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Unveiling the vulnerability of C57BL/6J female mice to HFpEF and its related complications

B. Srinivas[a], **K. Alluri**[a], **H. Peng**[b,c], **P.A. Ortiz**[b,c], **J. Xu**[d], **H.N. Sabbah**[d], **N.E. Rhaleb**[b,d], **K. Matrougui**[a,*]

[a]Department of Physiological Sciences, EVMS, Norfolk, VA 23501, USA

[b]Department of Physiology, Wayne State University, Detroit, MI 48202, USA

[c]Internal Medicine Department, Hypertension and Vascular Research, Detroit, MI 48202, USA

[d]Internal Medicine Department, Cardiovascular Medicine, Detroit, MI 48202, USA

Abstract

Introduction: The impact of female biological sex on the development of heart failure with preserved ejection fraction (HFpEF) and its associated kidney disease and vascular endothelial dysfunction is still controversial. Whether females are protected from HFpEF and associated complications is not well established. Previous studies report conflicting prevalence between genders. We hypothesize that female mice are unprotected from HFpEF and its associated kidney disease and vascular endothelial dysfunction.

Methods: Eight-week-old female mice were divided into four groups: control groups receiving a standard diet and water for either 5 or 16 weeks, and HFpEF groups fed a high-fat diet (HFD, Rodent Diet With 60 kcal% Fat) and N [w]-nitro-l-arginine methyl ester (L-NAME - 0.5 g/L) in the drinking water for 5 or 16 weeks. Various measurements and assessments were performed, including echocardiography, metabolic and hypertensive evaluations, markers of heart and kidney injury, and assessment of vascular endothelial function.

Results: Female mice with HFD and L-NAME developed HFpEF at 5 weeks, evidenced by increased E/E' ratio, reduced cardiac index, left ventricular mass, and unchanged ejection fraction. After 16 weeks, HFpEF worsened. Metabolic disorders, hypertension, lung wet/kidney

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*Corresponding author. NRHALEB1@hfhs.org (N.E. Rhaleb), matrouk@evms.edu (K. Matrougui).

CRediT authorship contribution statement

B. Srinivas: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation. **K. Alluri:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation. **H. Peng:** Methodology, Formal analysis, Data curation. **P.A. Ortiz:** Methodology, Formal analysis, Data curation. **J. Xu:** Methodology, Formal analysis, Data curation. **H.N. Sabbah:** Writing – review & editing, Methodology, Formal analysis, Data curation. **N.E. Rhaleb:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **K. Matrougui:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

Nothing to declare.

Disclosures

None.

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weight increase, exercise intolerance, and cardiac/renal injury markers were observed. Vascular endothelial dysfunction was associated with ER stress and fibrosis induction.

Conclusions: We found that female mice are susceptible to the development of HFpEF and its associated kidney disease and vascular endothelial dysfunction. Our data support the concept that the female sex does not protect from HFpEF and its associated kidney disease and vascular endothelial dysfunction when disease risk factors are present.

Keywords

HFpEF; Female mice; Vascular endothelia dysfunction; Kidney failure; Inflammation; ER stress

Heart failure with preserved ejection fraction (HFpEF) pathogenesis is a significant unmet need in cardiovascular medicine. The study aimed to determine the impact of HFpEF and its associated kidney disease and vascular endothelial dysfunction in female mice. Several risk factors can enhance the likelihood of developing HFpEF pathogenesis, and some of these risk factors may affect women. For instance, women who smoke, have high blood pressure, or have diabetes may have a higher risk of developing heart disease. The majority of patients with HFpEF are women, and clinical studies indicate a high prevalence of HFpEF pathogenesis in women [1,2]. An experimental study using a high-fat diet (HFD, Rodent Diet With 60 kcal% Fat, Research Diets Inc. Catalog# D12492i), and $N^{[w]}$-nitro-l-arginine methyl ester (L-NAME) to induce HFpEF showed that the female C57BL/6N mice are protected from HFpEF [3]. However, a recent study showed that female C57BL/6J mice develop HFpEF, and male hearts have more mitochondria and mitochondrial gene expression than female hearts, affecting HFpEF pathogenesis between males and females [4]. Dr. Tong et al. [3] showed that female C57BL/6N mice are protected from HFpEF. Our study demonstrates that females C57BL/6J are unprotected from HFpEF pathogenesis and could involve kidney and vascular endothelial dysfunction.

To induce HFpEF, we used a similar HFD composition and L-NAME dose as previously reported. Eight-week-old C57BL/6J (From Jackson Laboratory) female mice were randomly divided into four groups: Group 1 received a standard diet and water for 5 weeks (5-week control); Group 2 received an HFD (Rodent Diet With 60 kcal% Fat, Research Diets Inc. Catalog# D12492i) with L-NAME (0.5 g/L, Sigma-Aldrich) in the drinking water for 5 weeks (5-week HFpEF); Group 3 received standard diet and water for 16 weeks (16-week control); and Group 4 received HFD with L-NAME (0.5 g/L) in the drinking water for 16 weeks (16-week HFpEF). All experimental procedures followed the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the IACUC at EVMS and WSU (Norfolk, VA; Detroit, MI).

Echocardiography (Vevo 3100) data showed that female mice subjected to HFD and L-NAME for 5 weeks developed HFpEF, as evidenced by a significant increase in E/E' ratio, a decrease in cardiac index, an increase in left ventricular mass (LVM), and no change in ejection fraction (Fig. 1A). After 16 weeks of HFD and L-NAME, female mice developed a severe HFpEF as indicated by the further increase in E/E' ratio and LVM and decrease in cardiac index with no change in cardiac ejection fraction compared to 5 weeks of HFpEF (Fig. 1A). Many patients with HFpEF harbor the comorbidity of hypertension and

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metabolic diseases such as type 2 diabetes. [5] We observed that these mice developed a metabolic disorder and hypertension, as indicated by the significant increase in body weight, glucose tolerance test, insulin levels, and arterial blood pressure after 5-weeks of HFpEF (Fig. 1B). These results were associated with significant lung wet weight, kidney weight increase, and exercise intolerance (Fig. 1C). We further examined the heart and kidney failure/injury using specific markers such as Brain Natriuretic Peptide (BNP, MyBioSource, #MBS2700196), T cell Immunoglobulin and Mucin domain 1/Kidney Injury Molecules1, and Hepatitis A virus Cellular Receptor (Tim-1/KIM-1/HAVCR, Bio-techne, Cat No. MKM100), Liopcalin-2/Neutrophil Gelatinase Lipocalin associated (Lipocalin-2/NGAL, Bio-techne, Cat No. MLCN20), albuminuria, and glomerular filtration rate (GFR) in female control mice with 5-and-16-weeks of HFpEF. The results showed that BNP, a marker of cardiac hypertrophy, and Tim-1/KIM-1/HAVCR/Lipocalin-2/NGAL, markers of renal injury, were significantly increased in female mice with 5 weeks of HFpEF and further exacerbated in female mice with 16 weeks of HFpEF (Fig. 1D). Albuminuria and GFR were also affected (renal dysfunction) (Fig. 1D). We also examined vascular endothelial function in two vascular beds (mesenteric resistance arteries and aorta). Female mice with 5 weeks of HFpEF have impaired endothelium-dependent relaxation due to reduced eNOS and Akt phosphorylation, but their contractile response and endothelium-independent relaxation remain unaffected (Fig. 1E). At 5 weeks of HFpEF, cardiac ER stress CHOP was increased, and Xbp1s was decreased with no change in XBp1u and ATF6 expression (Fig. 1F). The mRNA of ER stress, inflammation, caspase 3 & 12, and TSP-1 were significantly increased in female mice with 16 weeks of HFpEF (Fig. 1F). Cardiac fibrosis increased slightly at 5 weeks of HFpEF but markedly increased at 16 weeks of HFpEF (Fig. 1F).

These findings have significant implications for female health. It is crucial to recognize the unique aspects of female biology and physiology and to address them in medical research and clinical practice. Therefore, acknowledging the significance of female biology and physiology is essential to advancing medical research and improving clinical outcomes for women. In summary, these results highlight the susceptibility of female mice to the development of HFpEF and its associated kidney disease and vascular endothelial dysfunction when disease risk factors are present.

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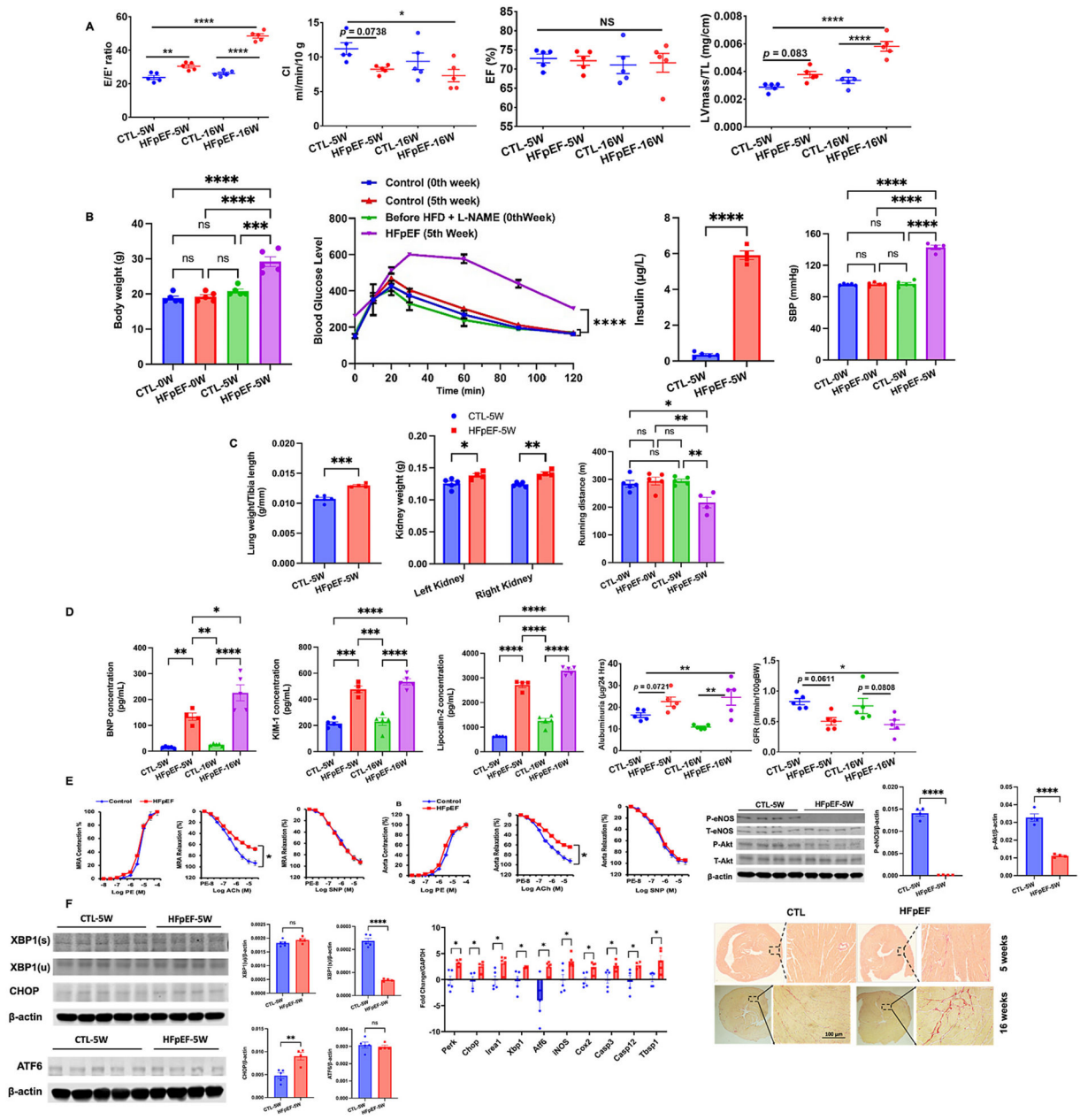

Fig. 1.

(A) Echocardiography shows cardiac E/E ratio, cardiac index (CI), left ventricular mass (LVM), and cardiac ejection fraction in female control mice and mice with 5 and 16 weeks of HFpEF (n = 5). (B) Body weight, glucose tolerance test, insulin level, and systolic blood pressure (SBP) in control female mice and female mice with 5 weeks of HFpEF (n = 4–5). (C) Lung wet weight/tibia length, kidney weight, and running distance in female control mice and mice with 5 weeks of HFpEF (n = 4–5). (D) Brain-Natriuretic-Peptide (BNP), Tim-1/KIM-1/HAVCR, Lipocalin-2/NGAL, albuminuria, and glomerular filtration rate (GFR) in female control mice and female mice with 5 and 16 weeks of HFpEF (n = 4–5). (E) Contraction response to phenylephrine (PE), endothelium-dependent and independent relaxation mesenteric resistance arteries and aorta of control and 5-weeks HFpEF female

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mice (n = 4–5) compared to the control. Western blot analysis and cumulative data for phosphorylated eNOS (P-eNOS), Akt (P-Akt), total eNOS (T-eNOS), and total Akt (T-Akt) in control female mice and female mice with 5-weeks of HFpEF (n = 4–5). (F) Cardiac ER stress Xbp1(s), Xbp1(u), CHOP, and ATF6 expression and cumulative data in female control mice and female mice with 5 weeks of HFpEF (n = 4–5). Cardiac mRNA qPCR data for ER stress (PERK, CHOP, IREa1, Xbp1, ATF6), inflammation (iNOS, Cox2), apoptosis (Caspase 3 and 12), and anti-angiogenic factor (thrombospondin 1: Tbsp1) in female control mice and female mice with 16 weeks of HFpEF (n = 5). Cardiac fibrosis in female control mice and mice with 5 and 16 weeks of HFpEF (n = 4–5). The software used for analysis is GraphPad Prism software version 10.1. Two-way followed by Tukey's multiple comparisons post hoc test was applied for (A), RM-One-way ANOVA followed by Tukey's multiple comparisons post hoc test and Paired t-tests or Unpaired t-tests for (B, C), Two-way ANOVA followed by Tukey's multiple comparisons post hoc test was applied for (D) and Paired t-tests or Unpaired t-tests was applied for (E, F). ns: not significant, RM: Repeated Measurement. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001, for CTL-0 W vs HFpEF-0 W, vs CTL-5 W vs HFpEF-5 W, CTL-16 W vs HFpEF-16 W (n = 4–5).