7-18-2023

Interleukin-1β Disruption Protects Male Mice From Heart Failure With Preserved Ejection Fraction Pathogenesis

Balaji K. Srinivas

Aya Bourdi

Jacob D. O'Regan

Kumar D. Malavalli

Nour-Eddine Rhaleb

See next page for additional authors

Authors

Balaji K. Srinivas, Aya Bourdi, Jacob D. O'Regan, Kumar D. Malavalli, Nour-Eddine Rhaleb, Souad Belmadani, and Khalid Matrougui

Journal of the American Heart Association

ORIGINAL RESEARCH

Interleukin-1β Disruption Protects Male Mice From Heart Failure With Preserved Ejection Fraction Pathogenesis

Balaji K. Srinivas, PhD; Aya Bourdi, BS; Jacob D. O'Regan, BS; Kumar D. Malavalli, PhD; Nour-Eddine Rhaleb, PhD; Souad Belmadani, PhD; Khalid Matrougui, PhD

BACKGROUND: Heart failure with preserved ejection fraction (HFpEF) is a significant unmet need in cardiovascular medicine and remains an untreatable cardiovascular disease. The role and mechanism of interleukin-1β in HFpEF pathogenesis are poorly understood.

METHODS AND RESULTS: C57/Bl6J and interleukin-1β$^{-/-}$ male mice were randomly divided into 4 groups. Groups 1 and 2: C57/Bl6J and interleukin-1β$^{-/-}$ mice were fed a regular diet for 4 months and considered controls. Groups 3 and 4: C57/Bl6 and interleukin-1β$^{-/-}$ mice were fed a high-fat diet with N[w]-nitro-l-arginine methyl ester (endothelial nitric oxide synthase inhibitor, 0.5 g/L) in the drinking water for 4 months. We measured body weight, blood pressure, diabetes status, cardiac function/hypertrophy/inflammation, fibrosis, vascular endothelial function, and signaling. C57/Bl6 fed a high-fat diet and N[w]-nitro-l-arginine methyl ester in the drinking water for 4 months developed HFpEF pathogenesis characterized by obesity, diabetes, hypertension, cardiac hypertrophy, lung edema, low running performance, macrovascular and microvascular endothelial dysfunction, and diastolic cardiac dysfunction but no change in cardiac ejection fraction compared with control mice. Interestingly, the genetic disruption of interleukin-1β protected mice from HFpEF pathogenesis through the modulation of the inflammation and endoplasmic reticulum stress mechanisms.

CONCLUSIONS: Our data suggest that interleukin-1β is a critical driver in the development of HFpEF pathogenesis, likely through regulating inflammation and endoplasmic reticulum stress pathways. Our findings provide a potential therapeutic target for HFpEF treatment.

Key Words: cardiac fibrosis ■ ER stress ■ HFpEF ■ inflammation ■ interleukin-1β ■ vascular endothelial function

Heart failure with preserved ejection fraction (HFpEF) is a systemic multiorgan disease, with a significant unmet need in cardiovascular medicine, and remains an untreatable cardiovascular disease. HFpEF prevalence is increasing, becoming the most common form of cardiovascular disease. HFpEF accounts for >50% of heart failure cases.[1–4] Understanding HFpEF will give us essential insights into the pathways and mechanisms of action involved in HFpEF pathogenesis. Almost all large-scale clinical trials to improve HFpEF have had neutral results.[5,6] Thus, there is a critical and immediate need to delineate cellular and molecular mechanisms and identify treatable targets to rescue vascular and cardiac function and structure in the setting of HFpEF pathogenesis. A significant number of patients with HFpEF harbor the comorbidities of hypertension and obesity with type 2 diabetes[7,8] that result in chronic cardiometabolic-physical stress. Many drugs have been tested in clinical trials to improve HFpEF, but most results were unsatisfactory, suggesting that HFpEF is a complex cardiovascular disease.[8] Conventional drugs against hypertension and diabetes do not have consistent evidence of improvements in HFpEF-induced pathogenesis.[8] Moreover, recent studies suggest that

Correspondence to: Khalid Matrougui, PhD, Department of Physiological Sciences, EVMS, Norfolk, VA 23501.
Email: matrouk@evms.edu

This article was sent to Rebecca D. Levit, MD, Associate Editor, for review by expert referees, editorial decision, and final disposition.

For Sources of Funding and Disclosures, see page 10.

JAHA is available at: www.ahajournals.org/journal/jaha

RESEARCH PERSPECTIVE

What New Question Does This Study Raise?

- The study raises whether interleukin-1β (IL-1β) plays a critical role in developing heart failure with preserved ejection fraction (HFpEF) pathogenesis through regulating inflammation and endoplasmic reticulum stress pathways. The genetic disruption of IL-1β protected mice from HFpEF pathogenesis. These findings offer a potential therapeutic target for HFpEF treatment, which is currently an unmet need in cardiovascular medicine.

What Question Should Be Addressed Next?

- The study raises whether interleukin-1β (IL-1β) plays a critical role in developing HFpEF pathogenesis through regulating inflammation and endoplasmic reticulum stress pathways. The genetic disruption of IL-1β protected mice from HFpEF pathogenesis. These findings offer a potential therapeutic target for HFpEF treatment, which is currently an unmet need in cardiovascular medicine.

Nonstandard Abbreviations and Acronyms

COX2	cyclooxygenase-2
eNOS	endothelial nitric oxide synthase
ER	endoplasmic reticulum
HFD	high-fat diet
HFpEF	heart failure with preserved ejection fraction
iNOS	inducible nitric oxide synthase
IRE1	inositol-requiring enzyme 1
L-NAME	N[w]-nitro-l-arginine methyl ester
MRAs	mesenteric resistance arteries
NF-κB	nuclear factor-κ B
TGF-β1	transforming growth factor beta 1

myocardial stiffness, inflammation, and microvascular endothelial dysfunction contribute to HFpEF pathogenesis.[9] Furthermore, high-mobility group protein B1 and mitochondrial hyperacetylation have been shown to contribute to HFpEF.[10] Recently, Dr. Hill laboratory generated a new preclinical mouse model of HFpEF that recapitulates the most features of HFpEF in patients.[11] Using this new mouse model of HFpEF induced by a high-fat diet and N[w]-nitro-l-arginine methyl ester (L-NAME) in drinking water for 5 weeks, Hill reported that an increase in iNOS (inducible nitric oxide synthase) expression dysregulates the endoplasmic reticulum (ER) stress inositol-requiring enzyme 1(IRE1)α- X-box binding protein 1 pathway as a mechanism in cardiomyocyte dysfunction.[11] Inflammation has long been considered an essential contributing factor in cardiovascular diseases. Our laboratory and others have previously reported that inflammation induction, immune regulatory T cells, and dendritic cells are crucial factors in hypertension- and diabetes-induced cardiovascular complications.[12–16] Patients with HFpEF display signs of chronic inflammation, and recent reports showed elevated circulating inflammatory biomarkers in HFpEF, such as CRP (C-reactive protein) and interleukin-1.[17,18] However, the role of interleukin-1β in the development of HFpEF and cardiovascular complications still remains unknown. Therefore, in this study, we sought to determine the role and underlying mechanism of interleukin-1β in HFpEF pathogenesis using C57/Bl6J and interleukin-1β$^{-/-}$ male mice fed with and without a high-fat diet and L-NAME in the drinking water for 4 months to induce chronic HFpEF.

METHODS

Transparency and Openness Promotion Statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

Mice

All the experimental procedures conformed to the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*. The procedures followed were in accordance with institutional guidelines. The Institutional Animal Care and Use Committee at the Eastern Virginia Medical School (Norfolk, VA) approved the experimental procedures. Male C57BL/6J and interleukin-1β$^{-/-}$ mice on the C57BL/6J background (8 weeks old) from Jackson Laboratory (Bar Harbor, ME) were housed in a temperature-controlled room (22 ± 1 °C) and exposed to 12 hours of the dark–light cycle with access to water and food.

HFpEF Induction

Group 1: C57BL/6J (n=5) and group 3: interleukin-1β$^{-/-}$ male mice (n=5) were fed a regular chow research diet and considered as control groups. Research Diet for the high-fat diet (HFD) groups with L-NAME (50 mg/100 mL, pH 7.4, CAS number: 51298–62-5, Sigma-Aldrich) (group 2: C57BL/6J+HFD+L-NAME [n=5]; group 4: interleukin-1β$^{-/-}$+HFD+L-NAME [n=5]) was supplied in the drinking water for the 4 months, and these were considered the HFpEF mice. We changed the water with L-NAME every other day. At the end of the treatment, mice were euthanized using isoflurane (5%) overdose, followed by heart excision when fully sedated.

Body Weight and Fasting Blood Glucose

We measured body weight in all groups of mice, and fasting blood glucose levels were measured at the end of the fourth month.

Glucose and Insulin Tolerance Test

For the HFpEF study, after 4 months, all mice were subjected to an intraperitoneal glucose tolerance test and insulin tolerance test.

Glucose Tolerance Test

Mice fasted overnight. Blood samples were obtained from the tail vein using an Ultra Touch glucometer (True Test Glucometer; NIPRO Diagnostics). Mice then received 2 g/kg D-glucose intraperitoneally, and tail vein lancing (using a small sterile needle) was used to draw the blood (≈2 μL) to determine the blood glucose levels. Blood glucose levels were measured with an Ultra Touch glucometer at 0, 10, 20, 30, 60, 90, and 120 minutes after the glucose injection.

Insulin Tolerance Test

Mice were fasted for 6 hours and then injected with 1 U/kg of insulin intraperitoneally. Using an Ultra Touch glucometer, blood glucose level was then determined at 0, 10, 20, 30, 60, and 90 minutes after insulin injection.

Echocardiography for HFpEF Study

Mice were anesthetized by 5% isoflurane and confirmed by lack of response to steady pressure on one of the hind paws. During echocardiography acquisition under body temperature-controlled conditions, isoflurane was reduced to 1% to 1.5% and adjusted to maintain a heart rate in the 415 to 460 beats/min range. Systolic ejection fraction and cardiac relaxation rate were measured in mice before and 4 months after HFD+L-NAME using an echocardiograph with a 15-MHz linear transducer (Acuson c256, Mountain View, CA).[19,20]

Tail Cuff Blood Pressure and Mice Preparation

Systolic blood pressure was measured noninvasively in conscious mice using the CODA tail-cuff blood pressure system[15] (Kent Scientific, Torrington, CT). Animals were placed in individual holders on a temperature-controlled platform (37 °C), and recordings were performed under steady-state conditions. Before testing, all mice were acclimated to systolic blood pressure measurements. During the HFD and L-NAME period, systolic blood pressure was measured once a month for 4 months. Arterial blood pressure measurements were performed at the same time of the day (between 9 AM and 11 AM) to avoid influence from the circadian cycle. The value of systolic blood pressure was obtained by estimating the average of 8 successful measurements. At the end of the fourth month, we euthanized the mice and harvested blood and tissues (heart, lung, kidney [left and right], mesenteric resistance arteries [MRAs], and thoracic aorta). Tibia length was used to determine organ hypertrophy. All the harvested tissues were immediately placed in cold PSS solution (NaCl 118 mmol/L; KCl 4.7 mmol/L; $CaCl_2$ 2.5 mmol/L; KH_2PO_4 1.2 mmol/L; $MgSO_4 \times 7H_2O$ 1.2 mmol/L; $NaHCO_3$ 25 mmol/L and glucose 11 mmol/L, pH=7.4) and processed appropriately for further studies. Blood samples were centrifuged at 24 147 *g* for 5 minutes at 4 °C to obtain plasma and immediately stored at −80 °C. Fixed heart, lung, kidney, and MRAs were also used to determine the fibrosis.

Exercise Exhaustion Test

After 3 days of acclimatization to treadmill exercise, an exhaustion test was performed in the experimental groups of mice. Mice ran (flat 180°) on the treadmill (Ugo Basile SRL, Model 47300-001, Italy), starting at a warm-up speed of 5 m/min for 4 minutes, after which the rate was increased to 14 m/min for 2 minutes. Every next 2 minutes, the speed was gradually increased by 2 m/min until the mouse was exhausted. Exhaustion was defined as the inability of the mouse to return to running within 10 seconds of direct contact with an electric-stimulus grid, and running distance was calculated.

Resistance and Conductance Artery Endothelial Function

MRAs and aorta reactivity in all groups of mice was evaluated as previously reported.[15,21] The MRAs and aorta from all groups of mice were immediately placed in cold PSS solution, carefully cleared of perivascular fat and connective tissue, and cut into rings (2 mm in length). The arteries were mounted in small vessels in a dual-chamber myograph (DMT myograph; AD Instruments Ltd., Oxford, UK) for measurement of isometric tension. After 30 minutes equilibration period in PSS solution bubbled with CO_2 at 37 °C (pH=7.4), arteries were stretched to their optimal lumen diameter for active tension development. After 1 hour of incubation time, artery rings were preconstricted with phenylephrine (10^{-8}–10^{-4} M) (Sigma-Aldrich P6126-50G, CAS 61-76-7), and when a steady maximal contraction was reached, cumulative dose-response curves were obtained for acetylcholine (10^{-8}–10^{-4} M) (Sigma–Aldrich, A6625-25G, Lot#BCBH3758V) and sodium nitroprusside (10^{-8}–10^{-4} M) (Sigma–Aldrich, S-0501, lot 81K3688). To determine the impact of inflammation and ER stress pathways on endothelial cell function, we performed experiments in which we isolated arteries

from each group and then incubated them with iNOS inhibitor (L-NIL hydrochloride [N^6-(1-Iminoethyl)-L-lysine hydrochloride]; CAS number: 150403–89-7, TOCRIS), dose 10 μmol/L and cyclooxygenase-2 (COX2) inhibitor (NS398 [N-(2-Cyclohexyloxy-4-nitrophenyl)] methanesulfonamide; CAS number:123653-11-2, TOCRIS, dose 10 μmol/L). After 30 minutes of incubation, we performed vascular contraction to phenylephrine and relaxation to acetylcholine and sodium nitroprusside on precontracted MRAs.

Measurement of Nitrate

The amounts of nitrate, the breakdown product of nitric oxide, were measured in the urine samples from all groups of mice using the nitrate assay kit (Cayman Chemicals, number 780001, batch 0600896) according to the manufacturer's protocol.

Interleukin-1β in Mouse Plasma

The amount of interleukin-1β was measured in the plasma samples from all groups of mice using the mouse interleukin-1β ELISA kit according to the manufacturer's protocol (ref: BMS6002, lot 308580–002, Invitrogen). Optical density at 450 nm was used to calculate the concentration of interleukin-1β in the samples, and results were expressed as pg/mL.

Measurement of Mouse Plasma Insulin

The quantitative determination of insulin level in mouse plasma was measured from all groups of mice using the mouse insulin ELISA kit according to the manufacturer's protocol. (Mercodia Mouse Insulin ELISA; ref: 10-1247-01, lot 29267). Optical density at 450 nm was used to calculate insulin concentration in the samples, and results were expressed as μg/L.

p65–Nuclear Factor-Kappa B Activity in Heart Tissues

ELISA-based estimations of the levels of p65–nuclear factor-kappa B (NF-κB), a critical regulator of inflammation, were recorded. The assessment protocol was followed per the manufacturer's protocol (p65–NF-κB, number NBP2-2966, Novus Biologicals). Optical density at 405 nm was used to calculate the concentration of protein present in the samples, and the results were represented as ng/mL protein.

Transforming Growth Factor Beta 1 in Mouse Plasma

The amount of transforming growth factor beta 1 (TGF-β1) was measured in the plasma samples from all groups of mice using the mouse TGF-β1 ELISA kit according to the manufacturer's protocol (Invitrogen, number BMS608-4). Optical density at 450 nm was used to calculate the concentration of TGF-β1 in the samples, and results were expressed as pg/mL protein.

Picrosirius Red Staining

Picrosirius Red staining was carried out to determine collagen deposition on the heart and aorta from all the groups of mice. Paraffin-embedded specimens of selected tissues were cut 4- to 5-μm thick; deparaffinized samples were rinsed with Milli-Q water 3 times. The paraffined samples were then incubated with the Picrosirius Red as per the manufacturer's protocol detailed in the kit (Novaultra Sirius Red STAIN Kit; catalog number: NC0249910). Finally, samples were dehydrated, mounted, and observed under the brightfield microscope (×20) (Olympus, IX73 U-TB190, Japan).

Quantitative Polymerase Chain Reaction

Total RNA isolated from C57BL/6J, C57BL/6J+HFD+L-NAME, interleukin-1$\beta^{-/-}$, and interleukin-1$\beta^{-/-}$+HFD+L-NAME male heart using MagMAX Total RNA Isolation kit (ref: AM1830, Lot 01257092; Thermo Fisher Scientific). RNA was reverse-transcribed using the High-Capacity cDNA Reverse Transcription kit (I script; Bio-Rad, number 1708840) following the manufacturer's instructions. Quantitative polymerase chain reaction was carried out using TaqMan Fast Advanced Mix (catalog number 4444556) with specific probes for the amplification for activating transcription factor 6 (Mm01295319_m1#PN2034377), protein kinase–like endoplasmic reticulum kinase (Eit2aK3Mm00438700_m1#PN435137), CCAAT/enhancer-binding protein (Ddit307294308_m1#PN4351370), IRE1 (Ern1 Mm00470233_m1#PN4351370), COX2 (Mm03294838_g1#4331182), iNOS (Mm00440502_m1 #PN4351370), and GAPDH (Mm99999915_g1#PN1996679). GAPDH was used for normalization. The relative mRNA expression was calculated using the $2^{-\Delta\Delta CT}$ method.

Western Blot Analysis

Western blot was used to identify specific proteins in lysates of mice MRA tissue as previously described.[12,15,22] The MRAs were isolated from all groups of mice and immediately stored at −80 °C. Tissue lysates were prepared by homogenization with an electrical homogenizer after adding ice-cooled tissue-protein extraction reagent (T-PER, Thermo Fisher; lot: WE320059, catalog number 78510), sonicated for 5 seconds, and centrifuged for 20 minutes at 24 147 *g*. Protein quantification was performed according to Pierce BCA Protein Assay Kit (product number 23225). We used specific antibodies against endothelial nitric oxide synthase (eNOS; Cell Signaling, D9A5L rabbit monoclonal antibodies; number 32027), phospho-eNOS (Ser1177; Cell

Signaling [C9C3] rabbit monoclonal antibodies, 9570), iNOS (Proteintech, number 18985-1-AP), anti-COX2 (Cayman; aa 584–598, number 160126), NLR family pyrin domain containing 3/Nacht leucine-rich repeat protein 3 (Novus Biotech; number NBP2-12446), X-box binding protein 1 (Abcam; number ab238456), anti–CCAAT/enhancer-binding protein (Cell Signaling; L63F7 mouse monoclonal antibodies number 2895), IRE1 (Abcam; number ab37073, lot GR3354835-4), activating transcription factor 6 (Abcam; number ab37149, lot GR3353621-3), and beta-actin (Santa Cruz; number SC47778). All dilutions were prepared according to the manufacturer's recommendation. The membranes were developed using the Odyssey imaging system LICOR, and the bands were quantified.

Statistical Analysis

Data are expressed as mean±SEM. Statistical calculations for significant differences were performed using 1-way or repeated-measure 2-way ANOVA plus Tukey's post hoc test for multiple comparisons as appropriate in all experiments. Comparisons were considered statistically significant when $P<0.05$. Statistical analyses were conducted using Prism version 9.5 (GraphPad Software).

RESULTS

Our data illustrated that C57/Bl6J male mice fed an HFD and L-NAME in the drinking water for 4 months develop HFpEF characterized by hypertension, obesity/type 2 diabetes, diastolic dysfunction, no change in cardiac ejection fraction, low exercise performance, glucose metabolism dysregulation, cardiac hypertrophy, lung edema, kidney weight, and insulin-level increase compared with C57/Bl6J and interleukin-1β$^{-/-}$ male mice fed regular chow for 4 months (Figure 1). Interestingly, our data uncovered that interleukin-1β$^{-/-}$ male mice are protected from HFpEF pathogenesis induced by an HFD and L-NAME in the drinking water for 4 months (Figure 1). Remarkably, after 4 months of an HFD and L-NAME, interleukin-1β$^{-/-}$ male mice did not develop hypertension and obesity/type 2 diabetes. Instead, they displayed improved cardiac relaxation function, exercise performance, and glucose metabolism (Figure 1). These data indicate the significant impact of interleukin-1β in the development of HFpEF.

It is well known that HFpEF is associated with vascular endothelial dysfunction. Our data showed endothelial dysfunction of large and resistance arteries (aortas and MRAs) in C57/Bl6J male mice fed an HFD and L-NAME compared with C57/Bl6J and interleukin-1β$^{-/-}$ male

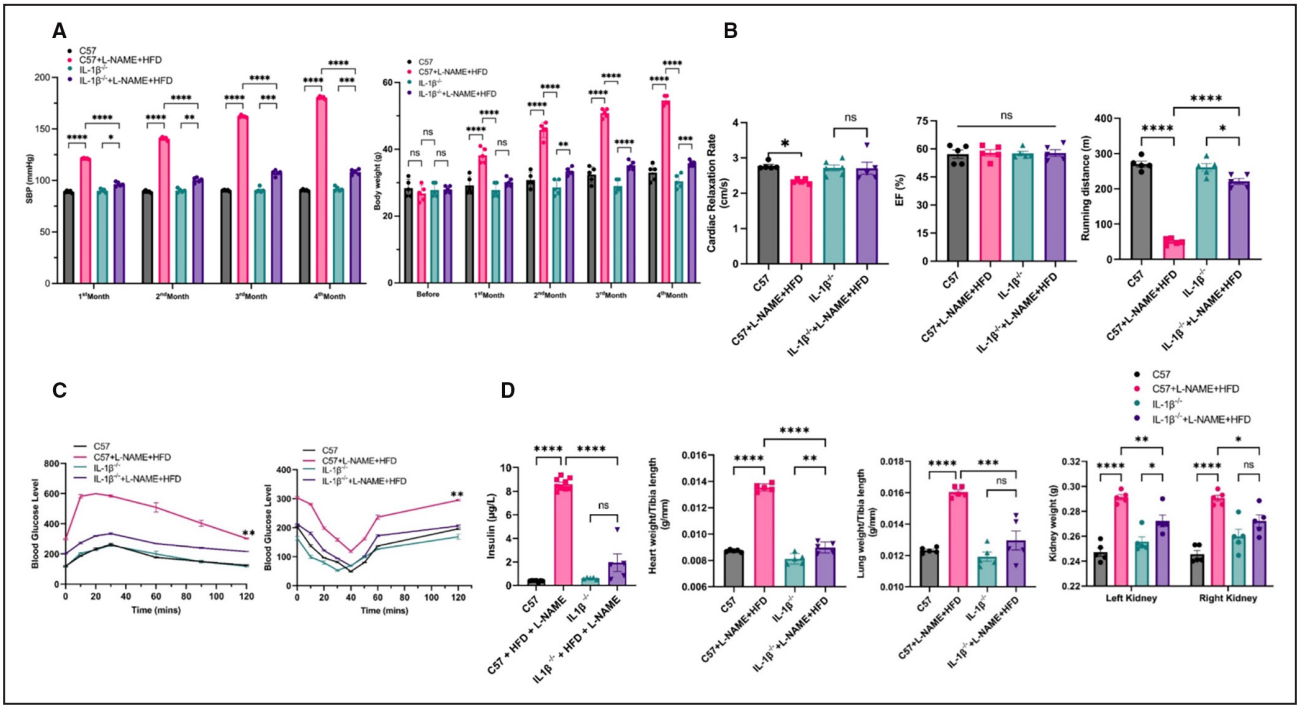

Figure 1. Heart failure with preserved ejection fraction (HFpEF) induction.
C57/Bl6J (C57) male mice fed a high-fat diet (HFD) and N[w]-nitro-l-arginine methyl ester (L-NAME) for 4 months developed HFpEF characterized by increased systolic arterial blood pressure (**A**); reduced cardiac relaxation but no change in cardiac ejection fraction, and reduced running distance; (**B**) impaired glucose and insulin metabolism (**C**); and increase in insulin level, heart, lung, and kidney weight (**D**) compared with male interleukin-1β$^{-/-}$ mice fed HFD and L-NAME for 4 months. Results are presented as mean±SEM. Repeated-measure 2-way ANOVA followed by Tukey's multiple comparisons post hoc test was applied for (**A**). One-way ANOVA followed by Tukey's multiple comparisons test for (**B** through **D**). ns indicates not significant; and SBP, systolic blood pressure. *$P<0.05$, **$P<0.01$, ***$P<0.001$, ****$P<0.0001$ for C57BL/6J vs C57/Bl6+HFD+L-NAME, vs interleukin-1β (IL-1 β) $^{-/-}$ vs IL-1β$^{-/-}$+HFD+L-NAME; (n=5).

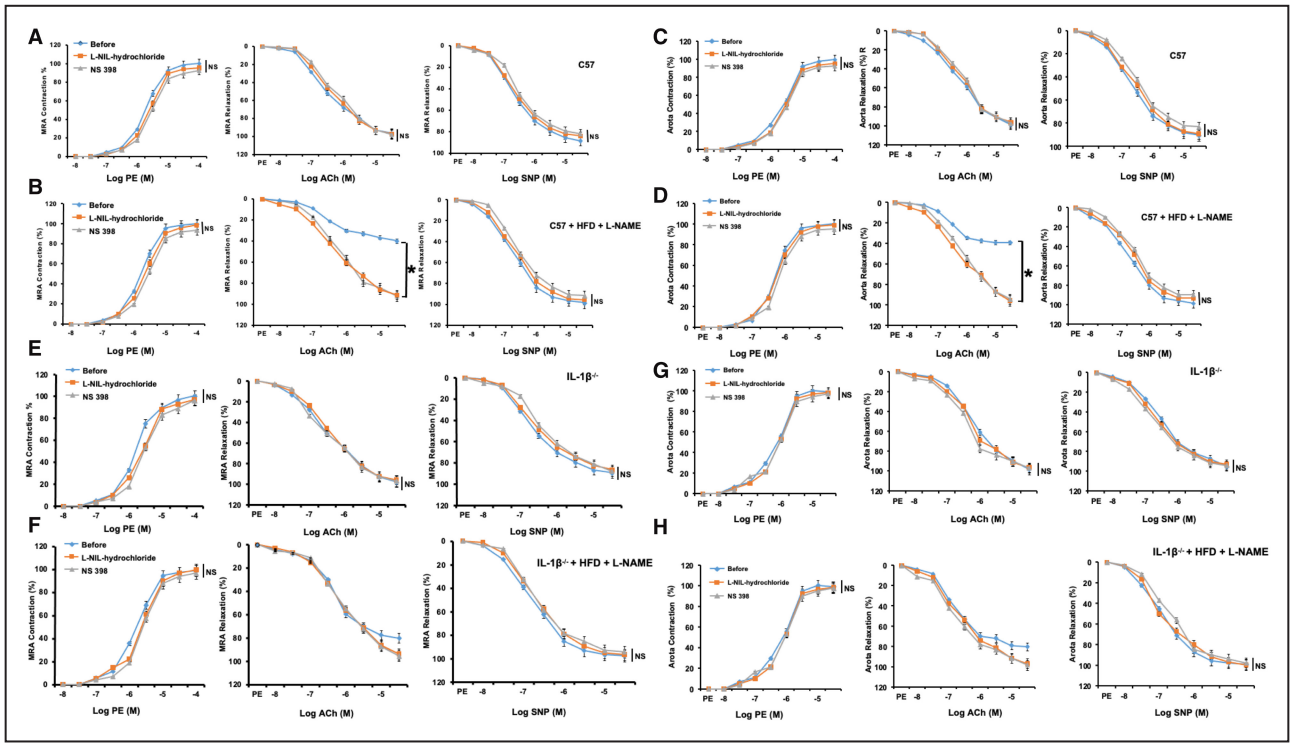

Figure 2. Mesenteric resistance artery (MRA) and thoracic aorta reactivity.
Showing contractility in response to sympathetic stimulation (phenylephrine [PE]) and endothelium-dependent and -independent relaxation in response to acetylcholine (ACh) and sodium nitroprusside (SNP) in C57BL/6J (C57), C57+high-fat diet (HFD)+N[w]-nitro-l-arginine methyl ester (L-NAME), interleukin-1β$^{-/-}$ (IL-1β$^{-/-}$), and interleukin-1β$^{-/-}$+HFD+L-NAME male mice MRAs (**A**, **B**, **E**, **F**) and aortas (**C**, **D**, **G**, **H**). MRA and aorta reactivity was also assessed in all groups of mice in the presence of inducible nitric oxide synthase (iNOS) inhibitor (L-NIL-hydrochloride) and cyclooxygenase-2 (COX2) inhibitor (NS 398) (**A** through **H**). *P<0.05 for C57/Bl6+HFD+L-NAME, vs C57. One-way ANOVA followed by Tukey's post hoc test was applied. *P<0.05 for C57 vs C57+HFD+L-NAME, vs IL-1β$^{-/-}$ vs IL-1β$^{-/-}$+HFD+L-NAME; (n=5).

mice fed regular chow for 4 months (Figure 2). The incubation of the arteries with iNOS or COX2 inhibitors (L-NIL hydrochloride and NS398, respectively) significantly improved vascular endothelium-dependent relaxation (Figure 2). Notably, the endothelial function was not impaired in large and resistance arteries from interleukin-1β$^{-/-}$ male mice fed an HFD and L-NAME in the drinking water for 4 months (Figure 2). Nitric oxide donor-induced endothelium-independent relaxation and phenylephrine-induced contraction were normal in groups fed an HFD and L-NAME (Figure 2). These data signify that HFpEF compromises the endothelial function rather than the response of vascular smooth muscle cells to nitric oxide.

Western blot analysis and kit assays show a decrease in eNOS phosphorylation and nitrate level in urine from male C57/Bl6J mice fed an HFD and L-NAME in the drinking water for 4 months compared with male C57/Bl6J and interleukin-1β$^{-/-}$ mice fed regular chow for 4 months (Figure 3A and 3B). Interestingly, eNOS phosphorylation and nitrate level were unaffected in interleukin-1β$^{-/-}$ male mice fed an HFD and L-NAME (Figure 3A and 3B).

Inflammation and ER stress are essential to cardiovascular complications. Here, we showed inflammation and ER stress induction in MRAs, indicated by the increase in inflammatory markers (iNOS, COX2, NLR family pyrin domain containing 3, NFκB, and interleukin-1β) and an increase in ER stress markers (CCAAT/enhancer-binding protein, activating transcription factor 6, IRE1, protein kinase–like endoplasmic reticulum kinase), in C57/Bl6J male mice fed a high-fat diet and L-NAME in the drinking water for 4 months compared with C57/Bl6J and interleukin-1β$^{-/-}$ male mice fed regular chow for 4 months (Figure 4). Interestingly, inflammation and ER stress inductions were blunted in interleukin-1β$^{-/-}$ male mice fed an HFD and L-NAME in the drinking water (Figure 4). On the other hand, our data show that ER stress splicing X-box binding protein 1 expression is decreased in C57/Bl6 subjected to a high-fat diet and L-NAME compared with interleukin-1β knockout mice (Figure 4).

It is well established that hypertension and metabolic diseases are associated with tissue fibrosis. In our model, we observed cardiac fibrosis and an increase in TGFβ1 level in C57/Bl6J male mice fed an HFD and

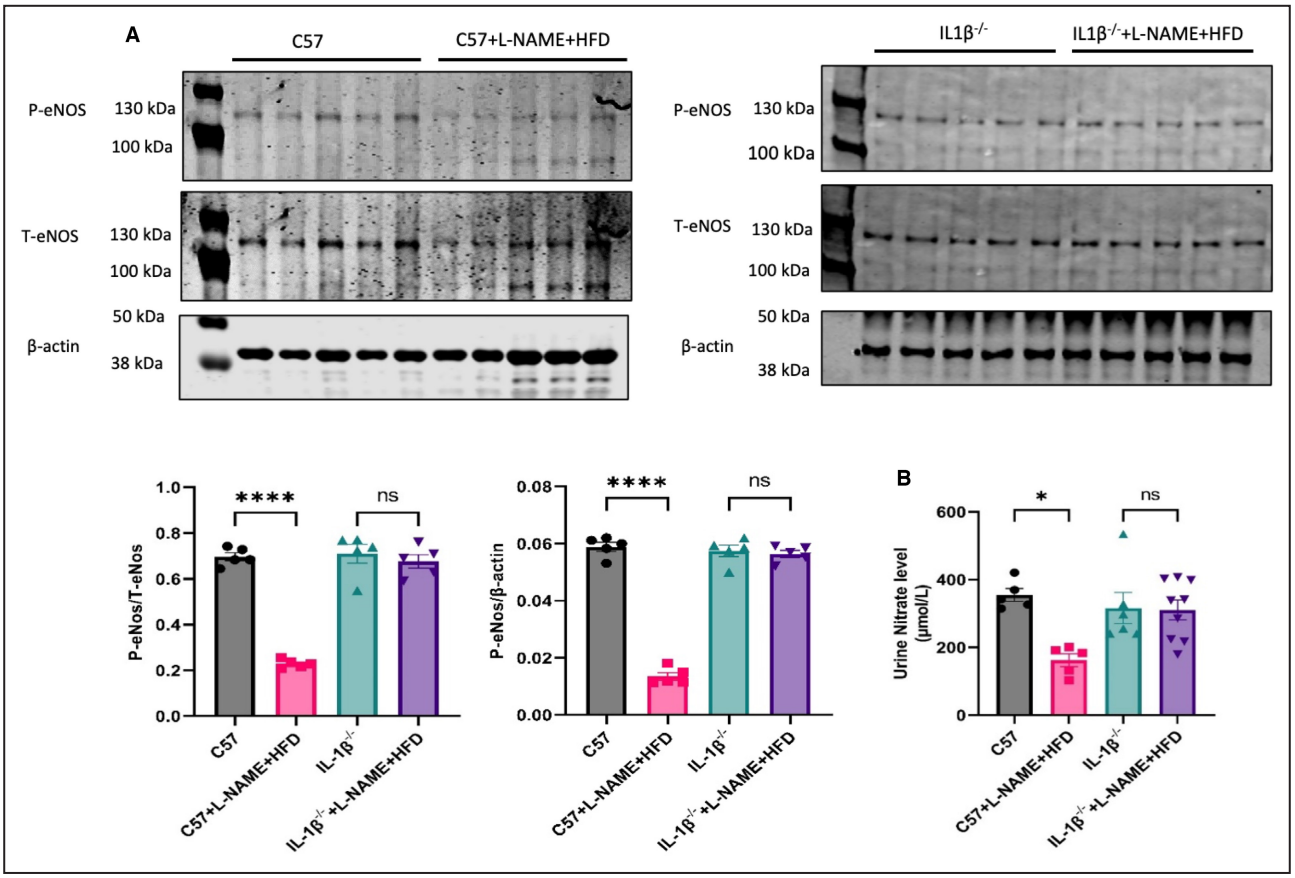

Figure 3. Western blot analysis and nitrate/nitrite levels.
Western blot analysis and cumulative data for phosphorylated endothelial nitric-oxide synthase (P-eNOS) in mesenteric resistance arteries (MRAs) isolated from C57/BL6J (C57), C57/BL6J+high-fat diet (HFD)+N[w]-nitro-l-arginine methyl ester (L-NAME), interleukin-1β$^{-/-}$ (IL-1β$^{-/-}$), and IL-1β$^{-/-}$+HFD+L-NAME male mice (n=5) (**A**) and nitrate/nitrite level in the urine (**B**) from all groups of mice. One-way ANOVA followed by Tukey's multiple comparisons post hoc test was applied for (**A** and **B**). *P<0.05, ****P<0.0001 for C57BL/6J vs C57/Bl6+HFD+L-NAME; (n=5). ns indicates not significant; and T-eNOS, total nitric oxide synthase.

L-NAME in the drinking water for 4 months compared with C57/Bl6J and interleukin-1β$^{-/-}$ male mice fed regular chow and interleukin-1β$^{-/-}$ male mice fed an HFD and L-NAME (Figure 5A and 5B).

DISCUSSION

HFpEF is a multifactorial disease characterized by cardiac diastolic relaxation dysregulation and vascular dysfunction with a major unmet need in cardiovascular medicine and remains untreatable. HFpEF prevalence is increasing and has become the most common form of heart failure due to the complications of the cardiovascular system. Understanding HFpEF pathogenesis will give us essential insights into the pathways and mechanisms of action involved in HFpEF. Inflammation has been considered a significant contributor to cardiovascular diseases for a long time. Patients with HFpEF show signs of chronic inflammation, and recent reports also showed elevated circulating inflammatory biomarkers in HFpEF, such as CRP and interleukin-1β.[17,18] In other animal models of HFpEF generated by a long-term HFD and desoxycorticosterone pivalate or high-salt diet for 28-weeks, the authors showed an increase in interleukin-1β associated with an exacerbation of mitochondrial hyperacetylation.[23]

It is well established that systemic inflammation is a significant culprit in cardiovascular disease development, including HFpEF.[24,25] Clinical trials reported that an interleukin-1β blockade in patients with obesity with HFpEF reduced N-terminal pro-B-type natriuretic peptide levels and improved exercise training.[26,27] However, recent clinical studies with anti-inflammatory agents have shown negative outcomes in patients with HFpEF.[26,27] In the present study, we aimed to determine the significant impact of interleukin-1β in HFpEF pathogenesis using the genetic approach by deleting the interleukin-1β gene. Our data uncovered a substantial role of interleukin-1β in HFpEF pathogenesis. Notably, male mice lacking the gene encoding for interleukin-1β (interleukin-1β$^{-/-}$ mice) are protected from HFpEF pathogenesis induced by an HFD and L-NAME in drinking water for 4 months, indicating that interleukin-1β is an essential mechanism in

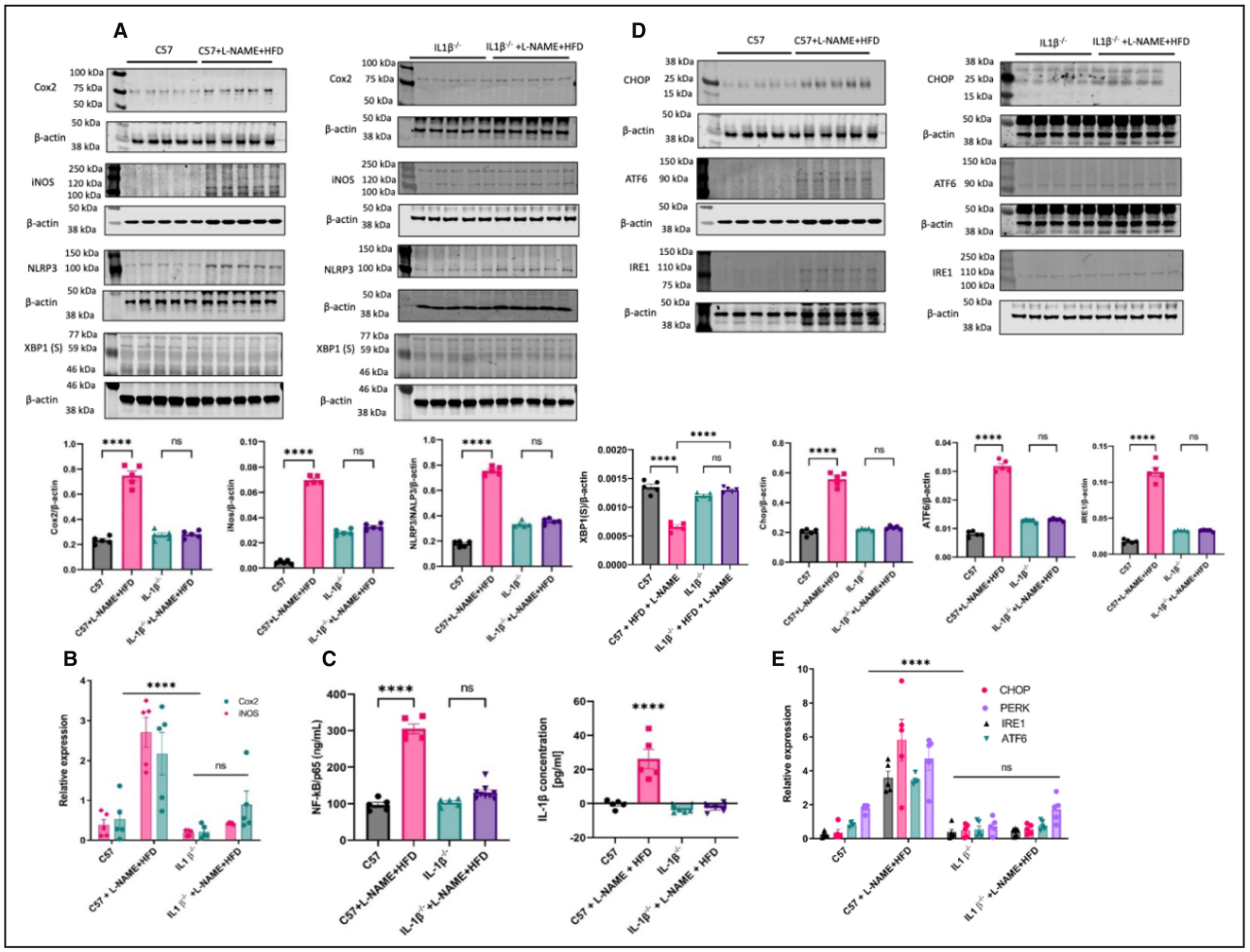

Figure 4. Western blot, quantitative polymerase chain reaction, nuclear factor-κ B (NF-κB/p65B), and interleukin-1β (IL-1β) analysis. Western blot analysis and cumulative data in mesenteric resistance arteries (MRAs) for COX2 (cyclooxygenase-2), iNOS (inducible nitric oxide synthase), NLRP3 (NLR family pyrin domain containing 3), and Xbp1s (X-box binding protein 1 spliced) (**A**) and mRNA level of iNOS and COX2 (**B**). Cardiac NF-κB activity and IL-1β level in the plasma in all the groups of mice (**C**). Western blot analysis and cumulative data in MRAs for endoplasmic reticulum (ER) stress CHOP (CCAAT/enhancer-binding protein), ATF-6 (activating transcription factor 6), and IRE1 (inositol-requiring enzyme 1) (**D**). Cardiac ER stress mRNA of PERK (protein kinase–like endoplasmic reticulum kinase), CHOP, IRE1, and ATF6 (**E**). One-way ANOVA followed by Tukey's multiple comparisons post hoc test was applied for (**A**) through (**E**). ****P<0.0001 for C57BL/6J vs C57/Bl6+HFD+L-NAME; (n=5). ns indicates not significant.

the development of HFpEF. Our data suggest that interleukin-1β is a potential therapeutic target to prevent and cure HFpEF pathogenesis.

Most patients with HFpEF harbor the morbidity of hypertension and obesity/type 2 diabetes/metabolic syndrome diseases.[7,8] Our preclinical mouse model of HFpEF recapitulates most of the clinical features of HFpEF in patients.[11] Using an HFD and L-NAME in the drinking water for 4 months, the C57/Bl6 male mice developed HFpEF, while interleukin-1β$^{-/-}$ male mice were protected, including the recovery of micro- and-macrovascular endothelial function. Interestingly, the deletion of interleukin-1β protected the function and structure of the cardiovascular system. Thus, vascular endothelial function and eNOS phosphorylation were not impaired in interleukin1β$^{-/-}$ male mice fed an HFD and L-NAME for 4 months. The protection of endothelial function could result from combining many factors, including reducing inflammation and ER stress in the aorta and MRAs. How the deletion of interleukin-1β reduces ER stress and inflammasome in HFpEF is still unknown. It has been shown previously that rats treated with L-NAME for 3 weeks exhibited reduced flow–induced dilation in mesenteric arteries, restored when treated with quinapril.[28] Moreover, as previously reported, in MRAs, there is the possibility of a switch of vasodilators involved in the endothelium-dependent relaxation that favors prostaglandin factors.[28] We also revealed that disrupting interleukin-1β blunted the induction of inflammation, ER stress, and cardiac and artery fibrosis and protected the metabolism from dysregulation. More importantly, interleukin-1β improved

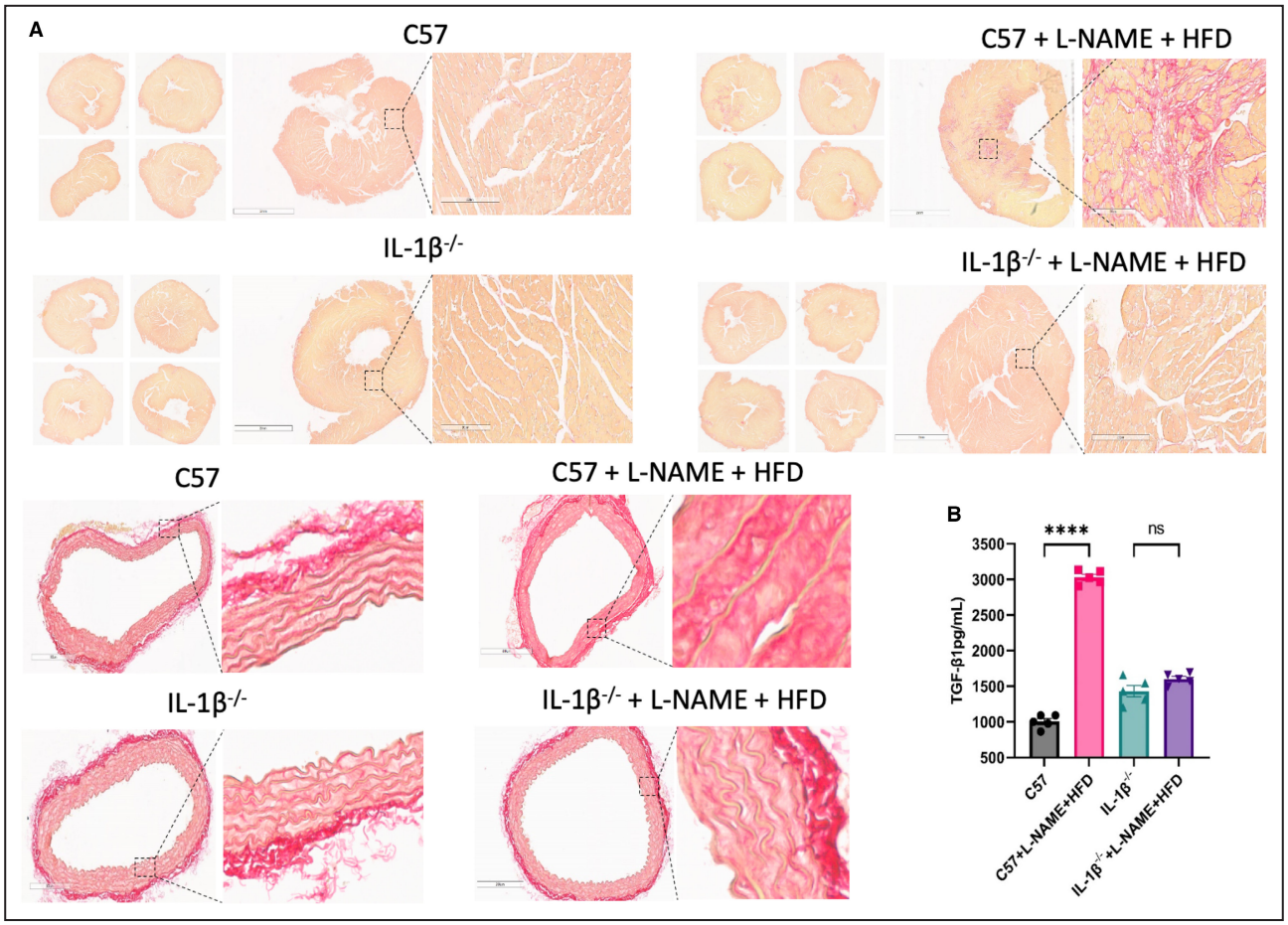

Figure 5. Cardiac fibrosis and TGF-β1 (transforming growth factor beta 1) analysis.
Picrosirius red staining on heart sections from C57/BL6J (C57), C57/BL6J+high-fat diet (HFD)+N[w]-nitro-l-arginine methyl ester (L-NAME), interleukin-1β (IL-1β$^{-/-}$), and IL-1β$^{-/-}$+HFD+L-NAME male mice (**A**) and TGF-β1 level in the plasma (**B**). One-way ANOVA followed by Tukey's multiple comparisons post hoc test was applied for (**B**). ****P<0.0001 for C57/BL6J vs C57/Bl6+HFD+L-NAME; (n=5). ns indicates not significant.

exercise tolerance, which is critical because limited exercise tolerance is a hallmark feature of HFpEF.[29]

The reciprocal interactions and mechanisms between inflammation and ER stress should be understood. Our data illustrated that deletion of interleukin-1β blunted the induction of inflammation and ER stress in response to the HFD and L-NAME. The results indicate that interleukin-1β, one of the inflammatory markers, controls the inflammatory markers (iNOS, Cox2, NF-κB, and inflammasome NLR family pyrin domain containing 3) and ER stress markers (protein kinase–like endoplasmic reticulum kinase/CCAAT/enhancer-binding protein, IRE1, and activating transcription factor 6). Our data also showed that X-box binding protein 1 level was reduced in male C57/Bl6J mice with HFpEF compared with C57/Bl6J without HFpEF and male interleukin-1β$^{-/-}$ mice with and without HFpEF. These data are in accordance with a previous study reporting similar data.[11] Previous studies reported that inhibition of interleukin-1β in vivo protected lungs from inflammation and improved survival in mice challenged with fatal influenza viral infection.[30] Another study showed that neutralizing interleukin-1β increases anti-inflammatory interleukin-10 plasma levels,[31] that could reduce inflammation and protect from HFpEF pathogenesis. A different study reported that neutralizing interleukin-1β improved cardiac remodeling. Still, it did not affect inflammasomes in hearts subjected to myocardial infarction,[32] while our research found that inflammasome (NLR family pyrin domain containing 3) was reduced in male interleukin-1β$^{-/-}$ mice subjected to an HFD and L-NAME for 4 months. The difference could be related to the approach of targeting interleukin-1β and the duration of the treatment.

The inflammation is a very complex process; we still do not know how interleukin-1β levels are increased in cardiovascular diseases such as HFpEF. Further studies are needed to uncover the first events in the induction of interleukin-1β in cardiovascular diseases. Other investigators and we reported that ER stress is involved in cardiovascular diseases.[14,33–35] Our data illustrate that disruption of interleukin-1β prevented ER stress induction in the MRAs, aorta, and heart. These

studies suggest that interleukin-1β directly or indirectly regulates ER stress induction. A previous study showed that ER stress inhibition with tauroursodeoxycholic acid reduced interleukin-1β levels and p65–NF-κb in the brains of mice with heart failure,[36] which suggests that ER stress regulates inflammation. In another study, interleukin-1β promoted ER stress induction in diabetic cardiomyopathy, indicating that ER stress induction is dictated by interleukin-1β.[37] All these data support that a potentially vicious cycle exists between interleukin-1β and ER stress induction. It is also well established that cardiovascular fibrosis is a feature of HFpEF.

Our study demonstrated that disruption of interleukin-1β blunted the induction of cardiac and vascular fibrosis and TGFβ1-level increase. At this stage, we do not know whether interleukin-1β is directly or indirectly, likely through ER stress or other inflammatory factors such as NF-κB, iNOS, or COX2, involved in fibrosis. Supporting our data, a previous study reported that interleukin-1β is involved in cardiac fibrosis through fibroblasts.[38] Whether through ER stress or other inflammatory factors like NF-κB, iNOS, or COX2, we still do not know if interleukin-1β is directly or indirectly involved in fibrosis.[39–41]

CONCLUSIONS

Our study highlighted the significant role of interleukin-1β in regulating arterial blood pressure, vascular endothelial function, inflammation, ER stress, and fibrosis in the setting of HFpEF pathogenesis. Therefore, modulating interleukin-1β could be a potential therapeutic strategy for HFpEF pathogenesis.

ARTICLE INFORMATION

Received February 4, 2023; accepted April 18, 2023.

Affiliations

Department of Physiological Sciences, EVMS, Norfolk, VA (B.K.S., A.B., J.D.O., K.D.M., S.B., K.M.); Hypertension & Vascular Research Division, Department of Internal Medicine, Henry Ford Health, Detroit, MI (N.-E.R.); and Department of Physiology, School of Medicine, Wayne State University, Detroit, MI (N.-E.R.).

Sources of Funding

This work is supported by NIH-HL150014 (Dr Matrougui), NIH-HL151616 (Dr Matrougui), and NIH-HL136456 (Dr Rhaleb).

Disclosures

None.

REFERENCES

1. Shah KS, Xu H, Matsouaka RA, Bhatt DL, Heidenreich PA, Hernandez AF, Devore AD, Yancy CW, Fonarow GC. Heart failure with preserved, borderline, and reduced ejection fraction: 5-year outcomes. *J Am Coll Cardiol*. 2017;70:2476–2486. doi: 10.1016/j.jacc.2017.08.074
2. Sharma K, Al Rifai M, Ahmed HM, Dardari Z, Silverman MG, Yeboah J, Nasir K, Sklo M, Yancy C, Russell SD, et al. Usefulness of coronary artery calcium to predict heart failure with preserved ejection fraction in men versus women (from the Multi-Ethnic Study of Atherosclerosis). *Am J Cardiol*. 2017;120:1847–1853. doi: 10.1016/j.amjcard.2017.07.089
3. Dunlay SM, Roger VL, Redfield MM. Epidemiology of heart failure with preserved ejection fraction. *Nat Rev Cardiol*. 2017;14:591–602. doi: 10.1038/nrcardio.2017.65
4. Yancy CW, Jessup M, Bozkurt B, Butler J, Casey DE Jr, Drazner MH, Fonarow GC, Geraci SA, Horwich T, Januzzi JL, et al. 2013 ACCF/AHA guideline for the management of heart failure: executive summary: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. *Circulation*. 2013;128:1810–1852. doi: 10.1161/CIR.0b013e31829e8807
5. Borlaug BA. Evaluation and management of heart failure with preserved ejection fraction. *Nat Rev Cardiol*. 2020;17:559–573. doi: 10.1038/s41569-020-0363-2
6. Cooper CJ, Murphy TP, Cutlip DE, Jamerson K, Henrich W, Reid DM, Cohen DJ, Matsumoto AH, Steffes M, Jaff MR, et al. Stenting and medical therapy for atherosclerotic renal-artery stenosis. *N Engl J Med*. 2014;370:13–22. doi: 10.1056/NEJMoa1310753
7. Shah SJ, Kitzman DW, Borlaug BA, van Heerebeek L, Zile MR, Kass DA, Paulus WJ. Phenotype-specific treatment of heart failure with preserved ejection fraction: a multiorgan roadmap. *Circulation*. 2016;134:73–90. doi: 10.1161/CIRCULATIONAHA.116.021884
8. Maryniak A, Maisuradze N, Ahmed R, Biskupski P, Jayaraj J, Budzikowski AS. Heart failure with preserved ejection fraction update: a review of clinical trials and new therapeutic considerations. *Cardiol J*. 2022;29:670–679. doi: 10.5603/CJ.a2022.0051
9. Franssen C, Chen S, Unger A, Korkmaz HI, De Keulenaer GW, Tschope C, Leite-Moreira AF, Musters R, Niessen HW, Linke WA, et al. Myocardial microvascular inflammatory endothelial activation in heart failure with preserved ejection fraction. *JACC Heart Fail*. 2016;4:312–324. doi: 10.1016/j.jchf.2015.10.007
10. Zhang XL, Wang TY, Chen Z, Wang HW, Yin Y, Wang L, Wang Y, Xu B, Xu W. HMGB1-promoted neutrophil extracellular traps contribute to cardiac diastolic dysfunction in mice. *J Am Heart Assoc*. 2022;11:e023800. doi: 10.1161/JAHA.121.023800
11. Schiattarella GG, Altamirano F, Tong D, French KM, Villalobos E, Kim SY, Luo X, Jiang N, May HI, Wang ZV, et al. Nitrosative stress drives heart failure with preserved ejection fraction. *Nature*. 2019;568:351–356. doi: 10.1038/s41586-019-1100-z
12. Radwan E, Mali V, Haddox S, El-Noweihi A, Mandour M, Ren J, Belmadani S, Matrougui K. Treg cells depletion is a mechanism that drives microvascular dysfunction in mice with established hypertension. *Biochim Biophys Acta Mol Basis Dis*. 2019;1865:403–412. doi: 10.1016/j.bbadis.2018.10.031
13. Kassan M, Wecker A, Kadowitz P, Trebak M, Matrougui K. CD4+CD25+Foxp3 regulatory T cells and vascular dysfunction in hypertension. *J Hypertens*. 2013;31:1939–1943. doi: 10.1097/HJH.0b013e328362feb7
14. Amin A, Choi SK, Galan M, Kassan M, Partyka M, Kadowitz P, Henrion D, Trebak M, Belmadani S, Matrougui K. Chronic inhibition of endoplasmic reticulum stress and inflammation prevents ischaemia-induced vascular pathology in type II diabetic mice. *J Pathol*. 2012;227:165–174. doi: 10.1002/path.3960
15. Matrougui K, Abd Elmageed Z, Kassan M, Choi S, Nair D, Gonzalez-Villalobos RA, Chentoufi AA, Kadowitz P, Belmadani S, Partyka M. Natural regulatory T cells control coronary arteriolar endothelial dysfunction in hypertensive mice. *Am J Pathol*. 2011;178:434–441. doi: 10.1016/j.ajpath.2010.11.034
16. Ali M, Mali V, Haddox S, AbdelGhany SM, El-Deek SEM, Abulfadl A, Matrougui K, Belmadani S. Essential role of IL-12 in angiogenesis in type 2 diabetes. *Am J Pathol*. 2017;187:2590–2601. doi: 10.1016/j.ajpath.2017.07.021
17. Bayes-Genis A, Cediel G, Domingo M, Codina P, Santiago E, Lupon J. Biomarkers in heart failure with preserved ejection fraction. *Card Fail Rev*. 2022;8:e20. doi: 10.15420/cfr.2021.37
18. Eirin A, Gloviczki ML, Tang H, Rule AD, Woollard JR, Lerman A, Textor SC, Lerman LO. Chronic renovascular hypertension is associated with elevated levels of neutrophil gelatinase-associated lipocalin. *Nephrol Dial Transplant*. 2012;27:4153–4161. doi: 10.1093/ndt/gfs370
19. Mali V, Haddox S, Belmadani S, Matrougui K. Essential role for smooth muscle cell stromal interaction molecule-1 in myocardial infarction. *J Hypertens*. 2018;36:377–386. doi: 10.1097/HJH.0000000000001518
20. Mali V, Haddox S, Hornersmith C, Matrougui K, Belmadani S. Essential role for EGFR tyrosine kinase and ER stress in myocardial

infarction in type 2 diabetes. *Pflugers Arch*. 2018;470:471–480. doi: 10.1007/s00424-017-2097-5
21. Kassan M, Galan M, Partyka M, Trebak M, Matrougui K. Interleukin-10 released by CD4(+)CD25(+) natural regulatory T cells improves microvascular endothelial function through inhibition of NADPH oxidase activity in hypertensive mice. *Arterioscler Thromb Vasc Biol*. 2011;31:2534–2542. doi: 10.1161/ATVBAHA.111.233262
22. McLellan AD, Kapp M, Eggert A, Linden C, Bommhardt U, Brocker EB, Kammerer U, Kampgen E. Anatomic location and T-cell stimulatory functions of mouse dendritic cell subsets defined by CD4 and CD8 expression. *Blood*. 2002;99:2084–2093. doi: 10.1182/blood.v99.6.2084
23. Deng Y, Xie M, Li Q, Xu X, Ou W, Zhang Y, Xiao H, Yu H, Zheng Y, Liang Y, et al. Targeting mitochondria-inflammation circuit by beta-Hydroxybutyrate mitigates HFpEF. *Circ Res*. 2021;128:232–245. doi: 10.1161/CIRCRESAHA.120.317933
24. Mishra S, Kass DA. Cellular and molecular pathobiology of heart failure with preserved ejection fraction. *Nat Rev Cardiol*. 2021;18:400–423. doi: 10.1038/s41569-020-00480-6
25. Hahn VS, Knutsdottir H, Luo X, Bedi K, Margulies KB, Haldar SM, Stolina M, Yin J, Khakoo AY, Vaishnav J, et al. Myocardial gene expression signatures in human heart failure with preserved ejection fraction. *Circulation*. 2021;143:120–134. doi: 10.1161/CIRCULATIONAHA.120.050498
26. Van Tassell BW, Arena R, Biondi-Zoccai G, Canada JM, Oddi C, Abouzaki NA, Jahangiri A, Falcao RA, Kontos MC, Shah KB, et al. Effects of interleukin-1 blockade with anakinra on aerobic exercise capacity in patients with heart failure and preserved ejection fraction (from the D-HART pilot study). *Am J Cardiol*. 2014;113:321–327. doi: 10.1016/j.amjcard.2013.08.047
27. Murphy SP, Kakkar R, McCarthy CP, Januzzi JL Jr. Inflammation in heart failure: JACC state-of-the-art review. *J Am Coll Cardiol*. 2020;75:1324–1340. doi: 10.1016/j.jacc.2020.01.014
28. Henrion D, Dechaux E, Dowell FJ, Maclour J, Samuel JL, Levy BI, Michel JB. Alteration of flow-induced dilatation in mesenteric resistance arteries of L-NAME treated rats and its partial association with induction of cyclo-oxygenase-2. *Br J Pharmacol*. 1997;121:83–90. doi: 10.1038/sj.bjp.0701109
29. Upadhya B, Haykowsky MJ, Eggebeen J, Kitzman DW. Exercise intolerance in heart failure with preserved ejection fraction: more than a heart problem. *J Geriatr Cardiol*. 2015;12:294–304. doi: 10.11909/j.issn.1671-5411.2015.03.013
30. Bawazeer AO, Rosli S, Harpur CM, Docherty CA, Mansell A, Tate MD. Interleukin-1beta exacerbates disease and is a potential therapeutic target to reduce pulmonary inflammation during severe influenza a virus infection. *Immunol Cell Biol*. 2021;99:737–748. doi: 10.1111/imcb.12459
31. Vromman A, Ruvkun V, Shvartz E, Wojtkiewicz G, Santos Masson G, Tesmenitsky Y, Folco E, Gram H, Nahrendorf M, Swirski FK, et al. Stage-dependent differential effects of interleukin-1 isoforms on experimental atherosclerosis. *Eur Heart J*. 2019;40:2482–2491. doi: 10.1093/eurheartj/ehz008
32. Toldo S, Mezzaroma E, Van Tassell BW, Farkas D, Marchetti C, Voelkel NF, Abbate A. Interleukin-1beta blockade improves cardiac remodelling after myocardial infarction without interrupting the inflammasome in the mouse. *Exp Physiol*. 2013;98:734–745. doi: 10.1113/expphysiol.2012.069831
33. Kassan M, Galan M, Partyka M, Saifudeen Z, Henrion D, Trebak M, Matrougui K. Endoplasmic reticulum stress is involved in cardiac damage and vascular endothelial dysfunction in hypertensive mice. *Arterioscler Thromb Vasc Biol*. 2012;32:1652–1661. doi: 10.1161/ATVBAHA.112.249318
34. Galan M, Kassan M, Kadowitz PJ, Trebak M, Belmadani S, Matrougui K. Mechanism of endoplasmic reticulum stress-induced vascular endothelial dysfunction. *Biochim Biophys Acta*. 2014;1843:1063–1075. doi: 10.1016/j.bbamcr.2014.02.009
35. Cicalese SM, da Silva JF, Priviero F, Webb RC, Eguchi S, Tostes RC. Vascular stress signaling in hypertension. *Circ Res*. 2021;128:969–992. doi: 10.1161/CIRCRESAHA.121.318053
36. Wei SG, Yu Y, Weiss RM, Felder RB. Endoplasmic reticulum stress increases brain MAPK signaling, inflammation and renin-angiotensin system activity and sympathetic nerve activity in heart failure. *Am J Physiol Heart Circ Physiol*. 2016;311:H871–H880. doi: 10.1152/ajpheart.00362.2016
37. Liu Z, Zhao N, Zhu H, Zhu S, Pan S, Xu J, Zhang X, Zhang Y, Wang J. Circulating interleukin-1beta promotes endoplasmic reticulum stress-induced myocytes apoptosis in diabetic cardiomyopathy via interleukin-1 receptor-associated kinase-2. *Cardiovasc Diabetol*. 2015;14:125. doi: 10.1186/s12933-015-0288-y
38. Frangogiannis NG. Cardiac fibrosis. *Cardiovasc Res*. 2021;117:1450–1488. doi: 10.1093/cvr/cvaa324
39. Kearney PM, Whelton M, Reynolds K, Muntner P, Whelton PK, He J. Global burden of hypertension: analysis of worldwide data. *Lancet*. 2005;365:217–223. doi: 10.1016/S0140-6736(05)17741-1
40. Murray CJ, Lopez AD. Measuring the global burden of disease. *N Engl J Med*. 2013;369:448–457. doi: 10.1056/NEJMra1201534
41. Kashyap S, Warner GM, Hartono SP, Boyilla R, Knudsen BE, Zubair AS, Lien K, Nath KA, Textor SC, Lerman LO, et al. Blockade of CCR2 reduces macrophage influx and development of chronic renal damage in murine renovascular hypertension. *Am J Physiol Renal Physiol*. 2016;310:F372–F384. doi: 10.1152/ajprenal.00131.2015