroxyanthranilate), and methylated ketone bodies (methylacetoacetate).

In HFpEF hearts, creatine and *trans*-hydroxyproline were extracted by both male and female HFpEF hearts (Figure 3H). Taurine, glutamate, and uridine were preferentially extracted by female HFpEF hearts and 2-aminoadipate and homocysteate preferentially by male HFpEF hearts (Figure 3H).

In all female hearts together (Figure 3I), *trans*-hydroxyproline and creatine were extracted by control and HFpEF hearts; orotate by control hearts; and taurine, uridine, and glutamate by HFpEF hearts.

In all male hearts together (Figure 3J), creatine and *trans*-hydroxyproline were extracted by control and HFpEF hearts and homocysteate and 2-aminoadipate preferentially by HFpEF hearts. Among male hearts, 19 metabolites were extracted by control but not HFpEF hearts, and these included the cardioprotective metabolite taurine,[36] cardiac substrate glutamate, ketogenic substrates (glyceraldehyde, glyceraldehyde-3-phosphate), TCA intermediates and related metabolites (succinate, arginosuccinate), nucleosides (hypoxanthine, inosine, xanthurenate), the polyhydroxy acid gluconolactone, and the glycosylated pyrimidine uridine.

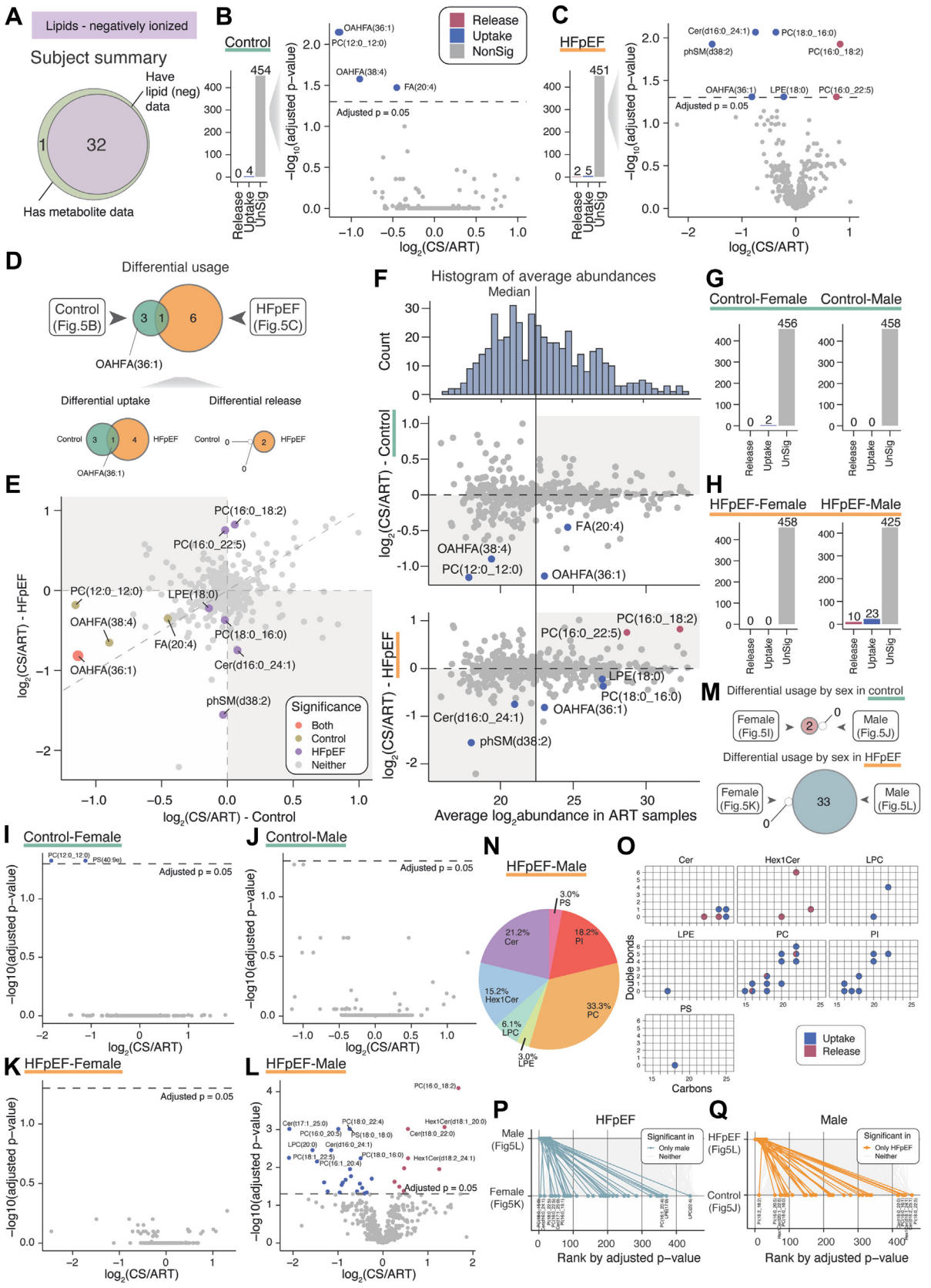
LIPID USE—POSITIVELY IONIZING LIPIDS. All 33 subjects had data for positively ionizing lipids (Figure 4A). Control hearts had no significantly extracted or released lipids (Figure 4B). Triacylglycerol (TG) (16:0_14:0_22:6) was significantly extracted (FC: 0.46; $P = 0.04$) by HFpEF myocardium (Figure 4C). There were many more lipids trending toward significant uptake and release although these did not reach significance, whereas in control subjects there was almost no change in lipid use (Figure 4C vs 4B), including TG (16:0_14:0_22:6) (Figure 4D). The significantly extracted TG (16:0_14:0_22:6) in HFpEF was a relatively higher circulating abundance compared to other lipids (Figure 4E).

Dividing healthy and HFpEF groups into female and male subgroups, it became apparent there was a significant interaction of sex and disease status, with many more significantly released and extracted lipids in male HFpEF hearts at these defined significance thresholds (Figure 4F vs 4G). There was one significantly extracted lipid in female HFpEF: TG (16:0_22:6_22:6) (Figures 4G and 4H). In comparison, there were 8 released and 12 extracted lipids in male HFpEF (Figures 4G and 4I). These included uptake of several TGs (16:0_18:1_22:5; 18:4_18:1_18:1; 29:0_18:2_18:2) and ceramide (Cer) (eg, d19:1_22:0) (Figure 4I). Significantly released lipids included phosphatidylinositols (PIs) (eg, 18:0_20:2), hexosylceramide (Hex1Cer) (eg, d18:2_24:0), and TGs (eg, 16:0_16:0_20:3) (Figure 4I). The breakdown of lipid use in male HFpEF (Figure 4J) reveals that TGs were used most, followed by Hex1Cer, Cer, phosphatidylcholines (PCs), lysophosphatidylcholine (LPCs), PIs, and a type of fatty ester called wax monoesters (WEs). Examining the double (unsaturated) bond content and carbon content of the uptake and release lipids (Figure 4K) revealed that extracted Cers were of lower double bond content, with carbons between 20 and 24; released Hex1Cer were of lower double bond content, with 23 or 24 carbons; extracted LPC had 20 carbons and 4 double bonds; extracted PCs had both short (with more double bonds) and longer carbon chains (with fewer double bonds); released PCs were 15 or 16 carbons in length and had lower double bond content; PIs had chain lengths of 17 to 20 carbons and low double bond content; released TGs had carbon lengths of 16 to 20 with fewer double bonds; extracted TGs were centered around 18 carbons and had a wide spread of double bond content; and extracted WEs had between 6 and 16 carbons with a mix of double bond content.

FIGURE 4 Continued

(A) Number of samples acquired. (B) Significant use breakdown in control subjects with corresponding volcano plot. (C) Use breakdown in HFpEF with corresponding volcano plot. (D) Log fold change of CS/ART in control vs HFpEF hearts. (E) Use (y-axis) vs arterial abundance (x-axis) for HFpEF. (F) Summary of use for female and male control subjects. (G) Summary of use for female and male HFpEF. (H) Volcano plot of $\log_2$(CS/ART) vs significant for female HFpEF. (I) Volcano plot of $\log_2$(CS/ART) vs significant for male HFpEF. (J) Pie chart of lipid class use in male HFpEF. (K) Breakdown of lipid classes and their significant use according to carbon chain length (x-axis) and double bond content (y-axis). (L) Schematic summary of differential use by sex in HFpEF. (M) Rank order P value (x-axis) vs sex (y-axis) in HFpEF. (N) Rank order *P* value (x-axis) vs disease status (y-axis) in male subjects. Cer = ceramide; Hex1Cer = hexosylceramide; LPC = lysophosphatidylcholine; PC = phosphatidylcholine; PI = phosphatidylinositol; pos = positively; TG = triacylglycerol; WE = wax monoester; other abbreviations as in Figures 1 and 2.

FIGURE 5 **Negatively Ionized Lipids**



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The stark difference in lipid use by sex is illustrated in Figure 4L. Rank order (Figure 4M) captures the sex interaction, revealing the most important sex interaction composed of PCs, Cers, PIs, and TG. Perturbation of lipid classes within male hearts according to disease status is illustrated in Figure 4N.

LIPID USE: NEGATIVELY IONIZING LIPIDS. Negatively ionizing lipids include several unique classes, including (*O*-acyl)-omega-hydroxy fatty acids (OAHFAs) and Hex1Cer. Negatively ionizing lipids were captured for 32 subjects compared to all 33 for metabolites and positively ionizing lipids (Figure 5A). Among these lipids, 4 lipids were significantly extracted in healthy hearts: OAHFA (36:1), PC (12:0_12:0), OAHFA (34:8), and FA (20:4) (Figure 5B). In HFpEF hearts, there were 5 significantly extracted: Cer (d16:0_24:1), phytosphingosine (sphingomyelin) (d38:2), PC (18:0_16:0), OAHFA (36:1), and lysophosphatidylethanolamine (LPE) (18:0) (Figure 5C). There were 2 significantly released lipids from HFpEF hearts, both PCs (16:0_18:2; 16:0_22:5) (Figure 5C).

As can be seen in Figure 5D, there was 1 significantly used lipid that overlapped in healthy control and HFpEF groups, OAHFA (36:1). The relative uptake release of lipids in both groups is illustrated in Figure 5E. The lipids extracted by healthy hearts were evenly split between lower and higher median abundance (Figure 5F), as was the case for HFpEF hearts; however, both PCs released by HFpEF hearts were of high abundance.

As for the positively ionizing lipids, uptake and release of negatively ionizing lipids had a major interaction of sex with disease state (Figures 5G and 5H). Female control hearts significantly extracted 2 lipids (Figure 5G), whereas female HFpEF did not use any lipids evident at our significance threshold. Male hearts have a major divergence of lipid use according to disease state (Figures 5G and 5H): male control hearts did not significantly use any lipids, yet male HFpEF hearts extracted 23 lipids and released 10 (Figures 5G and 5H). The interaction of disease sate and male sex is starkly illustrated using a comparison of the volcano plots in Figures 5I to 5L and summarized in Figure 5M. Among the negatively ionizing lipids, male HFpEF hearts used PCs the most, followed by Cer, PIs, Hex1Cer, LPCs, LPEs, and phosphatidylserines (PSs) (Figure 5N). Cers that were released had generally shorter carbon length and lower double bond content than those that were extracted (Figure 5O). Hex1Cers were released and were a mix of low and high double bond content with 20 to 23 carbons in length. PCs were both extracted and released by male HFpEF hearts and were a mixture of low and high double bond content, as well as shorter and longer carbon chain lengths. PSs without double bonds and shorter double bond content were extracted. The extracted LPE and PS each had lower carbon chain length and low double bond content (Figure 5N).

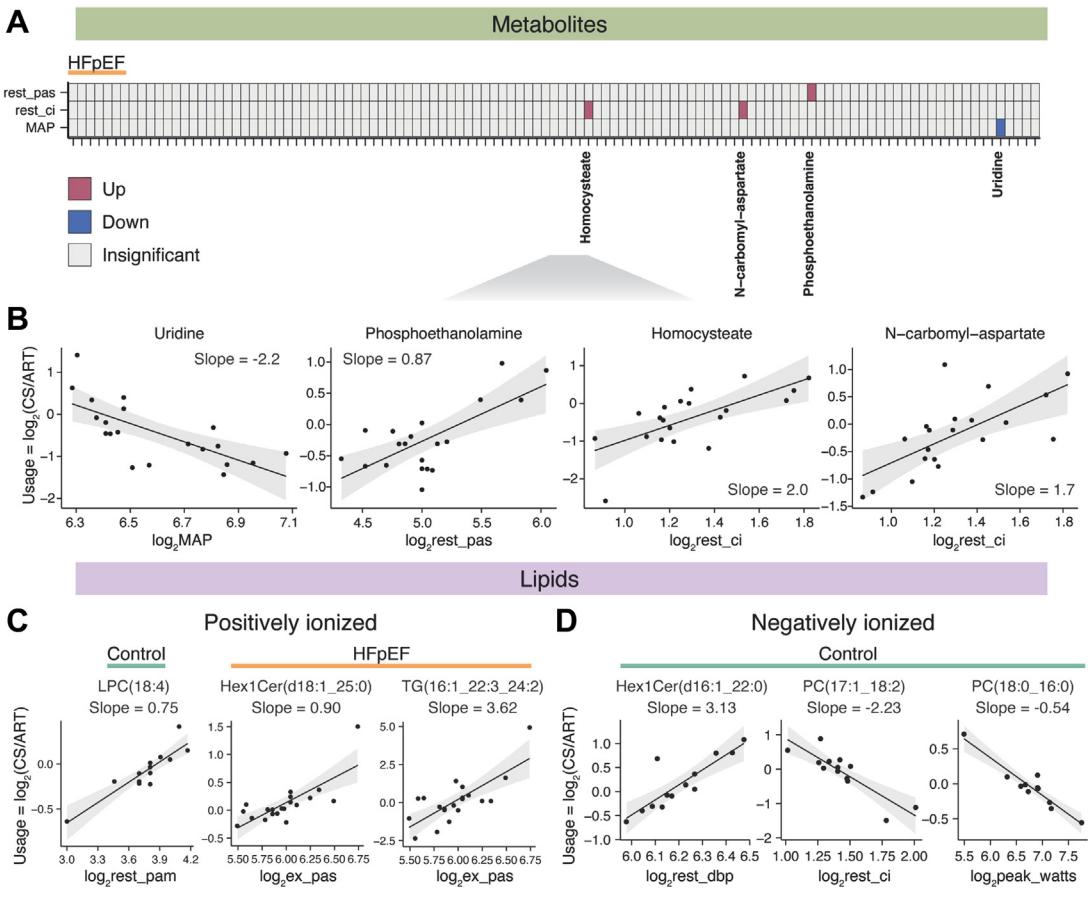
Plotting the rank order of *P* values illustrates the sex interaction, where there is mostly an opposite rank order—the most extreme cases being LPCs, LPEs, and PCs, which were highly ranked in male but not female hearts (Figure 5P). PC (18:0_16:0) and Cer (d16:0_24:1) were similarly ranked in male and female hearts (although only significant at the threshold in male hearts) (Figure 5). Within male hearts, there were lipids that were ranked in HFpEF but that ranked very low in healthy hearts, such as a PI, 2 Cers, a Hex1Cers, and a PC. The Cers in particular were highly extracted by HFpEF male hearts but not control male hearts (Figure 5Q).

RELATIONSHIP TO CARDIAC FUNCTIONAL PARAMETERS. We next determined metabolites whose use was associated with cardiac functional parameters within the HFpEF group (Figure 6A). We found significant relationships of metabolite use (log2CS/ART) with 3 functional parameters: resting mean arterial pressure (MAP), resting pulmonary arterial systolic pressure (rest_PAS), and resting cardiac index (Figure 6A). There was an inverse relationship between CS/ART for uridine and MAP, meaning that extraction of

FIGURE 5 Continued

(A) Number of samples acquired. (B) Significant use breakdown in control subjects with corresponding volcano plot. (C) Use breakdown in HFpEF with corresponding volcano plot. (D) Schematic of differential use with overlap, with uptake and release. (E) Log fold change of CS/ART in control vs HFpEF hearts. (F) Use (y-axis) vs arterial abundance (x-axis) for control and HFpEF hearts. (G) Summary of use for female and male control hearts. (H) Summary of use for female and male HFpEF hearts. (I) Volcano plot of $\log_2$(CS/ART) vs significant for female control hearts. (J) Volcano plot of $\log_2$(CS/ART) vs significant for male control hearts. (K) Volcano plot of $\log_2$(CS/ART) vs significant for female HFpEF hearts. (L) Volcano plot of $\log_2$(CS/ART) vs significant for male HFpEF hearts. (M) Schematic of differential use by sex in control and HFpEF hearts. (N) Pie chart of lipid class use in male HFpEF hearts. (O) Breakdown of lipid classes and their significant use according to carbon chain length (x-axis) and double bond content (y-axis). (P) Rank order *P* value (x-axis) vs sex (y-axis) in HFpEF. (Q) Rank order *P* value (x-axis) vs disease status (y-axis) in male subjects. FA = fatty acid; neg = negative; LPE = lysophosphatidylethanolamine; OAHFA = (*O*-acyl)-omega-hydroxy fatty acids; phSM = phytosphingosine (sphingomyelin); PS = phosphatidylserine; other abbreviations as in Figures 1, 2, and 4.

FIGURE 6 Relationship of Substrate Use to Invasive Hemodynamic Parameters at Rest and With Exercise



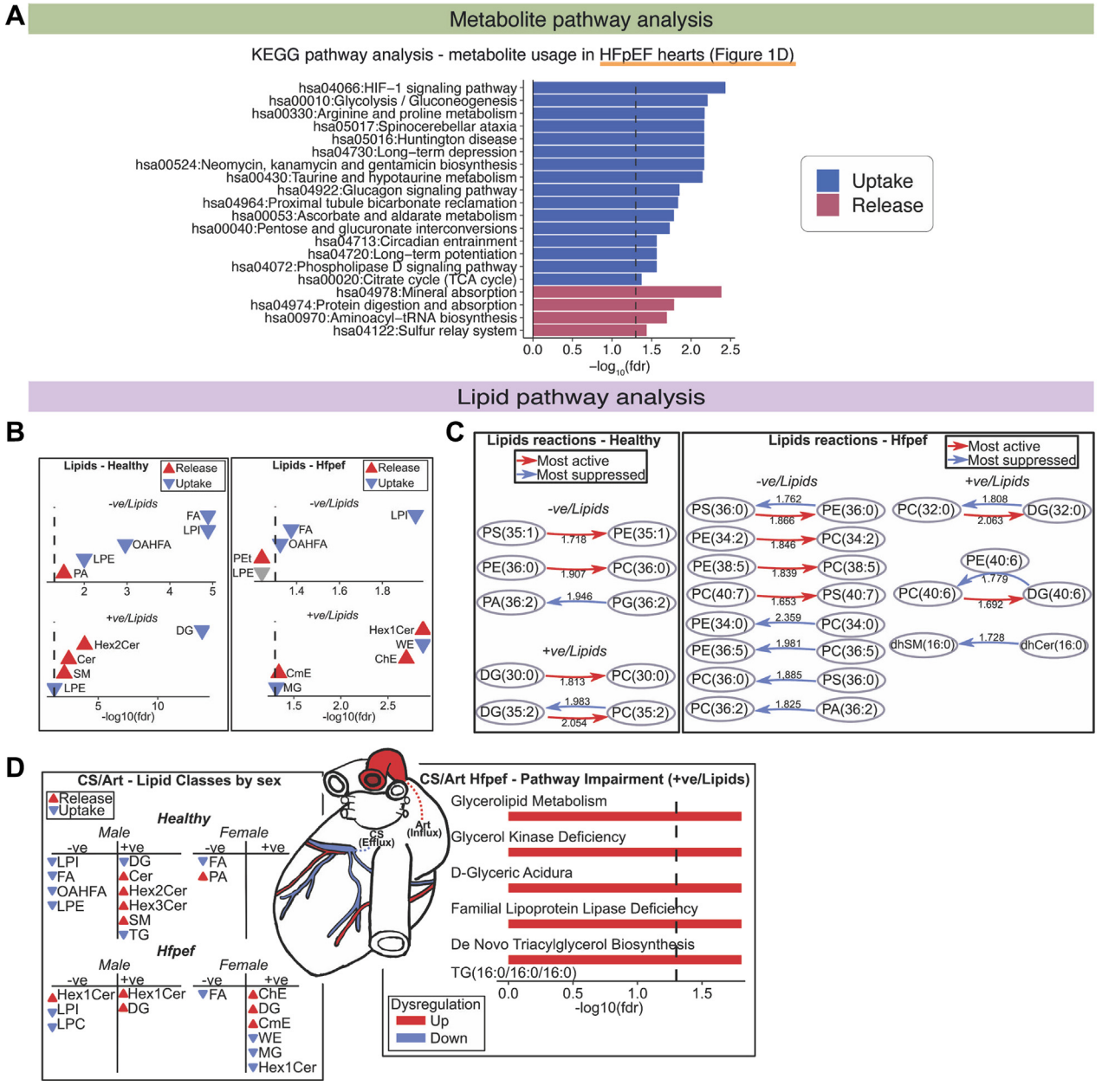
(A) Highlighted significant associations of metabolite use with hemodynamic parameters. (B) Correlation plots of hemodynamic parameters (x-axis) with metabolite use (y-axis). (C) Lipids—positively ionized—and their use correlation plots with hemodynamic parameters. (D) Negatively ionized lipids and their correlation plots with hemodynamic parameters. ex_pas = exercise pulmonary artery systolic pressure; MAP = mean arterial pressure; rest_ci = resting cardiac index; rest_pam = rest mean pulmonary artery pressure; rest_dbp = resting diastolic blood pressure; rest_pas = resting pulmonary systolic pressure; other abbreviations as in Figures 1, 4, and 5.

uridine was positively correlated with MAP (β = -2.2; P = 0.03) (Figure 6B). Extraction of phosphoethanolamine was negatively correlated with rest_PAS (β = 0.87; P = 0.035) (Figure 6B). Extractions of homocysteate (β = 2.0; P = 0.03) and N-carbamoylaspartate (β = 1.7; P = 0.03) were negatively correlated with resting cardiac index (Figure 6B).

Regarding positively ionizing lipids, control hearts extracted LPC (18:4) in close correlation with resting mean pulmonary arterial pressure (β = 0.75; P = 0.04) (Figure 6C). In HFpEF hearts, 2 lipids were released in close relationship with exercise parameters (Figure 6C). Negatively ionizing lipids were also related to exercise parameters in control hearts (Figure 6D). Hex1Cer (d16:1_22:0) was extracted with a strong inverse linear relationship to resting diastolic blood pressure. Two PCs (17:1_18:2 and 18:0_16:0) were released more with higher cardiac index or higher cardiac fitness per exercise watts achieved, respectively.

METABOLITE AND LIPID PATHWAY ANALYSES. To synthesize and summarize the described changes, we performed pathway analyses (Figure 7). Analysis of differential metabolite pathways revealed no significant metabolite pathway use in control hearts, but there were several in HFpEF (Figure 7A). There were many significant metabolite uptake pathways in HFpEF, including the HIF-1 signaling pathway, the glycolytic/gluconeogenic pathway, nitric oxide synthase-related arginine metabolism, and the TCA cycle (Figure 7A). Significant release metabolite pathways indicated loss of mineral absorption,

FIGURE 7 **Pathway Analyses**



(A) KEGG metabolite pathway use indicating uptake and release pathways. Lipid pathway analysis: (B) Lipid class release and uptake in (left) healthy hearts and (right) HFpEF hearts. (C) BioPAN analysis of lipid transitions in (left) healthy hearts and (right) HFpEF hearts. (D) Pathway lipid analysis, uptake and release, in (left) healthy male and female and (right) HFpEF male and female hearts, HFpEF pathway impairment. -ve = negative; +ve = positive; ChE = cholesterol ester; dh = dihydro; fdr = false discovery rate; KEGG = Kyoto Encyclopedia of Genes and Genomes; LPEt = lysophosphatidylethanols; LPI = lysophosphatidylinosine; MG = monoglyceride; TCA = tricarboxylic acid; tRNA = transfer RNA; other abbreviations as in Figures 1, 4, and 5.

evidence of protein catabolism, and decreased transfer ribonucleic acid biosynthesis (Figure 7A).

Analysis of lipid classes revealed that healthy hearts released phosphatidic acid, sphingomyelins, Cers, and Hex2Cers, whereas OAHFAs, FAs, LPEs, lysophosphatidylinositols, and diacylglycerol (DGs) were the lipid uptake classes (Figure 7B). Among HFpEF hearts, campesterol ester (CmE), cholesterol

ester, and Hex1Cer were significantly released lipid pathways/classes, whereas OAHFA, FA, lysophosphatidylinositol, and WE were significantly extracted (Figure 7B). WEs were taken up by HFpEF but not heathy hearts.

Next, we interrogated lipid species reaction curated on LIPID MAPS using BioPAN[32] to gain insight into key lipid transitions across the heart (Figure 7C). In healthy hearts, we identified active transitions from PS to phosphatidylethanolamine, PE to PC, and DG to PC (Figure 7C). In HFpEF hearts, we identified transitions of PE to PC, PC to PS, and inhibition of conversions to PE and PC. Additionally, we found transitions to DG, a storage lipid, that were not seen in healthy hearts (Figure 7C).

When examining lipid class interaction with sex, it can be seen that male healthy hearts had more flux in lipid classes than female, whereas female HFpEF had more than male HFpEF hearts (Figure 7D). Interestingly, there were several novel lipid classes unique to female HFpEF hearts, including cholesterol ester, CmE, WE, and monoglycerides (Figure 7D).

Further interrogation of the Small Molecule Pathway Database unveiled several lipid pathways impaired in HFpEF (Figure 7D), including upregulation of TG biosynthesis and storage, along with glycerolipid metabolism.

DISCUSSION

In this report, in a robustly defined HFpEF cohort, we present a descriptive, hypothesis-generating overview of cardiac substrate use in HFpEF and the relationship to invasive hemodynamic parameters, which needs to be validated in future work. HFpEF is a major HF phenotype commonly associated with complex physiologic and metabolic derangements. In this context, it was recently shown that the plasma and myocardial tissue concentrations of key metabolites correspond poorly in HFpEF.[5] For example, important cardiac substrates, such as medium- and long-chain acylcarnitines (MLACs) are depleted in the hearts but not the plasma of HFpEF and HFrEF patients.[5] To comprehensively integrate the metabolic and physiologic phenotype of the HFpEF myocardium, we directly assessed cardiac substrate use and invasively measured hemodynamic performance rather than via indirect approaches including circulating plasma nutrient levels.

FA USE IN THE HFpEF HEART. It has been proposed that FA metabolism increases systemically and in the heart in instances where glucose availability is decreased, such as in obesity and states of insulin resistance. However, recent work in clinical HFpEF has called this hypothesis into question, with little change observed in plasma MLACs and lower expression of FA-uptake genes in the HFpEF heart.[5] However, this recent study was predominantly conducted in patients with advanced obesity based on BMI and high rates of diabetes (40 kg/m^2 and 74%, respectively)[5] compared to our cohort (34 kg/m^2 and 30%, respectively), which is more comparable to previous reports.[37,38] We found that although FAs were still taken up by HFpEF hearts, uptake of FAs was not as significant as it was in healthy hearts; however, our data do suggest a relative preservation of FA uptake in patients with more modest obesity and insulin resistance compared to recent studies. Additionally, MLACs were mostly not used in our healthy and HFpEF hearts. However, short-chain acylcarnitines were nonsignificantly released by healthy hearts and nonsignificantly extracted by HFpEF hearts, with C4 butyrylcarnitine being the closest to significance after FDR adjustment (FC: 0.83; $P = 0.06$). This is again consistent with preservation of FA uptake in HFpEF hearts in our study. Unfortunately, the prior studies with comparable levels of obesity and diabetes used the fasting state and relied solely on peripheral plasma and serum metabolites, so the cardiac use could not be assessed. However, our data may suggest that at these BMI levels, with corresponding degrees of insulin resistance, upregulating FA oxidation is a reasonable potential therapeutic strategy in HFpEF to further augment the availability and oxidation of cardiac FAs. Although the HFpEF patients in this study were not taking sodium glucose co-transporter 2 inhibitors or glucagon-like peptide 1 receptor agonists, because the samples were collected before these therapies emerged for HFpEF, our findings are highly relevant for novel metabolic therapeutics. The attendant weight loss with glucagon-like peptide 1 receptor agonists therapy in particular could conceivably cause a shift from the cardiac lipid storage we describe to a state of lipolysis, promoting restoration of fatty acid oxidation.

GLUCOSE UPTAKE IN NONFASTED HUMAN CONTROL AND HFpEF HEARTS. It is known that the fasting state is a strong potential confounder of cardiac fuel use, with particular impact on carbohydrate metabolism, insulin levels, and insulin-facilitated glucose metabolism. This was highlighted as an important limitation in a recent comprehensive fuel analysis of failing and nonfailing hearts in fasting subjects, noting that future efforts need to be repeated in the nonfasting state.[4] In this regard, our study addresses this knowledge gap by assessing patients in the nonfasted

state after a light meal. To our knowledge, all previous studies of metabolomics in HF and HFpEF have been performed in the fasting state,[4,5,37,38] and thus, for the first time, we assessed cardiac metabolic pathways without the confounder of absent insulin-dependent uptake and metabolism. Therefore, we can be confident that the loss of glucose and fructose uptake by our HFpEF hearts (both extracted by healthy control hearts) was not an artifact of fasting but, in fact, a real finding.

TCA CYCLE INTERMEDIATES. It was previously observed that TCA cycle intermediates were lower in HFpEF myocardium compared with control, often interpreted as inadequate anaplerosis.[5] However, TCA cycle intermediates can also be taken from the circulation, and we found a significant uptake of the TCA cycle intermediates succinate and aconitate in control hearts, but not in HFpEF hearts, perhaps contributing to the lower levels of these intermediates previously observed in HFpEF myocardium.[5] Lactate and pyruvate are also entry points to the TCA cycle; however, both were also significantly taken up by HFpEF hearts, therefore not appearing to play a role in later TCA flux in the HFpEF heart. These data may also suggest that the increased reliance on the glycolytic intermediates, lactate and pyruvate, indicates a possible reduction in mitochondrial efficiency or inability to fully oxidize substrates through the TCA cycle and oxidative phosphorylation.[39] Assessing enzymatic activity of TCA proteins and fluxomic interrogation of HFpEF myocardial tissue are important next steps to address remaining gaps in knowledge.

BCAA AND NON-BCAA METABOLISM IN HFpEF. We did not detect any significant differences between healthy and HFpEF arterial levels of BCAAs, nor did we identify significant uptake or release of BCAAs in healthy or HFpEF hearts. This may be accounted for by the nonfasting state of our sample, because meal ingestion will influence all circulating amino acid levels, peaking at 1 or 2 hours after ingestion.[40] However, similar results were identified in recent work using fasting venous blood, with comparable prevalence of diabetes within the HFpEF group, where it was shown that there were changes in BCAAs in HFrEF compared to control subjects but not HFpEF compared to control subjects.[30]

Non-BCAAs were avidly taken up by both healthy and HFpEF hearts. For example, we did not see a difference in arterial concentrations between healthy and HFpEF hearts nor any limitation on cardiac uptake of glutamate, a nonessential amino acid, in either condition. In fact, glutamate was more avidly extracted in HFpEF hearts. Although glutamate and the glutamate-to-glutamine ratio are known to be associated with insulin resistance and risk of developing diabetes, these relationships were identified in the fasting state[41] and the lack of change of glutamate in our cohort may again be influenced by the nonfasting state and the lower prevalence of diabetes. However, again, the lack of change in glutamate is comparable to recent work using fasting samples in an HFpEF cohort with similar obesity and diabetes rates.[30] Nonetheless, recent work did identify an accumulation of BCAAs, with reduced distal catabolites, suggesting reduction of BCAA oxidation in that cohort that had more significant obesity and higher prevalence of diabetes.[5]

KETONE BODIES. We did not see a difference in either arterial levels of ketone bodies (3-hydroxybutyrate and acetoacetate) between heathy and HFpEF groups or extraction by healthy or HFpEF hearts. Methylated acetoacetate was extracted by healthy hearts but not by HFpEF hearts. Acetoacetate was nonsignificantly extracted by HFpEF hearts ($P = 0.07$). Therefore, whereas ketone extracted has been shown to be dramatically up-regulated in HFrEF,[4] we did not see this in HFpEF.

SEX INTERACTION. We determined a significant interaction of sex with disease state, although caution should be used when interpreting results in these smaller subsets. This was seen most dramatically in female vs male HFpEF lipid use. Sexual dimorphism in metabolism has long been recognized, with males and females exhibiting distinct preferences for energy substrates: males predominantly use carbohydrates, whereas females prefer lipids.[42] However, our results suggest a different pattern in the context of HFpEF. Female HFpEF hearts appeared significantly constrained with regard to lipid use and reverted to use of nontraditional lipids such as CmE, WS, and monoglycerides. In contrast, male HFpEF hearts significantly extracted various lipid classes, including sphingolipids (SMs and Cers), glycerolipids (TGs), and glycerophospholipid (PC), and released PIs and Hex1Cers. Most species preferentially extracted from the blood and released by the failing heart contained longer carbon chains, were saturated, or had only a few double bonds. Failing hearts often exhibit impaired energy production and use, leading to a shift in the types of lipids being used as energy substrates. Our data suggest decreased use of FAs, activation of lipid and TG storage pathways, and spillover of TGs into circulation. This is consistent with previous reports of intramyocardial lipid accumulation in HFpEF[43]

and suggests that activation of lipid storage pathways leads to accumulation of lipids locally in the myocardium.

RELATIONSHIP OF FUEL USE TO INVASIVE HEMODYNAMICS. For the first time to our knowledge, we report the relationship of fuel use to rest and exercise invasive hemodynamic parameters. Of all the hemodynamic parameters, pulmonary pressures and cardiac index had the greatest relationship to fuel use. This is likely a function of the arterial-venous pressure differential and cycle time through the coronary vasculature.

Uridine, a pyrimidine nucleoside with favorable effects on cardiac mitochondrial function in experimental models of diabetic cardiomyopathy,[44] was extracted by HFpEF hearts with a very strong linear relationship to MAP: the greater the MAP, the greater the extraction. This may indicate that extraction of some metabolites is consequent upon a pressure gradient. PE lipid extraction was negatively affected by elevated rest_PAS, which may indicate a systemic-pulmonary arterial gradient dependence for this lipid species. Intriguingly, the greater the cardiac index, the less the extraction of homocysteate and *N*-carbamoyl-aspartate, which may indicate less use of these substrates with better cardiac function. Pulmonary pressures negatively affected lipid extraction, and cardiac index positively affected lipid extraction. Although PCWP is a critical invasive parameter used to define HFpEF and was measured both at rest and during exercise, we did not find a significant relationship to metabolite or lipid extraction. This may suggest that although PCWP is an important physiologic parameter that is related to disease severity and outcome, it does not per se influence energetic aspects of the disease. Although these relationships to hemodynamic parameters are novel and interesting, they need to be replicated in future studies.

STUDY LIMITATIONS. Given the invasive nature of our study and the inclusion of a healthy control cohort, the sample sizes were relatively small, although they were carefully phenotyped. Importantly, and in contrast to other reports, catheterization studies were done in the nonfasting state and thus are more likely to reflect myocardial metabolism in the context of normal daily activity. However, meals were not standardized. We did not measure tissue metabolite levels, which could have informed gaps in carbon flux and metabolic turnover, although assessment in left ventricular tissue would be required to truly provide context relevant to HFpEF.

CONCLUSIONS

In a cohort of carefully phenotyped HFpEF patients and control subjects, we provide novel insights into human cardiac fuel use in the nonfasted state. In this state, we reveal that HFpEF hearts preserve some FA uptake, although much less than control hearts. In fact, HFpEF hearts demonstrate a transition to more complex lipid generation, storage, and spillover along with protein catabolism. Despite the known female mitochondrial preference for lipid substrates compared to male hearts, in the HFpEF disease state, female hearts used fewer lipids than male hearts, which were able to leverage an abundant range of lipid classes, with uptake/release corresponding to carbon chain and double bond content for Cers, PCs, and TGs. In general, pulmonary artery pressures and cardiac performance were related to lipid extraction, whereas MAPs were positively coupled to metabolite extraction. Future studies using concurrent transmyocardial sampling and hemodynamics during exercise will provide further insights into HFpEF pathophysiology and therapeutic target identification.

ACKNOWLEDGMENTS The expert technical assistance of Donna Vizi and Liz Dewar are acknowledged.

FUNDING SUPPORT AND AUTHOR DISCLOSURES

Samples were collected with support of a National Health & Medical Research Council (NHMRC) of Australia Project Grant (GNT1159721) to Drs Marques and Kaye. Dr Kaye is the recipient of an NHMRC Investigator Grant (Level 3). Dr O'Sullivan is supported by an National Heart Foundation (NHF) Future Leader Fellowship (Level 2) and an New South Wales early to mid career (NSW EMCR) award. Dr Marques is supported by a Senior Medical Research Fellowship from the Sylvia and Charles Viertel Charitable Foundation, a National Heart Foundation Future Leader Fellowship (105663), and NHMRC Emerging Leader Grant (GNT2017382). All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: HFpEF accounts for approximately 50% of all HF cases. The pathophysiology of HFpEF is complex, including both central and peripheral cardiovascular abnormalities together with noncardiovascular comorbidities. Elevated left atrial pressure, particularly during exertion, is a hallmark feature, resulting from impaired left ventricular diastolic performance. The contribution of altered myocardial metabolism to HFpEF is poorly understood. The current study demonstrates that a complex pattern of myocardial metabolic remodeling occurs in HFpEF, including less free fatty acid use with a concomitant transition to more complex lipid generation and protein catabolism.

TRANSLATIONAL OUTLOOK: Impaired left ventricular diastolic performance is a hallmark feature of HFpEF; however, the mechanism(s) responsible for this remain poorly understood. Altered myocardial energetics may contribute to the pathophysiology of HFpEF given the energy dependence of diastole. Our study identifies a complex pattern of altered myocardial metabolism, providing potential therapeutic targets.

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KEY WORDS cardiac energetics, heart failure with preserved ejection fraction, invasive hemodynamics, lipids

APPENDIX For supplemental Datasets and an expanded Methods section, please see the online version of this paper.