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RESEARCH LETTER

Genes of the Fatty Acid Oxidation Pathway are Upregulated in Female as Compared to Male Cardiomyocytes

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The nature and extent of molecular sex differences between healthy human male and female hearts are largely unknown. Knowledge of such differences in the cardiac transcriptome could provide insight into sex differences in both cardiac physiology and pathology.[1] Here, we combine bulk and single-nucleus RNA-sequencing data to demonstrate that genes of the fatty acid oxidation (FAO) pathway – the primary source of energy in the healthy heart – exhibit female-biased expression in cardiomyocytes. Using cardiac metabolomic data, we further identify increased flux and energetic utilization of free fatty acids in nonfailing female as compared to male hearts (Figure [A]). Overall, our results demonstrate that nonfailing, age-matched (Figure [B]) male and female human hearts exhibit fundamental differences in expression, flux, and energetic reliance on FAO.

We performed sex-biased gene expression analysis of bulk RNA-seq data from 560 donors in the Genotype Tissue Expression (GTEx) Project, through which we identified 2956 and 1107 significantly sex-biased genes (most of which were autosomal) in the left ventricle (LV) and right atrial appendage (RAA), respectively. Using gene set enrichment analysis, we observed a strong female-bias of oxidative phosphorylation in the LV and RAA (Figure [C]). Oxidative phosphorylation produces >90% of adenosine triphosphate in the healthy heart, primarily by utilizing fatty acids (Figure [D]). Consistently, we observed that the fatty acid metabolism pathway was significantly female-biased in the LV and RAA (Figure [C]). Performing gene set enrichment analysis using genes involved in either FAO (and therefore ATP production via oxidative phosphorylation) or fatty acid synthesis, we found that only the FAO gene set was significantly female-biased in both the LV and RAA (LV false discovery rate = 0.018; RAA false discovery rate < 2.2e-16; Figure [E through F]). Eighteen of 20 genes in the FAO pathway showed female-biased expression in the LV and RAA, with a strong correlation in sex bias fold-change between the 2 regions (Figure [G]).

Sex-biased analysis of bulk RNA-seq data can be confounded by sex differences in cellular composition, and we have previously reported that healthy female hearts have a higher proportion of cardiomyocytes than healthy male hearts.[2] To ensure that the female-bias of FAO and oxidative phosphorylation that we observed in bulk tissue is not because of an increased proportion of cardiomyocytes in females, we integrated 2 cardiac single-nucleus RNA-sequencing datasets that we previously generated from the 4 chambers of the heart of 12 male and 8 female donors without cardiac pathology.[2,3] We confirmed that females harbored a higher proportion of LV cardiomyocytes than males (Figure [H]), and we identified 8167 protein-coding genes – 97% of which

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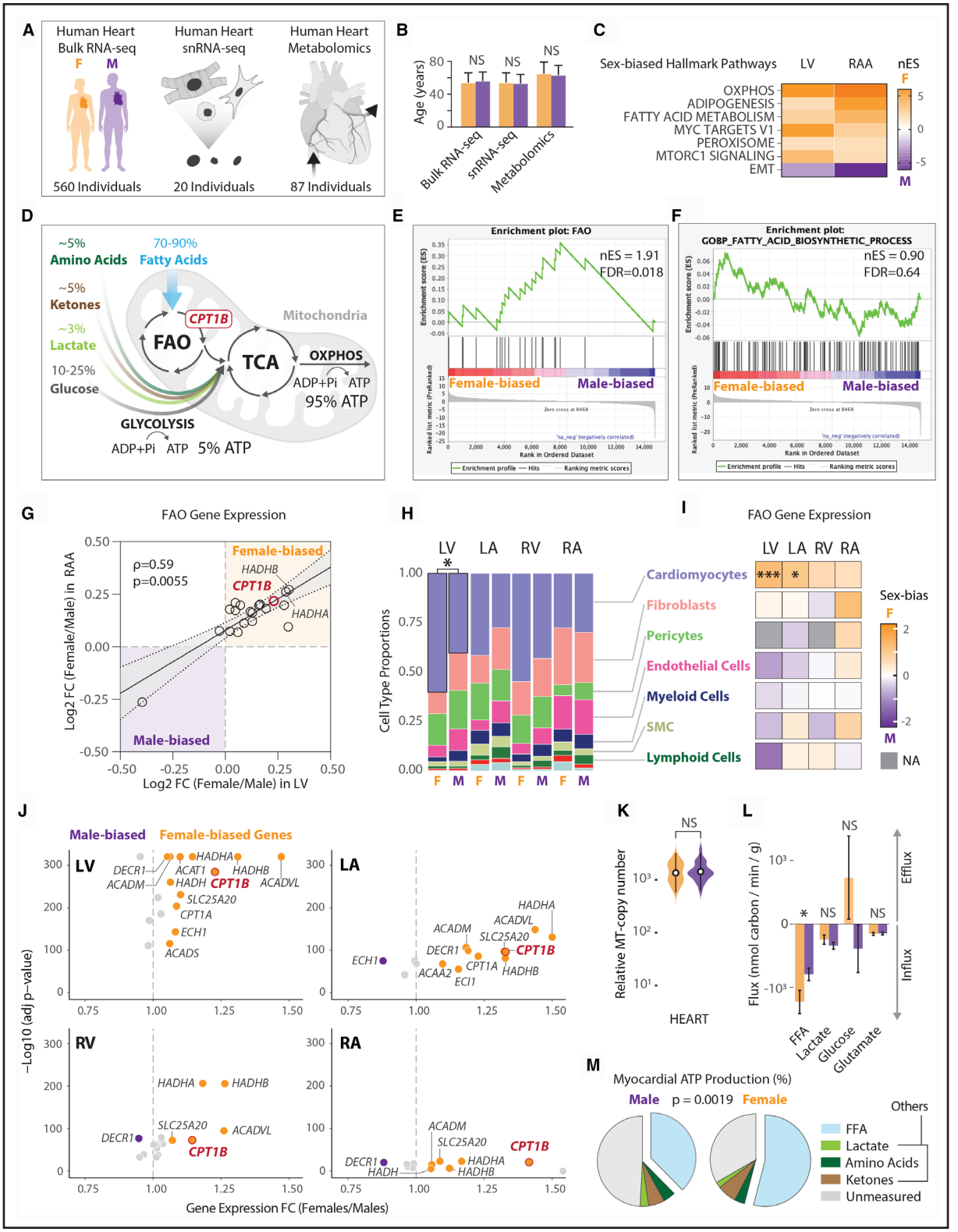

Figure. Nonfailing female human hearts exhibit increased expression, flux, and energetic reliance on fatty acid oxidation compared to age-matched, nonfailing male hearts.

A, Bulk RNA-sequencing, single nucleus RNA-sequencing (snRNA-seq) and metabolomic data used in this study to identify transcriptomic differences between nonfailing female (F; orange) and male (M; purple) human hearts. **B**, Average age ± SD of females and males in datasets used in this study. The sexes are age-matched in all datasets. **C**, Significantly female-biased and male-biased Hallmark Molecular Signature Database pathways (false discovery rate [FDR] < 0.05) shared between left ventricle (LV; n=428 donors) and right atrial appendage (RAA; n=429 donors) using gene set enrichment analysis (GSEA) on sex-biased genes identified from Gene Tissue Expression Consortium (GTEx) bulk RNA-seq data. **D**, Schematic of fuels consumed in cardiac ATP production. **E**, GSEA running rank plots of fatty acid oxidation (FAO) (*Continued*)

Figure Continued. and **F**, fatty acid synthesis (FAS) in LV using GTEx bulk RNA-seq data. **G**, Spearman correlation between sex bias of individual FAO genes in LV and RAA. **H**, Proportions of cardiac cell types in female (n=12) and male (n=8) heart chambers from snRNA-seq data. Cell types with low numbers (neuronal cells: red; adipocytes: light blue) are unlabeled due to space constraints. **I**, Sex bias of FAO pathway using GSEA across all cardiac chambers and cell types represented by at least 5000 nuclei. **J**, Volcano plots of FAO genes significantly differentially expressed between male (FC < 0.95, FDR < 0.05) and female cardiomyocytes (FC > 1.05, FDR < 0.05) in each heart region, with the enzyme *CPT1B* that catalyzes the rate-limiting step of FAO in red and nonsignificant genes in gray. **K**, Relative mitochondrial copy number (mtCN) measured by quantitative PCR of mitochondrially-encoded and nuclear-encoded genes in nondiseased female (n=12) and male (n=20) LV samples from GTEx. **L**, Myocardial fluxes of free fatty acids (FFAs), lactate, glucose, and glutamate (median values with 95% confidence intervals) in nonfailing female (n=34) and male hearts (n=53) calculated with data from Murashige et al[4] and Ngo et al. [5] Only FFAs shows a significant sex difference in myocardial flux (Welch's t-test, $P = 0.037$). **M**, Proportional contributions of classes of metabolites to myocardial ATP production in male and female myocardium (chi-squared proportion test, 2-tailed $P = 0.0019$). EMT indicates epithelial mesenchymal transition; FC, fold change; FDR, false discovery rate; LA, left atrium; LV, left ventricle; NA, not applicable; NS, not significant; nES, normalized enrichment score; OXPHOS, oxidative phosphorylation; RA, right atrium; RV, right ventricle; SMC, smooth muscle cells; and TCA, tricarboxylic acid cycle.*FDR < 0.05; **FDR < 0.01; ***FDR < 0.001. Created in BioRender. Chmatal, L. (2025). https://BioRender.com/n02z861

Nonstandard Abbreviations and Acronyms

FAO	fatty acid oxidation
LV	left ventricle
RAA	right atrial appendage

were autosomal – with significant sex-biased expression in at least 1 cardiac cell type.

Using gene set enrichment analysis, we found that the FAO pathway was significantly female-biased in LV and left atrium cardiomyocytes and nominally female-biased in right atrium and right ventricle cardiomyocytes (Figure [I]). As we were similarly powered to identify sex-biased genes in the left and right heart chambers, this stronger female bias in left atrium and LV cardiomyocytes may reflect the increased energetic demand of the left heart as compared to the right heart. No other cell type showed a consistent sex bias in FAO, including myeloid cells and fibroblasts that also utilize FAO for energy production. Inspection of each gene within the FAO pathway revealed strikingly concordant sex bias effect sizes across all regional cardiomyocytes, with 14 of 20 FAO genes expressed approximately 5% to 50% higher in female compared to male cardiomyocytes in at least 1 heart region (Figure [J]). These consistently female-biased genes encompass all major enzymes of the cardiac FAO pathway, including *CPT1B*, which catalyzes the rate-limiting step of FAO in the heart. To ensure this female-bias was not driven by sex differences in copy number of mitochondria – the site of FAO – we utilized quantitative polymerase chain reaction to calculate relative copy number of mitochondria from 20 male and 12 female nondiseased LV samples from GTEx and found no such sex difference (Figure [K]).

Finally, we re-analyzed our previously reported metabolomic data from the coronary sinus and radial artery of 34 female and 53 male individuals – all of whom had no history of heart failure or reduced ejection fraction but were undergoing elective catheter ablation of atrial fibrillation.[4] Using age- and sex-specific myocardial blood flow values,[5] we found that females exhibited significantly higher cardiac flux of free fatty acids than males (Figure [L]). We did not observe a significant sex difference in the cardiac flux of other metabolites. We also identified a significant sex difference in proportional use of fuels for cardiac ATP production (Figure [M]), with free fatty acids providing approximately 54% of ATP in the female heart and 38% of ATP in the male heart. Taken together with our transcriptomic analyses, we establish that the healthy human female heart exhibits higher expression and flux through the FAO pathway as well as increased energetic reliance on free fatty acid oxidation compared to the male heart.

ARTICLE INFORMATION

Data and materials are available at http://pagelabsupplement.wi.mit.edu/papers/Talukdar_et_al_2025/. No institutional review board approval was required for these analyses.

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Disclosures

None.

REFERENCES

1. Regitz-Zagrosek V, Gebhard C. Gender medicine: effects of sex and gender on cardiovascular disease manifestation and outcomes. *Nat Rev Cardiol*. 2023;20:236–247. doi: 10.1038/s41569-022-00797-4
2. Litviňuková M, Talavera-López C, Maatz H, Reichart D, Worth CL, Lindberg EL, Kanda M, Polanski K, Heinig M, Lee M, et al. Cells of the adult human heart. *Nature*. 2020;588:466–472. doi: 10.1038/s41586-020-2797-4
3. Reichart D, Lindberg EL, Maatz H, Miranda AMA, Viveiros A, Shvetsov N, Gärtner A, Nadelmann ER, Lee M, Kanemaru K, et al. Pathogenic variants damage cell composition and single cell transcription in cardiomyopathies. *Science*. 2022;377:eabo1984. doi: 10.1126/science.abo1984
4. Murashige D, Jang C, Neinast M, Edwards JJ, Cowan A, Hyman MC, Rabinowitz JD, Frankel DS, Arany Z. Comprehensive quantification of fuel use by the failing and nonfailing human heart. *Science*. 2020;370:364–368. doi: 10.1126/science.abc8861
5. Ngo V, Harel F, Finnerty V, Pelletier-Galarneau M. Characterizing normal values of myocardial blood flow and myocardial flow reserve evaluated by PET rubidium-82 imaging in patients with low risk of coronary artery disease. *J Nucl Med*. 2022;63(supplement 2):2462–2462.