ORIGINAL RESEARCH

Interaction of Obesity and Hypertension on Cardiac Metabolic Remodeling and Survival Following Myocardial Infarction

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BACKGROUND: Obesity and hypertension are risk factors for myocardial infarction (MI); however, their potential interactions on post-MI outcomes are unclear. We examined interactions of obesity and hypertension on post-MI function, remodeling, metabolic changes, and recovery.

METHODS AND RESULTS: Male and female C57BL/6J mice were provided standard chow or high-fat/fructose diet for 8 weeks and then infused with angiotensin II for 2 weeks to induce hypertension. MI was then induced by surgical ligation of the left coronary artery for 7 days. Obesity alone did not cause cardiac injury or exacerbate hypertension-induced cardiac dysfunction. After MI, however, obese-normotensive mice had lower survival rates compared with chow-fed mice (56% versus 89% males; 54% versus 75% females), which were further decreased by hypertension (29% males; and 35% females). Surviving obese-normotensive males displayed less left ventricular dilation and pulmonary congestion compared with chow-fed males after MI; hypertension reversed left ventricular dilation because of high-fat/fructose diet and promoted significant pulmonary congestion compared with chow-fed controls. Obese-normotensive males displayed higher left ventricular α-MHC (alpha-myosin heavy chain) protein, phosphorylated Akt (protein kinase B) and AMPK (adenosine-monophosphate activated kinase), PPAR-γ (peroxisome proliferator activated receptor gamma), and plasma adiponectin levels after MI, indicating favorable contractile and metabolic changes. However, these favorable contractile and metabolic changes were attenuated by hypertension. Obese-hypertensive males also had lower levels of collagen in the infarcted region, indicating decreased ability to promote an adaptive wound healing response to MI.

CONCLUSIONS: Obesity reduces post-MI survival but is associated with improved post-MI cardiac function and metabolism in surviving normotensive mice. When hypertension accompanies obesity, favorable metabolic pathways associated with obesity are attenuated and post-MI cardiac function and remodeling are adversely impacted.

Key Words: angiotensin II ■ cardiac hypertrophy ■ heart failure ■ metabolic syndrome

Approximately 1 million people in the United States suffer a myocardial infarction (MI) every year.[1] Of patients who survive MI, ≈25% develop heart failure, which has a 5-year mortality of ≈50%.[2] Obesity and hypertension are major components of the metabolic syndrome and highly prevalent risk factors for cardiovascular disease, including MI.[3–8] However, few studies have focused on the interaction of these comorbidities and the mechanisms by which they affect post-MI remodeling and recovery. Although obesity is a major cause of primary hypertension,[9] some studies in patients have reported that mild to moderate obesity is paradoxically associated with improved post-MI outcomes and long-term survival.[10,11] It is controversial, however, whether the apparent paradoxical effect of

CLINICAL PERSPECTIVE

What Is New?

- In obese mice, hypertension negates paradoxically improved outcomes and left ventricle metabolic changes and promotes adverse remodeling and outcomes, potentially through impaired adipokine signaling.

What Are the Clinical Implications?

- Our results indicate that in obese populations, patients with hypertension may be at significantly higher risk than normotensive patients for adverse myocardial infarction outcomes, and that therapies targeting metabolic or adipokine signaling pathways may differentially affect these populations.

Nonstandard Abbreviations and Acronyms

ACC	acetyl-CoA carboxylase
AMPK	adenosine-monophosphate activated kinase
Ang II	angiotensin II
HFFD	high-fat/fructose diet
MHC	myosin heavy chain
PDH	pyruvate dehydrogenase
PGC-1α	peroxisome proliferator-activated receptor gamma coactivator 1-alpha
PPAR-γ	peroxisome proliferator activated receptor gamma

obesity to improve post-MI outcomes even exists and the potential mechanisms involved are unknown.[12,13] For example, in type III (extreme) obese populations with higher a prevalence of hypertension, the obesity paradox disappears.[10]

While some experimental studies have investigated the effects of obesity on post-MI remodeling in mice, the results have ranged from detrimental to beneficial, and have usually ignored the confounding effects of hypertension,[14–17] as diet-induced obesity produces little to no changes in blood pressure in mice.[18] However, hypertension is a major risk factor for MI, is highly prevalent with obesity, and is associated with impaired post-MI left ventricular (LV) function and increased mortality.[19] Obesity and hypertension are also associated with shifts in cardiac metabolism that eventually contribute to dysfunction.[20] Thus, we sought to investigate post-MI outcomes (survival, functional, and LV remodeling) in obese normotensive and hypertensive adult mice. We hypothesized that the presence of hypertension in obesity would exacerbate adverse cardiac remodeling and outcomes after MI.

METHODS

All data and supporting materials have been provided with the published article.

Experimental Design

Adult (12–24 weeks) male and female C57BL/6J mice were used for this study. All procedures involving animals were approved by the Institutional Animal Care and Use Committee at the University of Mississippi Medical Center. Two separate cohorts of mice were used. For the first cohort (cohort 1), mice were fed normal chow or a high-fat/fructose diet (HFFD) starting at 8 to 16 weeks of age for 8 weeks to produce obesity; in the final 2 weeks, mice were chronically infused with saline or angiotensin II (Ang II) to induce hypertension. The average age was not different among groups. The experimental groups for cohort 1 were: chow+saline, HFFD+saline, chow+hypertension, HFFD+hypertension. For cohort 2, MI was induced following the normal chow, HFFD, and HFFD+hypertension protocols. The experimental groups for the second cohort were: chow+saline+MI, HFFD+saline+MI, HFFD+hypertension+MI.

High-Fat Fructose Diet

Mice were fed a high fat diet (D12451; 45% kcal% fat, Research Diets) supplemented with 30% fructose (F0127; Sigma-Aldrich) in the drinking water. Control mice received a normal chow diet (Envigo #8640, 5% fat) and tap water. Body weight was assessed at the start of the study before high fat feeding and terminally before euthanasia. Body fat and lean mass composition was assessed at the end of the experiments by magnetic resonance imaging (EchoMRI 4-in-1; Echo Medical System; Houston, TX).

Angiotensin II Model of Hypertension

Mice were chronically infused for 14 days with Ang II(A9525, Sigma-Aldrich; 1.0 μg/kg per minute). Osmotic mini-pumps (Alzet 2002) were loaded with Ang II dissolved in sterile saline and equilibrated overnight. Mice were anesthetized with 2% isoflurane, and mini-pumps were surgically implanted subcutaneously. Control mice were infused with an equivalent volume of sterile saline. Systolic blood pressure was assessed by tail cuff plethysmography (MRBP Systems; IITC Life Science) in a dark chamber at 37°C 2 weeks after pump implantation. Mice were acclimated to the plethysmography system for 3 days before taking measurements.

The average of 3 to 5 tracings was used as the systolic blood pressure value.

Myocardial Infarction

MI was induced by surgical ligation of the left anterior descending coronary artery as previously described.[21,22] Briefly, mice were anesthetized with 2% isoflurane, a tracheotomy was performed, and mice were intubated and ventilated for the duration of the procedure. The LV was exposed through the third and fourth ribs, and the artery was ligated with an 8-0 suture. MI was confirmed by LV blanching and ECG ST-segment elevation. For analgesia, mice were administered buprenorphine (0.05 mg/kg) immediately before surgery, and 6 and 24 hours after surgery.

Echocardiography

LV function was assessed by echocardiography (VEVO 2100/3100; VisualSonics; Toronto, CA) either before euthanasia for cohort 1 (no MI) or before MI surgery (baseline) for cohort 2, and again at 7 days post-MI. Baseline echocardiograms from cohort 2, including mice that died during or after MI surgery, were combined with cohort 1. Mice were anesthetized under 2% isoflurane for the procedures. Both long-axis and short-axis images were obtained and averaged over 3 cardiac cycles and processed using VEVO Lab software. LV volumes and ejection fraction were calculated from long-axis images using the LV trace function. LV dimensions, fractional shortening, and wall thickness were calculated from short-axis images. Average wall thickness was calculated as the average of the posterior and anterior walls, and relative wall thickness was calculated as the average wall thickness divided by the end-diastolic diameter. Longitudinal, circumferential, and radial strain were assessed using speckle tracking analysis. LV dyssynchrony was assessed by the standard deviation of the time to peak for the longitudinal and radial strain, normalized to the R-R interval.[23,24]

Histology and Infarct Size Measurement

Hearts were removed, and the LV separated from the right ventricle (RV). LV mid-sections were fixed overnight in zinc formalin, sectioned at 5 µm, and stained with picrosirius red to assess collagen content. Infarct size was assessed in whole heart images as the area occupied by the collagenous scar using ImageJ software. Images (40×) were obtained using the Mantra Quantitative Pathology Imaging System (Perkin Elmer) and analyzed by inForm software (Perkin Elmer) to assess collagen content in the remote and infarcted LV.

LV Proteins

LV protein expression was assessed by Western blot analysis. LV tissue from the remote area of MI hearts or base region of no-MI hearts (~15 mg) was homogenized in T-PER buffer with protease and phosphatase inhibitors, and protein concentration was determined using a bicinchoninic acid assay. Protein (10 µg) was then separated by SDS-PAGE and transferred to nitrocellulose membranes, which were blocked for 1 hour at room temperature and probed overnight at 4°C with the following antibodies: α-MHC (alpha-myosin heavy chain, 1:1000; Abcam #50967), β-MHC (beta-myosin heavy chain, 1:5000; Abcam #11083), phospho-Akt (phospho-protein kinase B, serine 473; 1:1000; Cell Signaling Technologies #9271), phospho-AMPK (phospho-adenosine-monophosphate activated kinase threonine-172, 1:1000; Cell Signaling Technologies #2535), AMPK (1:1000; Cell Signaling Technologies #5831), phospho-acetyl-CoA carboxylase (phospho-ACC, serine 79; 1:1000; Cell Signaling Technologies #11818), ACC (1:1000; Cell Signaling Technologies #3662), phospho-pyruvate dehydrogenase (phospho-PDH, serine 293; 1:1000; Cell Signaling Technologies #31866), PDH (1:1000; Abcam #155096), PPAR-γ (peroxisome proliferator activated receptor gamma, 1:1000; Abcam #59256), and PGC-1α (peroxisome proliferator-activated receptor gamma coactivator 1-alpha,1:1000; Abcam #54481). Membranes were then incubated with secondary antibody (IRDye 8000CW Donkey Anti-Mouse or Anti-Rabbit immunoglobulin G; LiCor) for 1 to 2 hours at a 1:10 000 dilution and visualized using an Odyssey CLx Imaging System (LiCor). Results were normalized total protein (Revert 700 Total Protein Stain Kit; Li-Cor #P/N 926-11010).

Measurement of Plasma Lipids and Adipokines

Mice were anesthetized with 2% isoflurane and administered heparin (4 U/g body weight) for 5 minutes. Blood was collected from the carotid artery before euthanasia, and plasma was collected and snap frozen. Plasma measurements were performed by the University of Mississippi Medical Center Analytical and Assay Core. Plasma lipids, including total cholesterol, high-density lipoprotein, low-density lipoprotein, and triglycerides were assessed using a Vet Axcel Chemistry Analyzer. Plasma leptin and adiponectin was assessed by ELISA.

Statistical Analysis

Data are presented as mean±SEM. Survival rate was analyzed by Kaplan−Meier survival analysis and compared by the log-rank test. Comparisons of no

MI groups and group interactions were performed by 2-way ANOVA. Comparisons of MI groups were performed by 1-way ANOVA followed by Tukey post hoc analysis. Interactions and group comparisons between males and females were performed by 2-way ANOVA for MI groups and 3-way ANOVA for no MI groups. A value of $P<0.05$ was considered statistically significant.

RESULTS

Obesity-Hypertension Phenotypes

Changes in body weight, body composition, and systolic blood pressure are summarized in Table 1. Male and female mice fed an HFFD gained significantly more body weight and body fat composition, as assessed by EchoMRI, compared with chow controls (no effect of hypertension). HFFD and HFFD+hypertension males gained significantly more body weight than HFFD and HFFD+hypertension females (significant interaction of sex), but males and females had similar levels of body fat composition in chow, HFFD, and HFFD+hypertension groups. As expected, male and female hypertension and HFFD+hypertension groups showed significant increases in systolic blood pressure, which was not affected by HFFD.

Heart, Lung, and Spleen Morphometric Parameters of Obese-Hypertensive Mice

Changes in LV, RV, lung, and spleen mass (normalized to tibia length) are summarized in Table 2. HFFD alone did not alter normalized LV, RV, lung, or spleen mass in either males or females as compared with chow controls. As expected, hypertension led to a significant increase in LV mass in chow-fed and HFFD-fed male and female mice. Normalized LV mass was significantly greater in all male groups compared with females (significant interaction of sex). No significant differences were observed in RV mass among any groups. In males, hypertension increased total (wet) and dry lung mass in chow-fed mice but not in HFFD-fed mice compared with chow and HFFD normotensive controls. In females, hypertension did not affect normalized wet or dry lung mass in chow-fed mice but increased dry lung mass in HFFD-fed mice compared with chow and HFFD normotensive controls. Spleen mass was not different among male groups but was significantly increased in HFFD+hypertension females compared with chow.

LV Function, Metabolism, and Structural Remodeling in Obese-Hypertensive Mice Without MI

LV function was assessed by echocardiography after 8 weeks of normal chow or HFFD and 2 weeks of hypertension (Figure 1). End-diastolic volume was significantly decreased in HFFD+hypertension males compared with chow and HFFD controls; in females, hypertension alone decreased end-diastolic volume compared with chow controls, which was attenuated by HFFD (Figure 1A). There was a significant interaction between HFFD and hypertension in females ($P=0.004$), and also a significant interaction of sex ($P<0.0001$). Ejection fraction was not markedly different between groups, although in male mice there was a significant decrease in HFFD+hypertension compared with hypertension alone (Figure 1C). Hypertension decreased end-diastolic diameter in both males and females compared with chow and HFFD; in females, this was partially attenuated by HFFD (Figure 1D; significant interaction between HFFD and hypertenstion [$P=0.043$], and significant interaction of sex [$P<0.0001$]). Hypertension also decreased end-systolic diameter in females compared with chow (Figure 1E). No differences in fractional shortening were observed among groups (Figure 1F). In males, hypertension led to concentric hypertrophy in chow- and HFFD-fed mice, as assessed by increases in average wall thickness (Figure 1G) and relative wall thickness (Figure 1H) compared with chow controls. In females, hypertension also led to concentric hypertrophy in chow- and HFFD-fed mice compared with controls; however, this effect

Table 1. Body Weight, Fat Mass, and Blood Pressure

	Male				Female			
	Chow	HFFD	Hypertension	HFFD+Hypertension	Chow	HFFD	Hypertension	HFFD+Hypertension
Change BW, g	3.1±0.5	11.8±1.0*	1.0±0.6*	9.6±0.6*,†	2.3±0.3	5.0±0.7*	2.8±0.4	6.3±0.3*
Body fat, %	8.8±0.8	26.5±1.8*	NA	22.7±2.1*	10.6±0.8	19.4±0.9*	NA	21.8±1.8*
SBP, mm Hg	105±4	102±3	155±7*	143±4*	112±4	102±3	158±7*	145±3*,‡

Change in body weight was measured as the initial body weight (beginning of the study) subtracted from the final body weight (at euthanasia). Group numbers: n=17, chow male; n=18, HFFD male; n=10, hypertension male; n=18, HFFD+hypertension male; n=16, chow female; n=10, HFFD female; n=8, hypertension female; n=16, HFFD+hypertension female. HFFD indicates high fat/fructose diet; and SBP, systolic blood pressure.

*$P<0.05$ vs chow control.

†$P<0.05$ vs HFFD.

‡$P<0.05$ vs hypertension.

Table 2. Morphometric Parameters of Obese-Hypertensive Mice Without MI

	Male				Female			
	Chow	HFFD	Hypertension	HFFD+Hypertension	Chow	HFFD	Hypertension	HFFD+Hypertension
LV mass	5.7±0.2	6.1±0.2	7.3±0.1*	6.7±0.2*	4.6±0.1	4.7±0.2	5.3±0.1*	5.5±0.1*
RV mass	1.18±0.08	1.06±0.03	0.95±0.05	0.96±0.05	0.79±0.03	0.76±0.04	0.82±0.06	0.89±0.04
Wet lung mass	8.5±0.5	8.7±0.2	9.9±0.3*	9.2±0.4†	7.7±0.3	7.6±0.1	7.9±0.2	8.6±0.5
Dry lung mass	1.84±0.08	1.93±0.04	2.19±0.06*	2.07±0.08†	1.52±0.07	1.72±0.04	1.73±0.05	1.86±0.08*
Edema Index	0.78±0.00	0.78±0.00	0.78±0.00	0.78±0.00	0.80±0.01	0.77±0.00	0.78±0.00	0.79±0.01
Spleen mass	5.4±0.4	5.6±0.3	5.1±0.2	5.1±0.3	4.6±0.3	5.8±0.4	5.9±0.5	7.3±1.0*

Left ventricular, right ventricular, lung, and spleen masses (mg) were normalized to tibia length (mm). Group numbers: n=17, chow male; n=18, high-fat/fructose diet male; n=10, hypertension male; n=18, high-fat/fructose diet+hypertension male; n=16, chow female; n=10, high fat/fructose diet female; n=8, hypertension female; n=16, high-fat/fructose diet+hypertension female. HFFD indicates high-fat/fructose diet; LV, left ventricular; and RV, right ventricular.

*P<0.05 vs chow.

†P<0.05 vs hypertension.

was slightly attenuated by HFFD (significant interaction; $P=0.004$). HFFD also decreased heart rate in normotensive and hypertention males (Figure 1I; significant interaction between HFFD and hypertension; $P=0.04$), while no differences were observed in females (Figure 1I).

We assessed the impact of obesity and hypertension on post-MI MHC isoforms, including α-MHC and β-MHC (Figure S1A). There were no significant differences in LV α-MHC expression among groups, although HFFD tended to decrease α-MHC in females ($P=0.06$) compared with chow. Furthermore, there were no significant differences in LV β-MHC expression in males, which exhibited high variation. In females, however, hypertension increased β-MHC expression; which was attenuated by HFFD (significant interaction between HFFD and hypertension; $P=0.03$). Hypertension induced LV fibrosis in males and females, but was not significantly altered by HFFD (Figure S1B).

We also assessed expression and phosphorylation of key cardiac metabolic enzymes, including ACC and PDH, and AMPK. Although there were some strong trends, there were no significant differences among groups (Figure S2), indicating that neither obesity nor hypertension significantly altered the cardiac structural or metabolic environment before MI. Representative full blots are displayed in Figure S3.

Hypertension Promotes Development of Congestive Heart Failure in Obese Mice After MI

Survival over the first 7 days after MI was compared among groups (Figure 2A). The survival rate in males was 89% for the chow+MI, 56% for HFFD+MI, and 29% in HFFD+hypertension+MI, which was significantly decreased relative to the chow-fed group. In females, the survival rate after MI was 75% for chow-fed, 54% for HFFD, and 35% for HFFD+hypertension. In the small number of chow-fed males and females that did not survive for at least 7 days, 100% of the animals died of LV rupture (Figure 2B; n=1 males and n=2 females). The LV rupture rate after MI in HFFD-fed males and females was 29% and 0%, and in HFFD+hypertension males and females was 11% and 8%, suggesting that the higher rate of death after MI in HFFD and HFFD+hypertension mice was mainly because of other causes such as congestive heart failure. No differences in infarct size were observed among any groups (Figure 2C).

Morphometric parameters of mice 7 days after MI are summarized in Table 3. LV mass was similar in all male groups, indicating that hypertension impaired the ability of the LV to undergo further hypertrophy after MI in obese males. In females, however, HFFD+hypertension+MI significantly increased LV mass compared with chow+MI and HFFD+MI groups, indicating that hypertension promoted additional hypertrophy after MI (significant interaction of sex; $P<0.0001$). RV mass was significantly elevated in HFFD+hypertension+MI males compared with chow+MI controls, but not different among female groups. The edema index, an indicator of water content in the lung, was not different among groups. Wet and dry lung masses were significantly elevated in HFFD+hypertension+MI males compared with both chow+MI and HFFD+MI groups, suggesting pulmonary fibrotic and vascular remodeling and progression to congestive heart failure.[25] Although there was a trend, the different groups of females did not show statistically significant changes in lung wet or dry mass. Spleen mass was not different among groups. Both HFFD+MI and HFFD+hypertension+MI males and females showed similar degrees of hyperlipidemia, including elevated total cholesterol; however, only high-density lipoprotein and triglycerides were elevated in males (Table S1).
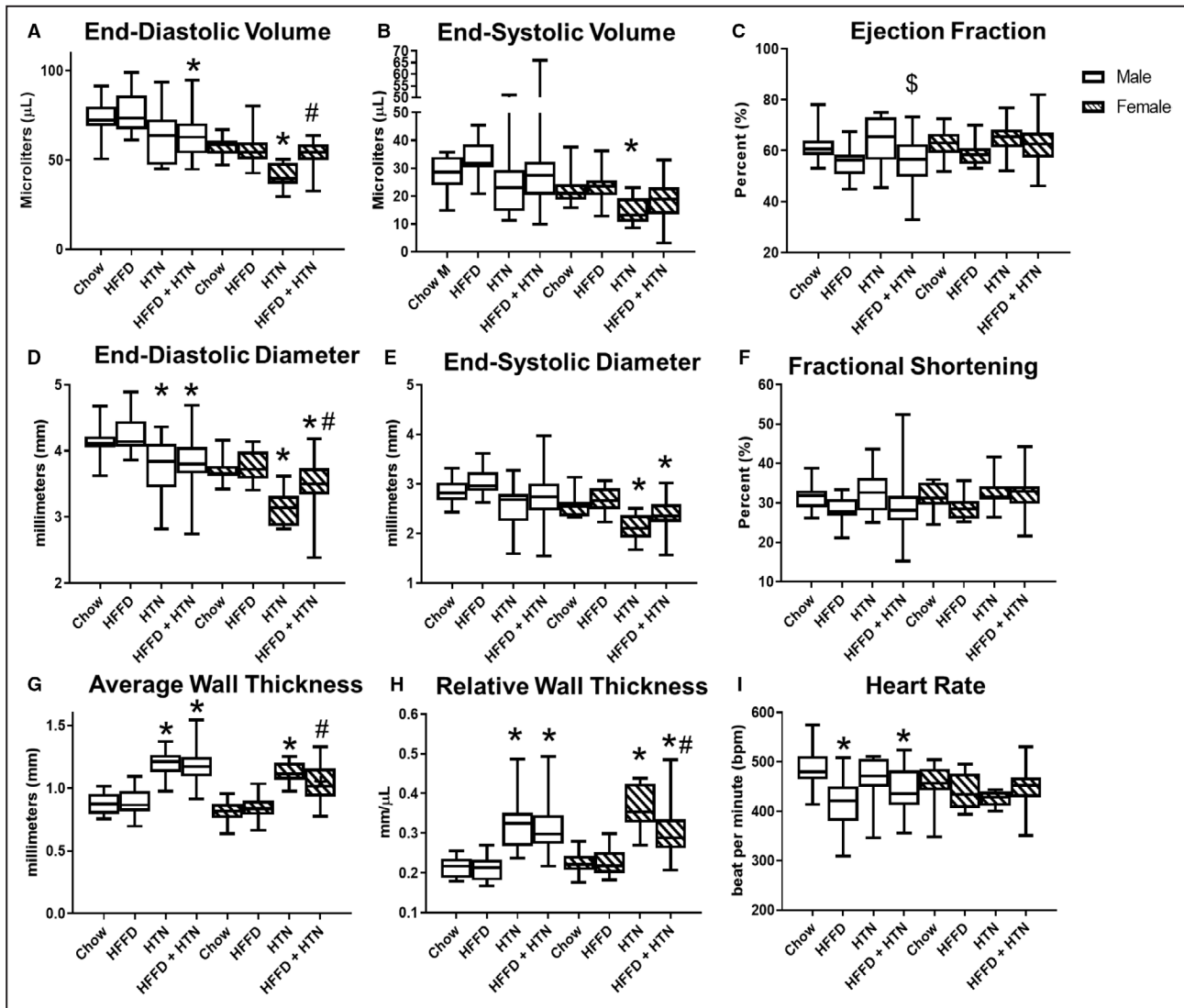
Figure 1. Cardiac function in male and female control, obese-normotensive, and obese-hypertensive mice.
A, In males, end-diastolic volume was decreased only by high-fat/fructose diet (HFFD)+HTN compared with chow (open bars). In females (shaded bars), end-diastolic volume and end-systolic volume (**B**) were decreased in HTN but not HFFD+HTN compared with chow. **C**, Ejection fraction was lower in HFFD+HTN males compared with HFFD alone but was unchanged in females. **D**, End-diastolic diameter was decreased in HTN and HFFD+HTN males; HFFD partially attenuated HTN-induced decreases in end-diastolic diameter in females. **E**, End-systolic diameter was not different among groups in males, but was decreased in HTN and HFFD+HTN females. **F**, Fractional shortening was not different among groups in either males or females. **G**, Average wall thickness (averaged from posterior and anterior) and relative wall thickness (**H**) was increased in both HTN and HFFD+HTN males and females but was partially attenuated by HFFD in females. **I**, Heart rate was decreased in both HFFD and HFFD+HTN males. *$P<0.05$ vs chow, #$P<0.05$ vs HTN, $\$$$P<0.05$ vs HFFD. Group numbers: n=17, chow male; n=18, HFFD male; n=10, HTN male; n=18, HFFD+HTN male; n=16, chow female; n=10, HFFD female; n=8, HTN female; n=16, HFFD+HTN female. HFFD indicates high-fat/fructose diet; and HTN, hypertension.

Post-MI Echocardiography Parameters

LV function was assessed by echocardiography at post-MI day 7 before euthanasia (Figure 3). In males, HFFD+MI had decreased LV dilation compared with chow+MI as assessed by decreased end-diastolic and systolic volumes (Figure 3A and 3B), whereas this effect of HFFD was not observed in females. HFFD+hypertension+MI males had decreased EF compared with HFFD+MI alone, but not chow+MI (Figure 3C). No differences in end-diastolic or systolic diameter (Figure 3D and 3E) or fractional shortening (Figure 3F) were observed among groups. No differences in thickness of the anterior wall (ie, the infarcted wall) were noted (Figure 3G), but HFFD+hypertension+MI males and females had increased posterior wall thickness compared with chow+MI and HFFD+MI males and females (Figure 3H), which may explain why the rupture rate was lower in these groups. HFFD+hypertension+MI
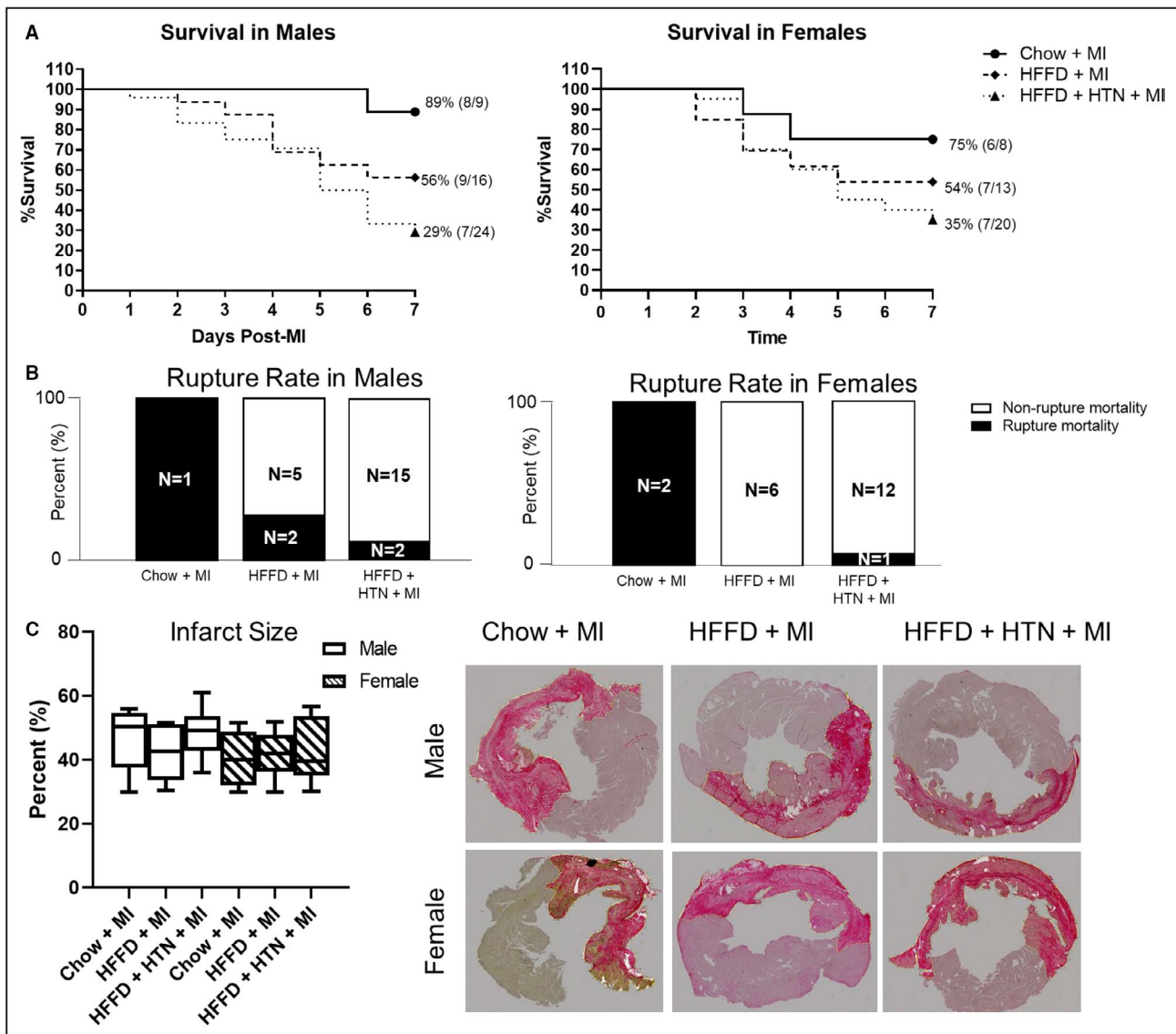
Figure 2. Post-myocardial infarction (MI) survival, rupture rate, and infarct size.
A, Survival was decreased in high-fat/fructose diet (HFFD)+MI males and females compared with chow+MI controls, and further decreased in HFFD+HTN+MI groups. **B**, Rupture rates for chow+MI controls were 100%; the majority of deaths for HFFD+MI and HFFD+HTN+MI groups were non-rupture. **C**, Infarct sizes (red area) were similar for all groups. Group numbers: n=8, chow+MI male; n=7, HFFD+MI male; n=7, HFFD+HTN+MI male; n=5; chow+MI female; n=6, HFFD+MI female; n=7, HFFD+HTN+MI female. HFFD indicates high-fat/fructose diet; HTN, hypertension; and MI, myocardial infarction.

males and females also showed decreased heart rate relative to chow-fed mice (Figure 3I). No significant differences in the longitudinal, circumferential, or radial strain, or LV dyssynchrony, were observed among groups after MI (Figure S4).

Hypertension Impairs Sarcomere Remodeling and Reparative Fibrosis in Obese Mice Following MI

We assessed the impact of obesity and hypertension on post-MI MHC isoforms, including α-MHC and β-MHC (Figure 3A). In males, HFFD+MI males

showed significantly increased expression of α-MHC compared with chow+MI; however, this effect was not observed in HFFD+hypertension+MI, whose expression was similar to chow controls, indicating that hypertension impaired HFFD-induced increases in post-MI α-MHC expression. No differences in α-MHC were observed among the different groups of females, and expression of β-MHC was not significantly different among groups in either males or females. We assessed collagen content in LV mid-section in both the infarct and remote regions (Figure 4B). In the infarct region, collagen was significantly decreased in HFFD+hypertension+MI males

Table 3. Post-MI Morphological Parameters

	Male			Female		
	Chow+MI	HFFD+MI	HFFD+Hypertension+MI	Chow+MI	HFFD+MI	HFFD+Hypertension+MI
LV mass	7.7±0.3	7.4±0.2	8.4±0.4	5.8±0.1	6.5±0.2	7.3±0.3*
RV mass	1.3±0.0	1.2±0.1	1.6±0.1†	1.2±0.1	1.2±0.1	1.3±0.1
Lung mass	14±1	11±1*	24±2*,†	11±1	14±2	16±3
Dry lung mass	2.7±0.2	2.3±0.1	5.2±0.5*,†	2.2±0.1	2.8±0.3	3.6±0.6
Edema Index	0.80±0.00	0.77±0.01	0.78±0.01	0.80±0.00	0.78±0.03	0.78±0.01
Spleen mass	5.6±0.5	5.6±0.5	4.9±0.6	6.3±0.3	7.7±0.4	7.6±0.7

Left ventricular, right ventricular, lung, and spleen masses (mg) were normalized to tibia length (mm). Edema index was calculated as the ratio of wet lung mass to total lung mass: edema index=(Lung mass−Dry lung mass)/Lung mass. Group numbers: n=8, chow+MI male; n=7, high-fat/fructose diet+MI male; n=7, high-fat/fructose diet+hypertension+MI male; n=6, chow+MI female, n=7 high-fat/fructose diet+MI female, n=6, high-fat/fructose diet+hypertension+myocardial infarction female. HFFD indicates high-fat/fructose diet; LV, left ventricle; MI, myocardial infarction; and RV, right ventricle.

*$P<0.05$ vs chow+MI.

†$P<0.05$ vs HFFD+MI.

compared with chow+MI and HFFD+MI groups, but not females (significant interaction of sex; $P<0.0001$). No differences in collagen content in the remote LV were observed among any of the groups.

## Hypertension Impairs Metabolic Remodeling in Post-MI Hearts of Obese Mice

We assessed the impact of obesity and hypertension on cardiac expression of key metabolic regulatory proteins and enzymes, including Akt, AMPK, ACC, PDH, PPAR-γ, and PGC-1α (Figure 5). HFFD+MI males showed a significant increase in phosphorylation of AMPK after MI compared with chow+MI, which was negated by hypertension, while no differences were observed among female groups (Figure 5A). Both HFFD+MI males and females had significantly elevated phosphorylation of Akt compared with chow+MI controls; which was higher in HFFD+MI males than females (Figure 4B; significant interaction of sex; $P<0.0001$). However, this effect was attenuated in both HFFD+hypertension+MI males and females. In males, inhibitory phosphorylation of ACC at serine 79 was significantly increased in HFFD+MI versus chow+MI controls, which was attenuated by hypertension. Total ACC expression was not changed (Figure 5C). In females, phosphorylation of ACC (normalized to total protein) and total expression of ACC were increased in HFFD+MI, but this effect was attenuated by hypertension (Figure 5D; significant interaction of sex; $P<0.0001$). Phosphorylation of ACC normalized to total ACC was not different in females. In males, a trend for increased inhibitory phosphorylation of PDH at serine 293 was observed in HFFD+hypertension+MI mice compared with chow+MI ($P=0.05$), indicating that glucose oxidation may be impaired in HFFD+hypertension+MI males; however, total expression was not significantly different; and total expression and phosphorylation of

PDH were not significantly different among groups in females (Figure 5D). Male HFFD-fed mice also had increased PPAR-γ expression, which was attenuated by Ang II; no differences in PGC-1α were observed in any male or female groups (Figure 5E). To assess potential upstream mediators of LV metabolic reprogramming after MI, we measured the adipokines leptin and adiponectin (Figure 5F). Leptin was elevated in HFFD+MI males and females compared with chow+MI controls. Leptin trended to be further increased in HFFD+hypertension+MI males ($P=0.056$ compared with HFFD+MI) but was not significantly elevated in HFFD+hypertension+MI females compared with chow+MI controls. In all 3 groups, plasma leptin was significantly lower in females than males (significant interaction of sex; $P=0.02$). Adiponectin was significantly increased in HFFD+MI males compared with chow+MI controls, but this effect was attenuated in the HFFD+hypertension+MI group. In females, adiponectin was significantly decreased in HFFD+hypertension+MI compared with both chow+MI and HFFD+MI controls. Adiponectin was significantly higher in all female groups compared with males (significant interaction of sex; $P<0.0001$).

DISCUSSION

The major new finding of our study is that obesity alone, in the absence of hypertension, increased post-MI mortality and that the presence of hypertension further increased mortality after MI, with the majority of deaths occurring because of congestive heart failure rather than rupture of the LV. We chose the 7-day time point to assess changes in mortality, as the majority of post-MI deaths in mice occur by day 7,[26] as well as maturation of the infarct collagenous scar tissue and maximal decreases in LV function.[27,28] In surviving mice, obesity-hypertension was associated with adverse remodeling, including exacerbated LV hypertrophy and pulmonary injury, as evidenced
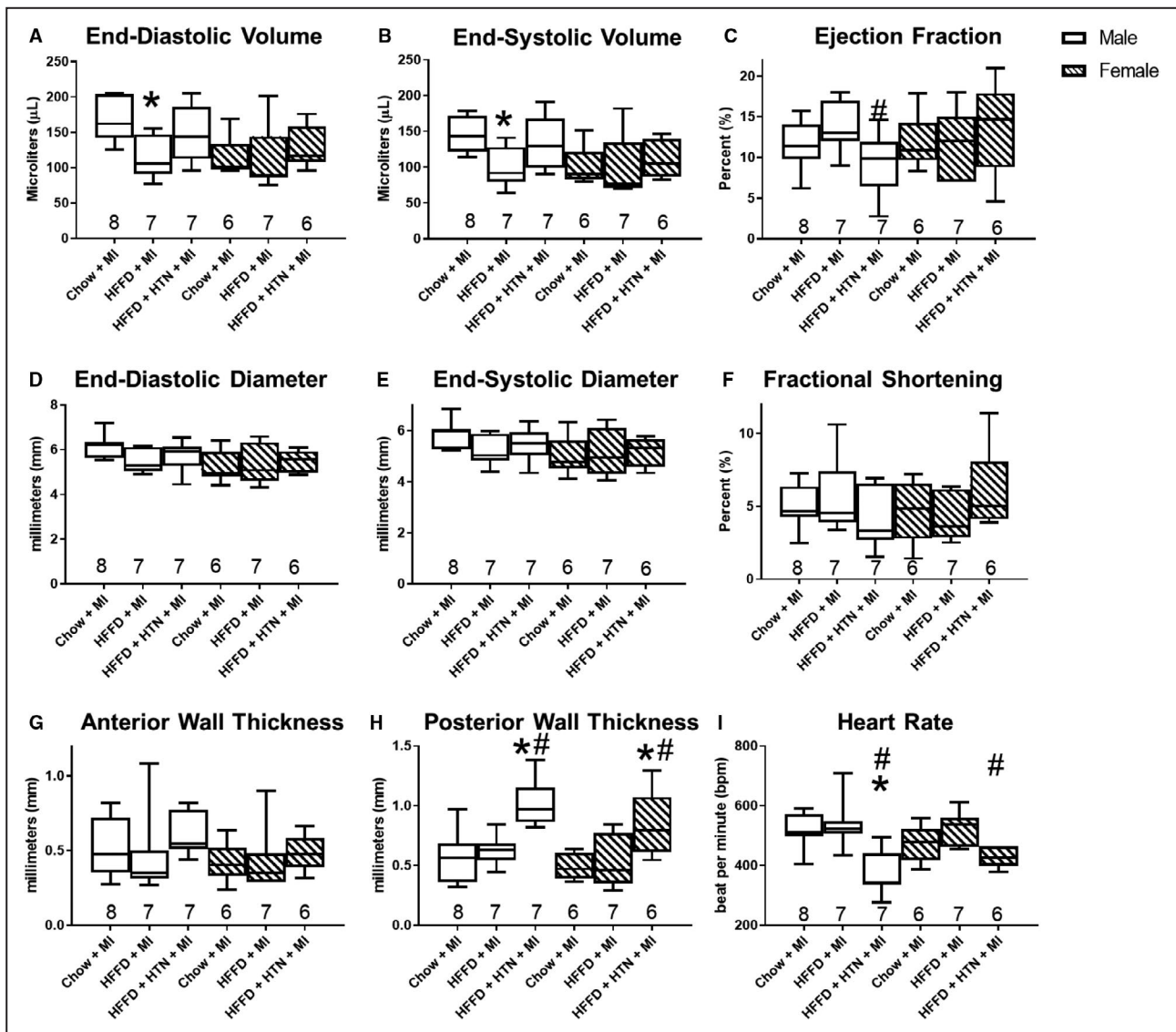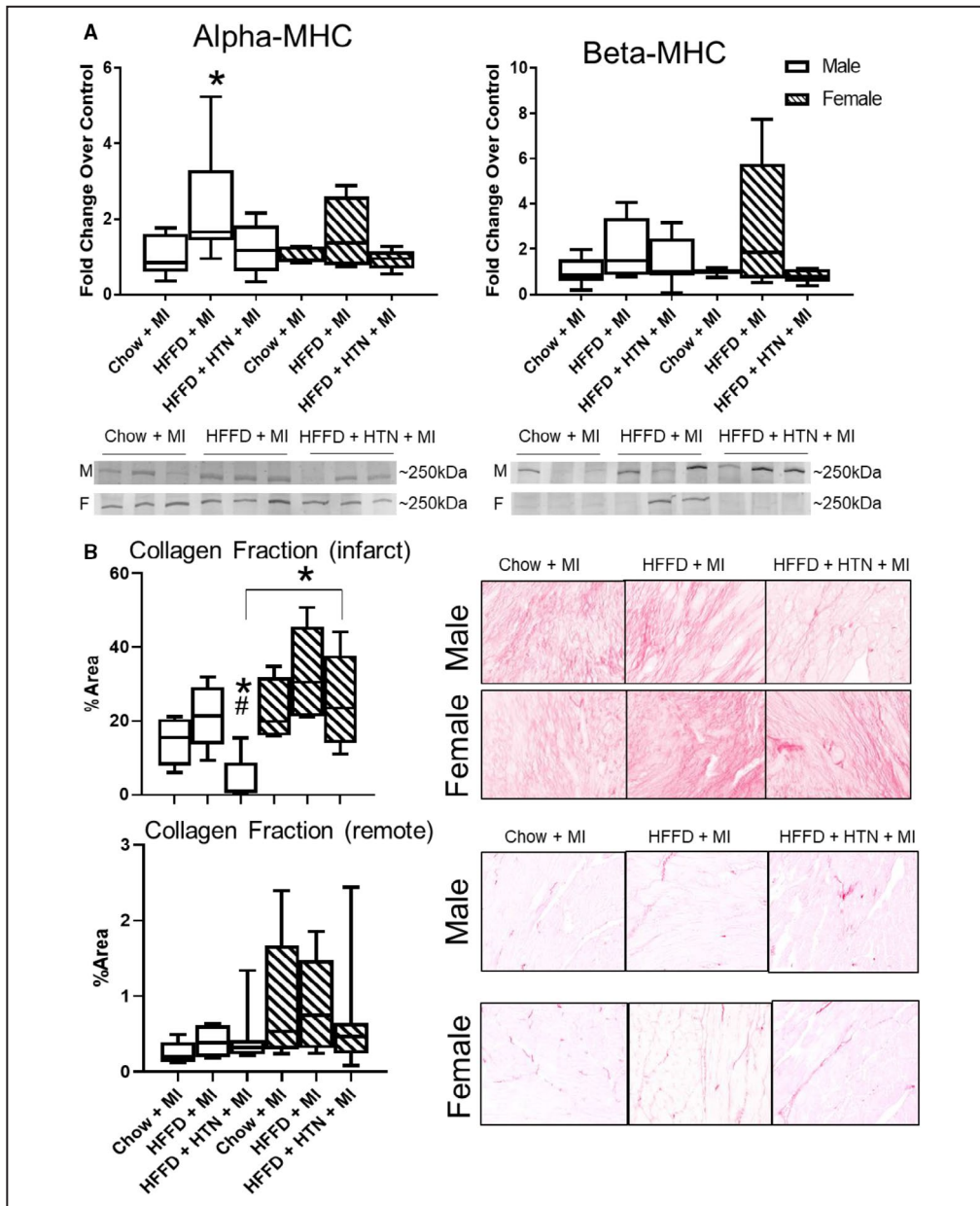
Figure 3. Cardiac function in male and female control, obese-normotensive, and obese-hypertensive mice 7 days after myocardial infarction (MI).

A, End-diastolic volume and (**B**) end-systolic volume was decreased in high-fat/fructose diet (HFFD)+myocardial infarction (MI) males compared with chow+MI, but not HFFD+hypertension+MI males. **C**, Ejection fraction was decreased in HFFD+hypertension+MI males compared with HFFD+MI males. **D** through **G**, No differences in end-diastolic diameter, end-systolic diameter, fractional shortening, or anterior wall thickness were detected among groups in either males or females. **H**, Posterior wall thickness (non-infarcted wall) was increased in HFFD+hypertension+MI males and females compared with chow+MI. **I**, Heart rate was significantly decreased in HFFD+hypertension+MI males and females compared with chow+MI. *$P<0.05$ vs chow+MI, #$P<0.05$ vs HFFD+MI. Group numbers: n=8, chow+MI male; n=7, HFFD+MI male; n=7, HFFD+HTN+MI male; n=6, chow+MI female; n=7, HFFD+MI female; n=6, HFFD+HTN+MI female. HFFD indicates high-fat/fructose diet; HTN, hypertension; and MI, myocardial infarction.

by the increase in lung dry weight.[25] Our results indicate that obesity-hypertension negatively impacts the post-MI remodeling response in adult male and female mice.

In the absence of hypertension, obesity was associated with some favorable metabolic and functional effects on the post-MI heart in males, despite decreasing survival. Obese males displayed upregulation of cardioprotective signaling pathways, including AMPK and PPAR-γ. However, these effects were negated when hypertension was present. Because hypertension is often associated with obesity in humans, but not in many mouse models of obesity, our findings also suggest that the impact of prior hypertension and associated cardiac remodeling should be considered when assessing the impact of obesity on MI outcomes. Despite some favorable metabolic and functional effects on the heart after MI, the overall effect of obesity is to increase mortality, especially when hypertension coexists.
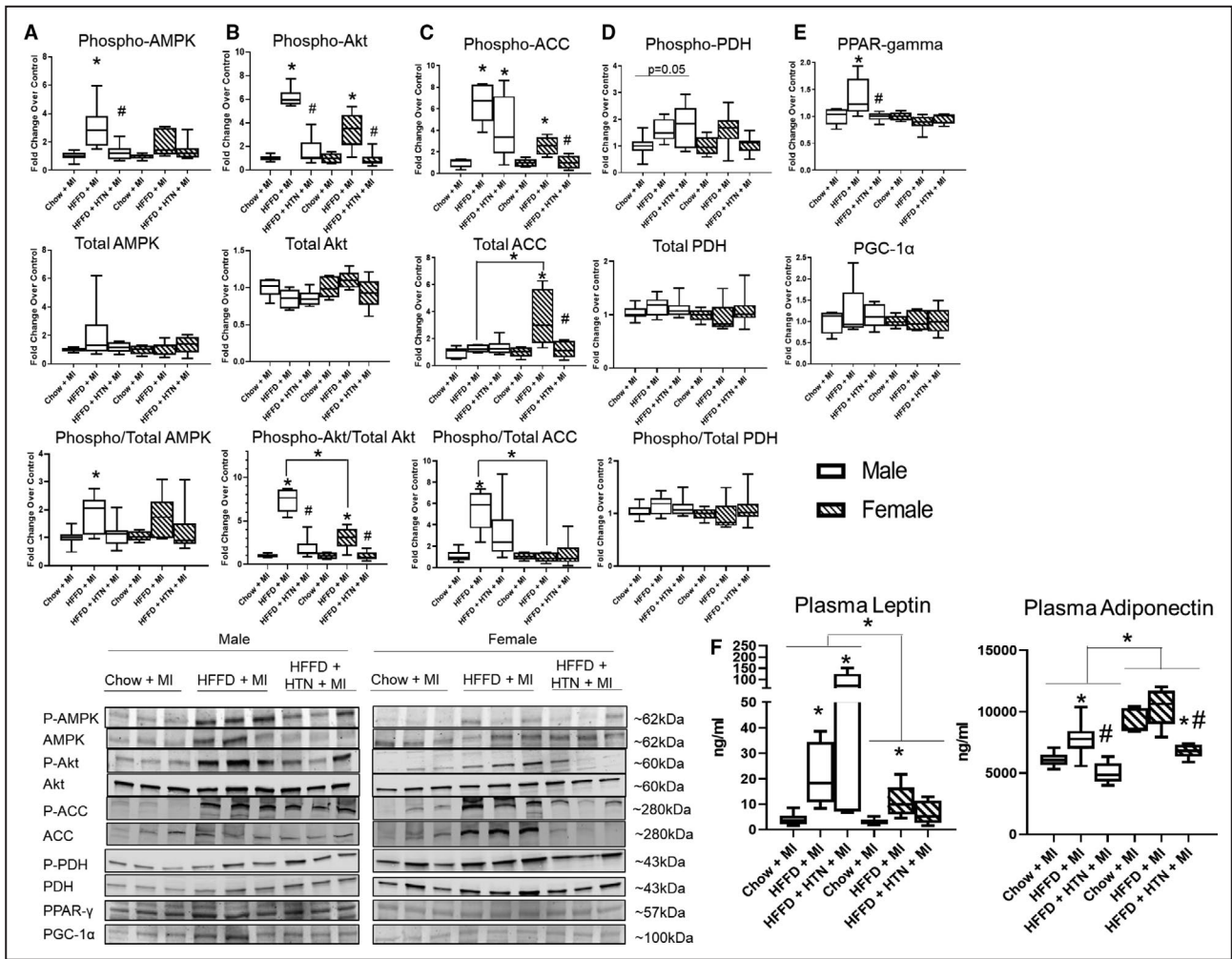
Figure 4.   Left ventricle myosin heavy chain expression and collagen content in male and female control, obese-normotensive, and obese-hypertensive mice 7 days after myocardial infarction (MI).

A, Left ventricle expression of alpha-myosin heavy chain was significantly compared with chow+MI, which was attenuated by high-fat/fructose diet (HFFD)+hypertension+MI. No significant differences were observed in beta-myosin heavy chain expression in either males or females. **B**, Collagen deposition in the infarct region was impaired in HFFD+hypertension+MI males compared with chow+MI and HFFD+MI controls; no differences were observed in infarct collagen content in female groups. No differences in remote left ventricle collagen content were observed among groups. *$P<0.05$ vs chow+MI. #$P < 0.05$ vs HFFD + MI. Group numbers: n=8, chow+MI male; n=7, HFFD+MI male; n=7, HFFD+HTN+MI male; n=6, chow+MI female; n=7, HFFD+MI female; n=6, HFFD+HTN+MI female. HFFD indicates high-fat/fructose diet; HTN, hypertension; MHC, myosin heavy chain; and MI, myocardial infarction.

Obesity, which has reached epidemic proportions in the United States and many other countries, has been associated with cardiac injury and increased risk of MI in humans.[10,20] However, an obesity paradox has been observed in which obese patients surviving MI may

display better long-term outcomes.[10–12] The mechanisms responsible for this apparent protection are unknown, and are controversial. Some studies have suggested that inclusion of younger subjects with mild forms of obesity may explain the improved outcomes

Figure 5. Left ventricle metabolic signaling pathways in male and female control, obese-normotensive, and obese-hypertensive mice 7 days after myocardial infarction (MI).

A, High-fat/fructose diet (HFFD)+MI increased AMPK (adenosine-monophosphate activated kinase) phosphorylation in males compared with chow+MI, which was attenuated by HFFD+HTN+MI. No changes in total AMPK levels were observed among groups. **B**, HFFD+MI increased Akt (protein kinase B) phosphorylation in males and females compared with chow+MI controls, which was attenuated by HFFD+HTN+MI; no changes in total Akt were observed. **C**, Increased acetyl CoA carboxylase phosphorylation was observed in both HFFD+MI and HFFD+HTN+MI males compared with chow+MI; while total acetyl CoA carboxylase was unchanged. In females, HFFD+MI increased acetyl CoA carboxylase phosphorylation and total acetyl CoA carboxylase compared with chow+MI, which was attenuated by HFFD+HTN+MI. **D**, No significant differences were observed in pyruvate dehydrogenase phosphorylation or total pyruvate dehydrogenase levels among groups. **E**, HFFD+MI increased PPAR-γ (peroxisome proliferator activated receptor gamma) in males compared with chow+MI, which was attenuated by HFFD+HTN+MI. No differences in PGC-1α (peroxisome proliferator-activated receptor gamma coactivator 1-alpha) were observed among groups. **F**, Plasma leptin was significantly increased in HFFD+MI males and females; this effect trended to be further increased in HFFD+HTN+MI males but was attenuated in HFFD+HTN+MI females. Plasma adiponectin was significantly increased in HFFD+MI males, which was attenuated by HFFD+HTN+MI. In females, plasma adiponectin was significantly decreased by HFFD+HTN+MI compared with chow+MI and HFFD+MI controls. *$P<0.05$ vs chow+MI, #$P<0.05$ vs HFFD+MI. Group numbers: n=8, chow+MI male; n=7, HFFD+MI male; n=7, HFFD+HTN+MI male; n=6, chow+MI female; n=7, HFFD+MI female; n=6, HFFD+HTN+MI female. ACC indicates acetyl CoA carboxylase; AMPK, adenosine-monophosphate activated kinase; HFFD, high-fat/fructose diet; HTN, hypertension; MI, myocardial infarction; PDH, pyruvate dehydrogenase; PGC-1α— peroxisome proliferator-activated receptor gamma coactivator 1-alpha; and PPAR-γ, peroxisome proliferator activated receptor gamma.

and apparent paradox.[10] Studies in mice that have investigated the impact of obesity and potential mechanisms of the obesity paradox on post-MI outcomes have produced highly variable results.[14–17,29] Poncelas et al reported that chronic high-fat feeding in B6D2F1

mice attenuated ischemia-reperfusion induced LV dilation and pulmonary congestion measured 7 days after the procedure.[14] Heaberlin et al reported that obese and diabetic mice heterozygous for the spontaneous yellow mutation (A^y) (*KKAy*) were partially protected

from LV contractile dysfunction and inflammation, but not dilation or pulmonary congestion, 7 days following permanent MI, and overall mortality was increased in obese mice.[16] Brainard et al reported that chronic high fat feeding for 6 months did not exacerbate nor protect against cardiac dysfunction following MI,[17] whereas Thakker et al reported that high-fat feeding for 6 months exacerbated cardiac dysfunction and remodeling after ischemia-reperfusion injury.[15] Some of these discrepancies may relate to use of non-reperfused or reperfused models of ischemic injury.

Our results corroborate some of these findings, including reduced survival in obese mice after MI. However, in obese male mice that survived for at least 7 days after MI we observed better cardiac function and a more favorable cardiac metabolic profile compared with lean male mice. One possible explanation for these differences is survivor bias, in which only the obese males with the best cardiac function and most favorable cardiac metabolic profile may have survived. Thus, it is difficult to assess whether the survivors are really representative of the changes occurring in most of the obese males which did not survive the MI.

The mechanisms responsible for potential effects of obesity on recovery of cardiac function have not, to our knowledge, be previously assessed. We found that obese males had a significant increase in α-MHC expression, the major MHC isoform in the rodent heart,[30,31] after MI compared with mice fed a normal chow. Furthermore, there were no changes in α-MHC expression at baseline (ie, in mice before MI) in obese-normotensive or obese-hypertensive mice, indicating that α-MHC was induced only after MI in HFFD-fed mice. Increased α-MHC expression is associated with improved function and may be induced by cardioprotective measures such as exercise, while decreased expression of α-MHC coincides with impaired cardiac function and onset of heart failure.[30,31] MHC isoform switching toward α-MHC may be a compensatory response for shifting metabolic demands, as the alpha isoform predominates over the beta isoform when fatty acid beta-oxidation predominates over glycolysis.[32] While we did not directly measure cardiac metabolic flux in this study, we found that inhibitory phosphorylation of ACC was increased in post-MI hearts of obese male mice, while total ACC expression was not changed, indicating inhibition of fatty acid synthesis and a shift toward fatty acid oxidation, which has been associated with improvements in post-MI LV function.[29] Thus, the altered metabolic environment in the post-MI heart of obese-normotensive males may have driven the increase in α-MHC expression.

We observed activation of cardioprotective signaling pathways in the post-MI heart of obese-normotensive male mice, including Akt, AMPK, and PPAR-γ. Akt is a member of the myocardial insulin signaling cascade, and is activated directly by insulin stimulation in the heart, while impaired Akt phosphorylation is a hallmark of myocardial insulin resistance.[33,34] Akt activation has also been observed in obese rats following MI,[34] indicating that obesity may paradoxically improve insulin signaling in the ischemic heart. Whether this occurs with more extreme levels of obesity remains to be investigated. AMPK has several cardioprotective actions in the ischemic heart, including upregulation of glucose oxidation and glycolytic metabolism and inhibition of fatty acid synthesis via phosphorylation of ACC, which was also observed in obese-normotensive mice.[35] Obesity also increased expression of PPAR-γ in the post-MI male heart. PPAR-γ is activated by ligands such as fatty acids,[36] and exerts multiple cardioprotective effects, including protection against oxidative stress and inflammation, and enhancing glycolytic and lipid/oxidative metabolism.[37]

We also observed differences in circulating adipokines after MI, including leptin and adiponectin, which may contribute to changes in insulin sensitivity and myocardial remodeling. Leptin is secreted by adipose tissue and correlates with increased body weight, particularly fat mass.[38] Although increased leptin may raise blood pressure, the effect of higher leptin levels on the heart after MI is still uncertain; some studies have also reported that high leptin levels improve cardiac function and protect against obesity-induced or ischemic injury,[39,40] However, plasma leptin was not significantly different among HFFD+MI or HFFD+hypertension+MI males, although it was slightly attenuated in HFFD+hypertension+MI females. We also observed significant differences in plasma adiponectin, which was increased in HFFD+MI males but attenuated in the HFFD+hypertension+MI group, mirroring the differences we saw in Akt, AMPK, and PPAR-γ activation. Adiponectin has insulin-sensitizing and cardioprotective properties, including activation of AMPK.[41] Although plasma adiponectin is typically decreased in obese humans, studies have shown that it is elevated in mice during the early stages of diet-induced obesity, then progressively decreases.[42] Thus, obesity may paradoxically offer some cardioprotection in the post-MI heart of surviving males via increased insulin signaling and activation of cardioprotective metabolic signaling pathways.

While we observed activation of some cardioprotective pathways and mild preservation of cardiac function in surviving obese-normotensive males, we also found that hypertension completely negated any potential beneficial effects of obesity on recovery of cardiac function in surviving male mice after MI. In many, if not most, humans with obesity, hypertension is a comorbidity.[43] Hypertension is also a major risk factor for MI and worsens post-MI outcomes.[19] Although obesity is a primary cause of hypertension in humans,[9] it often

fails to reliably produce substantial hypertension in rodents.[18,44,45] Thus, we induced hypertension by chronic Ang II infusion, a model that has been widely used to rapidly and reliably produce sustained elevations in arterial pressure in mice.[18,46] While obesity alone showed some cardioprotective benefits in post-MI hearts of surviving males, the presence of Ang II-induced hypertension negated these effects and induced significantly higher mortality and pulmonary congestion. This finding is consistent with observations in hypertensive human patients who often develop heart failure following MI.[19] Furthermore, although hypertension promoted fibrosis in the naïve heart, it impaired deposition of reparative collagen in the infarcted area, which protects against rupture and dilation, and normalizes wall stress,[47] in obese males. We observed little heart rupture in HFFD+hypertension+MI mice, but they were more likely to die of heart failure and pulmonary edema. The increased wall thickness in these groups may have also decreased the incidence of rupture, while increasing heart failure incidence. Thus, further studies may be necessary to determine mechanisms of hypertension-induced derangements in reparative fibrosis. Importantly, our model of obesity-hypertension did not significantly impair cardiac function or promote excessive cardiac injury before MI, indicating that adverse effects were unmasked after MI. Thus, when considering the impact of obesity-hypertension, it becomes evident that obesity is a major risk factor and comorbidity for patients with MI, even without preexisting cardiac abnormalities. Whether these adverse effects are attributable to an inability of the hypertensive heart to adapt to further stress, the persistent elevation of cardiac afterload, or both, remains to be determined.

We observed some sex differences in our study. Although males and females had similar post-MI survival rates in all 3 groups, surviving obese-normotensive (HFFD+MI) females did not display the same decrease in LV dilation, pulmonary congestion, and upregulation of favorable metabolic signaling pathways. Obese-hypertensive females also had more hypertrophy (significantly elevated LV mass) after MI compared with chow-fed controls, which was not observed in males. While this did not translate into changes in post-MI LV function or remodeling, it does indicate that hypertension may adversely affect LV remodeling post-MI differently than in males by promoting excessive hypertrophy, which may protect against rupture in the short-term but is associated with increased long-term risk for development of heart failure.[48,49] We also observed a sex difference in deposition of collagen in the infarct region, which was not altered in females but significantly impaired in obese-hypertensive males. There were also differences in LV metabolic pathways, as obese-normotensive males displayed activation of AMPK and ACC inhibitory phosphorylation, as well as expression of PPAR-γ, which was not observed in females. This discrepancy could be related to sex differences in the circulating adipokines leptin and adiponectin, which have been found to differ in men and women with metabolic syndrome.[50] In our study, leptin levels were overall lower in females while adiponectin was higher in females regardless of diet or hypertension, despite similar body fat percentages between males and females. However, obese-normotensive females did not display the same elevations in plasma adiponectin after MI as males, which may be partially responsible for the protection observed in males, and reflecting the clinical scenario in which women with metabolic syndrome display lower adiponectin levels than men.[50] Thus, there may be sex differences in the mechanisms that govern post-MI remodeling in obese-hypertensive males and females, which may be explained by changes in adipokines. However, we realize that these potential mechanisms are purely observational and more direct experiments are required to examine sex differences in post-MI remodeling during obesity-hypertension.

One limitation of our study was that the age of our mice was relatively young, as the adverse impact of obesity on post-MI outcomes is highly pronounced in aged mice.[51] Thus, it is possible that we may not have observed some of the favorable cardiac effects of obesity had we used older mice. Another possible explanation is that our model of obesity was not severe enough to adequately mimic human extreme (class III) obesity, in which the obesity paradox is lost.[10] Although we chose the 7-day time point to assess survival rates and capture the peak of MI-induced LV dysfunction, further remodeling and healing of the infarct and remote regions occurs for up to 28 days post-MI in mice.[28] Thus, longer studies are required to assess whether obesity exerts cardioprotection in surviving mice during long-term healing after MI. We did not include a chow+hypertension+MI group (lean hypertensive), as we were mainly interested in answering whether hypertension negates paradoxical improvements in post-MI function during obesity. There is also potential for some of our tests to be underpowered, as our sample sizes were relatively small (n=6–8). Despite the robust changes in some of our parameters with this number, increased numbers may be required to detect more subtle changes in future studies.

In conclusion, our studies indicate that obesity and hypertension both increase mortality after MI. Obesity-hypertension exacerbates adverse LV remodeling after MI, in both male and female mice, and hypertension negates any potential beneficial effects of obesity on cardiac metabolism in surviving mice. Since obesity and hypertension are major risk factors for MI and most obese humans are also hypertensive, it is important to further investigate mechanisms by which obesity and hypertension interact to alter cardiac metabolism

and remodeling in ischemic as well as in non-ischemic hearts.

ARTICLE INFORMATION

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Disclosures

None.

Supplementary Material

Table S1
Figures S1–S4

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SUPPLEMENTAL MATERIAL

**Table S1. Total cholesterol was significantly elevated in both HFFD + MI and HFFD + HTN + MI males and females compared to Chow + MI controls.**

|	Male			Female		
	Chow + MI	HFFD + MI	HFFD + HTN + MI	Chow + MI	HFFD + MI	HFFD + HTN + MI
Cholesterol (mg/dL)	84±5	146±14*	162±33*	65±4	101±8*	103±13*
HDL (mg/dL)	43±2	64±6*	62±9*	32±1	44±4	41±6
LDL (mg/dL)	12±2	18±3	17±3	12±2	19±4	19±3
Triglycerides (mg/dL)	46±10	108±15*	98±13*	61±6	69±12	43±9

HDL and triglycerides were also significantly elevated in HFFD + MI and HFFD + HTN + MI males compared to Chow + MI controls. *$p < 0.05$ versus Chow + MI. MI=myocardial infarction; HFFD=high fat/fructose diet; HTN=hypertension; mg/dl=milligrams per deciliter; HDL=high density lipoprotein; LDL=low density lipoprotein. Group numbers: n=8 Chow + MI male, n=7 HFFD + MI male, n=7 HFFD + HTN + MI male, n=6 Chow + MI female, n=7 HFFD + MI female, n=6 HFFD + HTN + MI female.

Figure S1. Left ventricle myosin heavy chain expression and collagen content in in male and female control, obese, and obese-hypertensive mice 7 days after myocardial infarction.
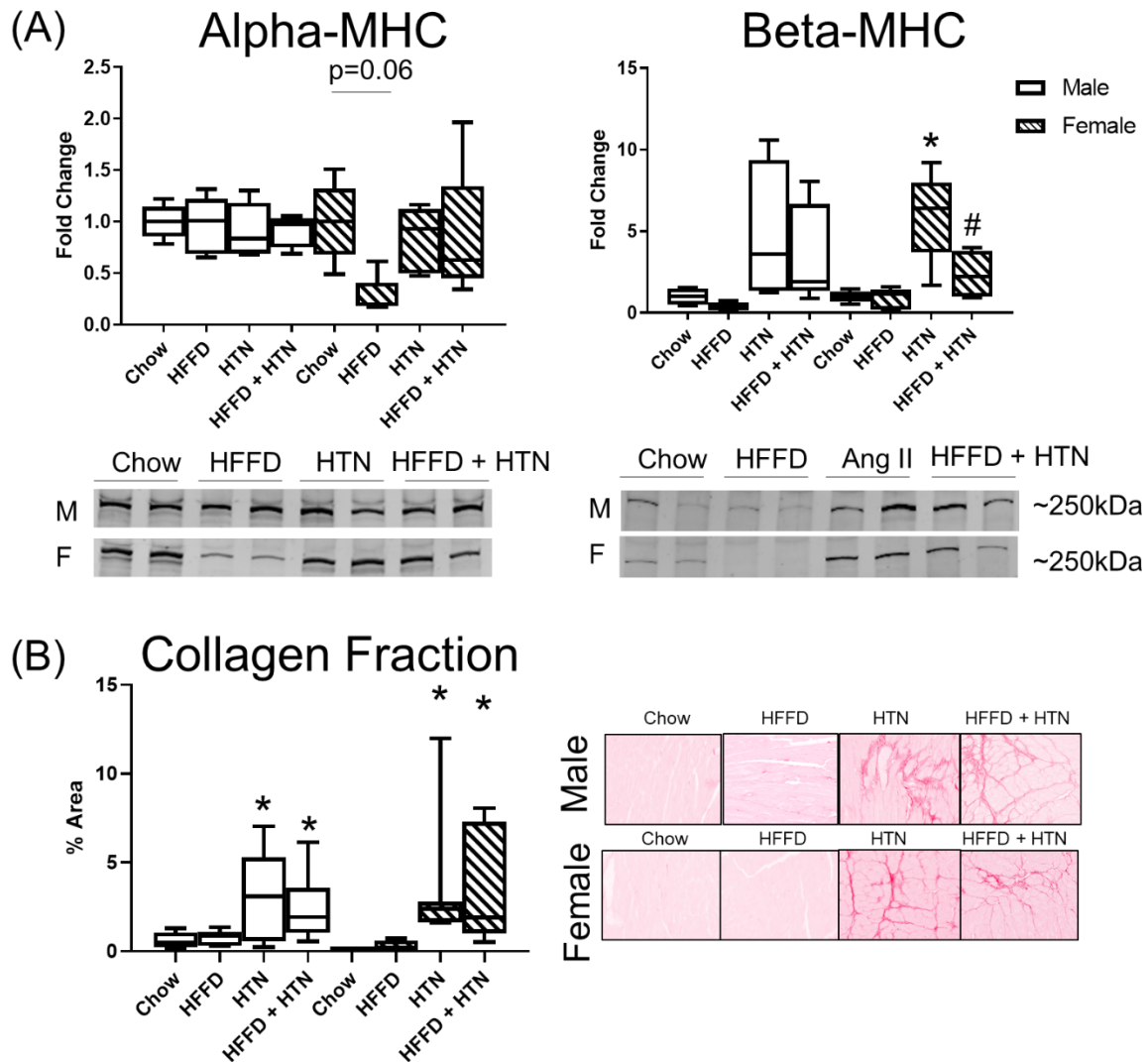

(A) Alpha-myosin heavy chain (α-MHC) expression was not significantly different among groups. Beta-myosin heavy chain (β-MHC) was not different in males. In females, HTN significantly increased β-MHC compared to chow, which was attenuated by HFFD + HTN. (C) LV collagen content was elevated by HTN and HFFD + HTN compared to chow in both males and females. *p<0.05 versus Chow, #p<0.05 versus HFFD + HTN. N=6 per group.

Figure S2. Left ventricle metabolic signaling pathways in in male and female control, obese-normotensive, and obese-hypertensive mice without myocardial infarction.
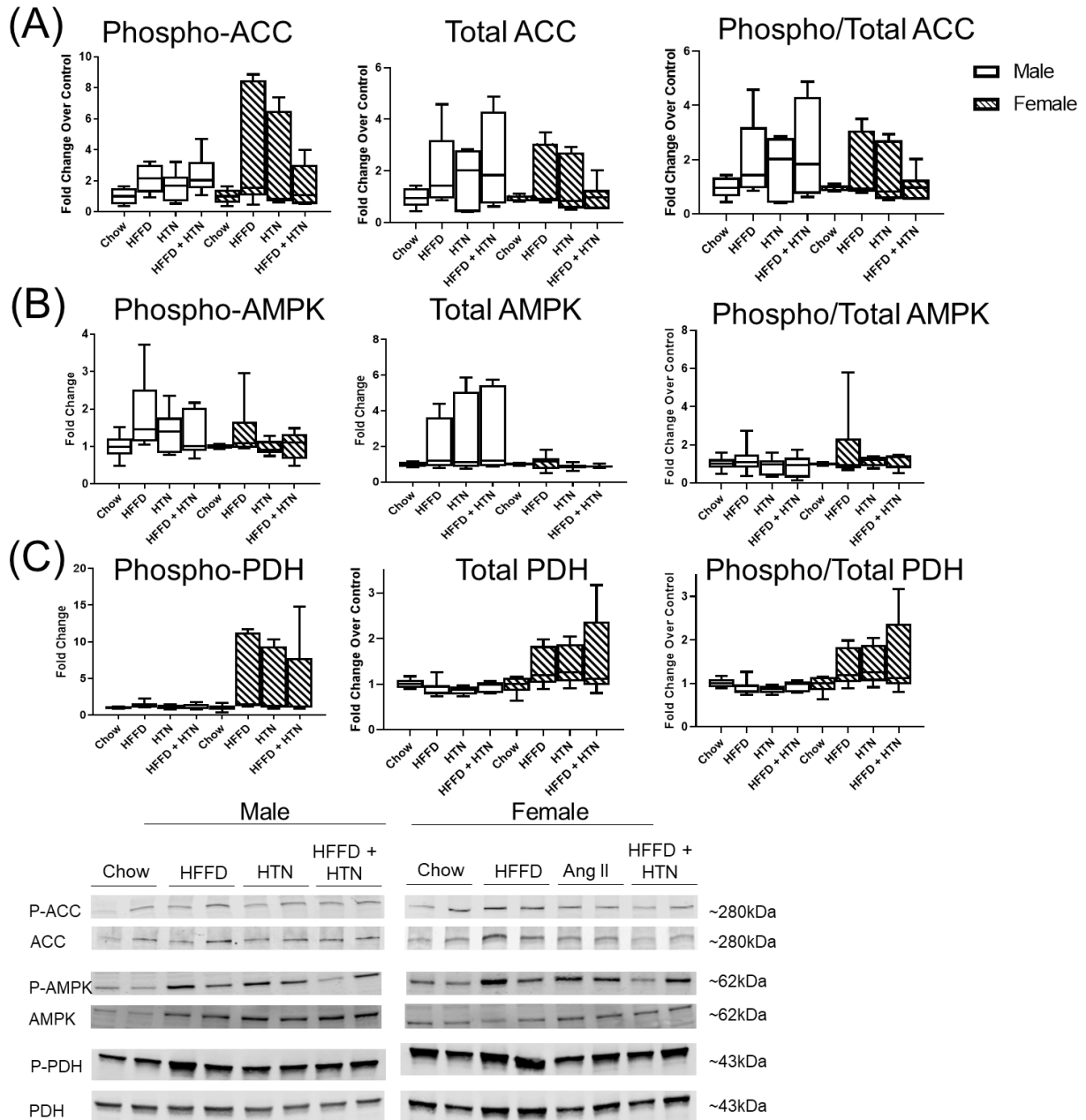
Phosphorylation and total levels of ACC (A), AMPK (B), and PDH (C) was not statistically different among groups. N=6 per group.

Figure S3. Representative full Western blots.
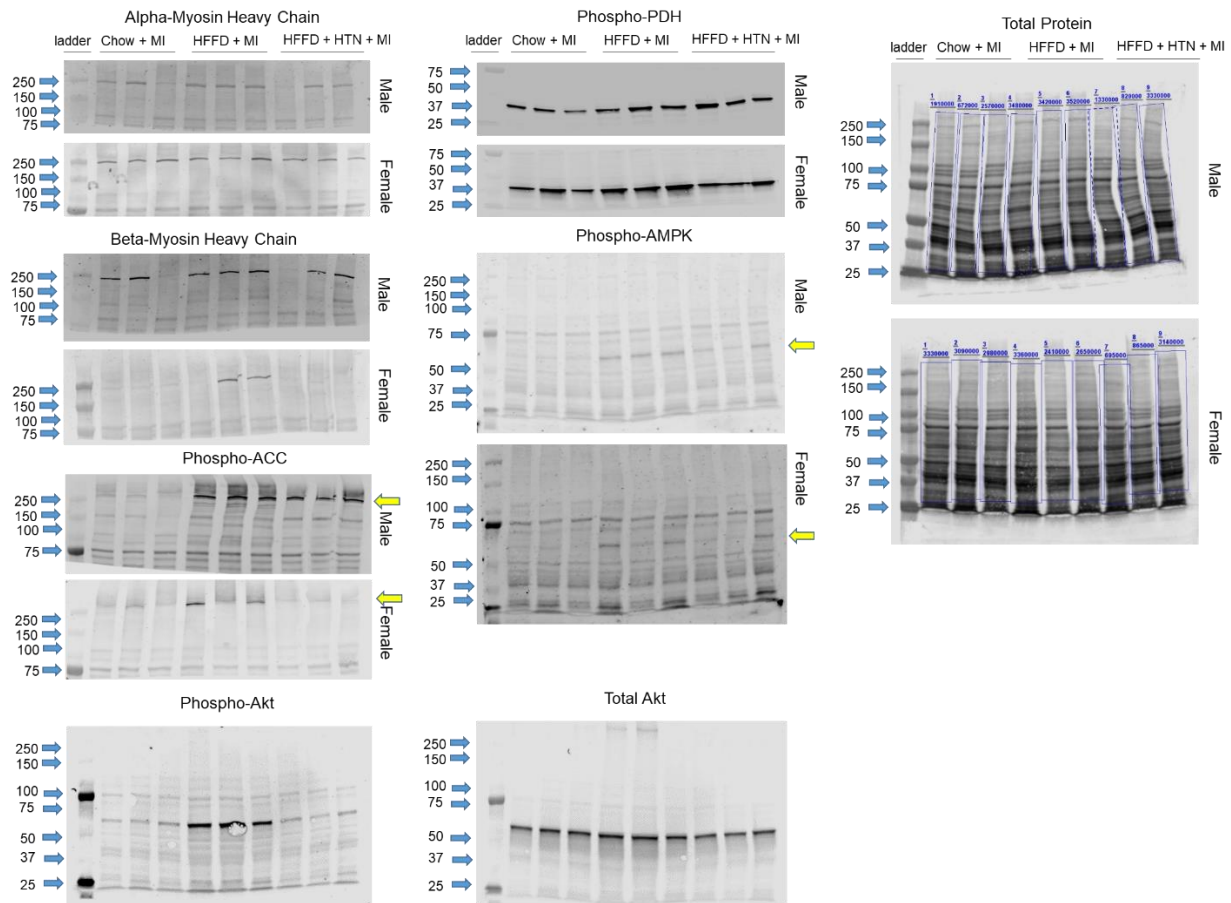
Molecular markers are denoted by blue arrows and are measured in kilodaltons (kDa). Some blots were cut in half to measure high molecular weight and low molecular weight proteins simultaneously (i.e. alpha- and beta-myosin heavy chains and ACC measured in top half, PDH measured bottom half).

Figure S4. No significant differences were observed in longitudinal, circumferential, or radial strain among groups.
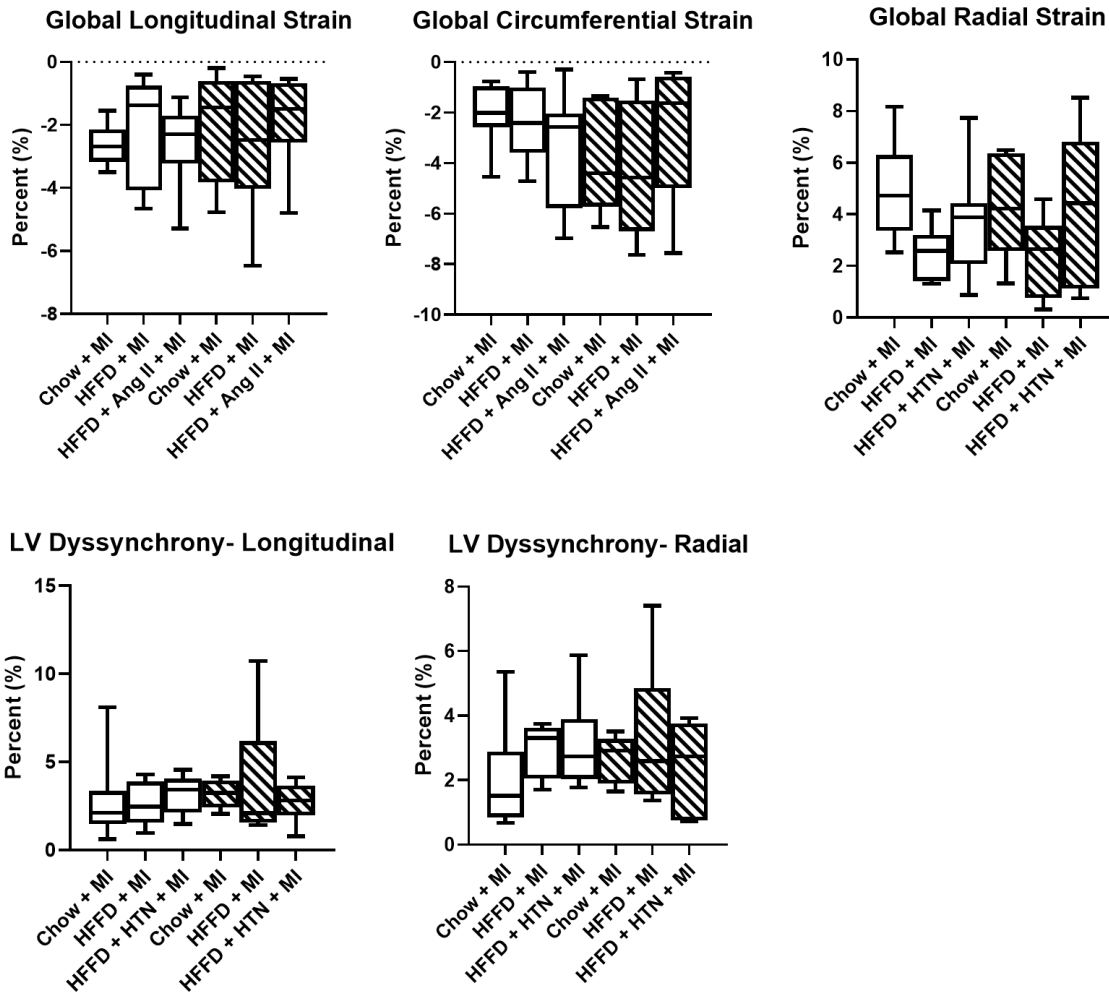
No differences were observed in LV longitudinal or radial dyssynchrony. Group numbers: n=8 Chow + MI male, n=7 HFFD + MI male, n=7 HFFD + HTN + MI male, n=6 Chow + MI female, n=7 HFFD + MI female, n=6 HFFD + HTN + MI female.