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LEADING EDGE IN TRANSLATIONAL RESEARCH

Empagliflozin Prevents Worsening of Cardiac Function in an Experimental Model of Pressure Overload-Induced Heart Failure

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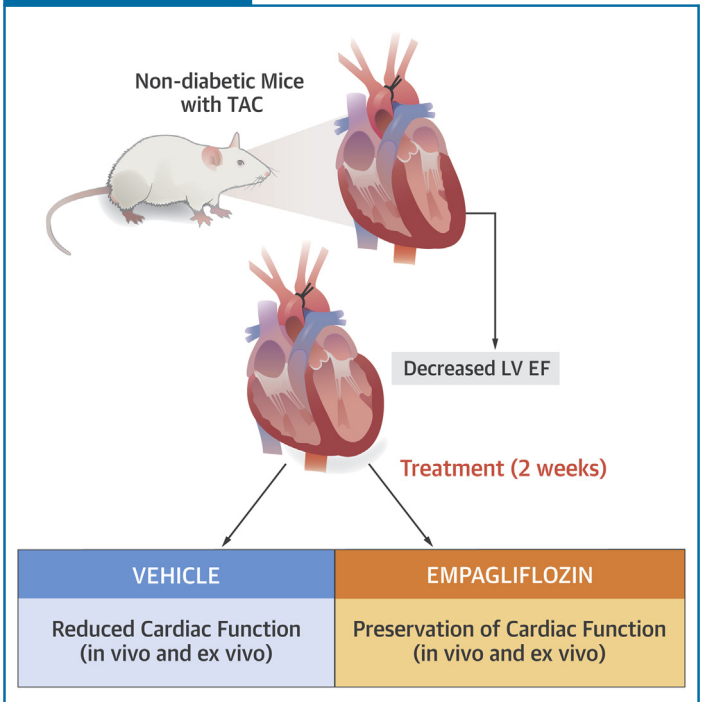
VISUAL ABSTRACT



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HIGHLIGHTS

- **Although empagliflozin markedly reduces heart failure and cardiovascular-related deaths in diabetic patients, whether empagliflozin improves cardiac outcomes in the absence of diabetes is unknown.**
- **Nondiabetic mice subjected to pressure overload exhibited a decline in LV function, both in vivo and ex vivo.**
- **Nondiabetic mice with significantly reduced LV function treated with empagliflozin demonstrated systolic function that was preserved compared to that in vehicle-treated mice, which continued to worsen.**
- **Preserved cardiac function in empagliflozin-treated mice with heart failure was sustained ex vivo in the absence of ketones or hemodynamic changes.**

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All authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the *JACC: Basic to Translational Science* author instructions page.

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ABBREVIATIONS AND ACRONYMS

HF = heart failure

LV = left ventricular

SGLT2 = sodium/glucose cotransporter 2

TAC = transverse aortic constriction

SUMMARY

This study sought to determine whether the sodium/glucose cotransporter 2 (SGLT2) inhibitor empagliflozin improved heart failure (HF) outcomes in nondiabetic mice. The EMPA-REG OUTCOME (Empagliflozin, Cardiovascular Outcome Event Trial in Type 2 Diabetes Mellitus Patients) trial demonstrated that empagliflozin markedly prevented HF and cardiovascular death in subjects with diabetes. However, despite ongoing clinical trials in HF patients without type 2 diabetes, there are no objective and translational data to support an effect of SGLT2 inhibitors on cardiac structure and function, particularly in the absence of diabetes and in the setting of established HF. Male C57Bl/6 mice were subjected to either sham or transverse aortic constriction surgery to induce HF. Following surgery, mice that progressed to HF received either vehicle or empagliflozin for 2 weeks. Cardiac function was then assessed in vivo using echocardiography and ex vivo using isolated working hearts. Although vehicle-treated HF mice experienced a progressive worsening of cardiac function over the 2-week treatment period, this decline was blunted in empagliflozin-treated HF mice. Treatment allocation to empagliflozin resulted in an improvement in cardiac systolic function, with no significant changes in cardiac remodeling or diastolic dysfunction. Moreover, isolated hearts from HF mice treated with empagliflozin displayed significantly improved ex vivo cardiac function compared to those in vehicle-treated controls. Empagliflozin treatment of nondiabetic mice with established HF blunts the decline in cardiac function both in vivo and ex vivo, independent of diabetes. These data provide important basic and translational clues to support the evaluation of SGLT2 inhibitors as a treatment strategy in a broad range of patients with established HF.

Recent findings from the EMPA-REG OUTCOME (Empagliflozin, Cardiovascular Outcome Event Trial in Type 2 Diabetes Mellitus Patients) trial demonstrated that the sodium/glucose cotransporter 2 (SGLT2) inhibitor empagliflozin markedly reduced cardiovascular death and heart failure hospitalization in subjects with type 2 diabetes (1).

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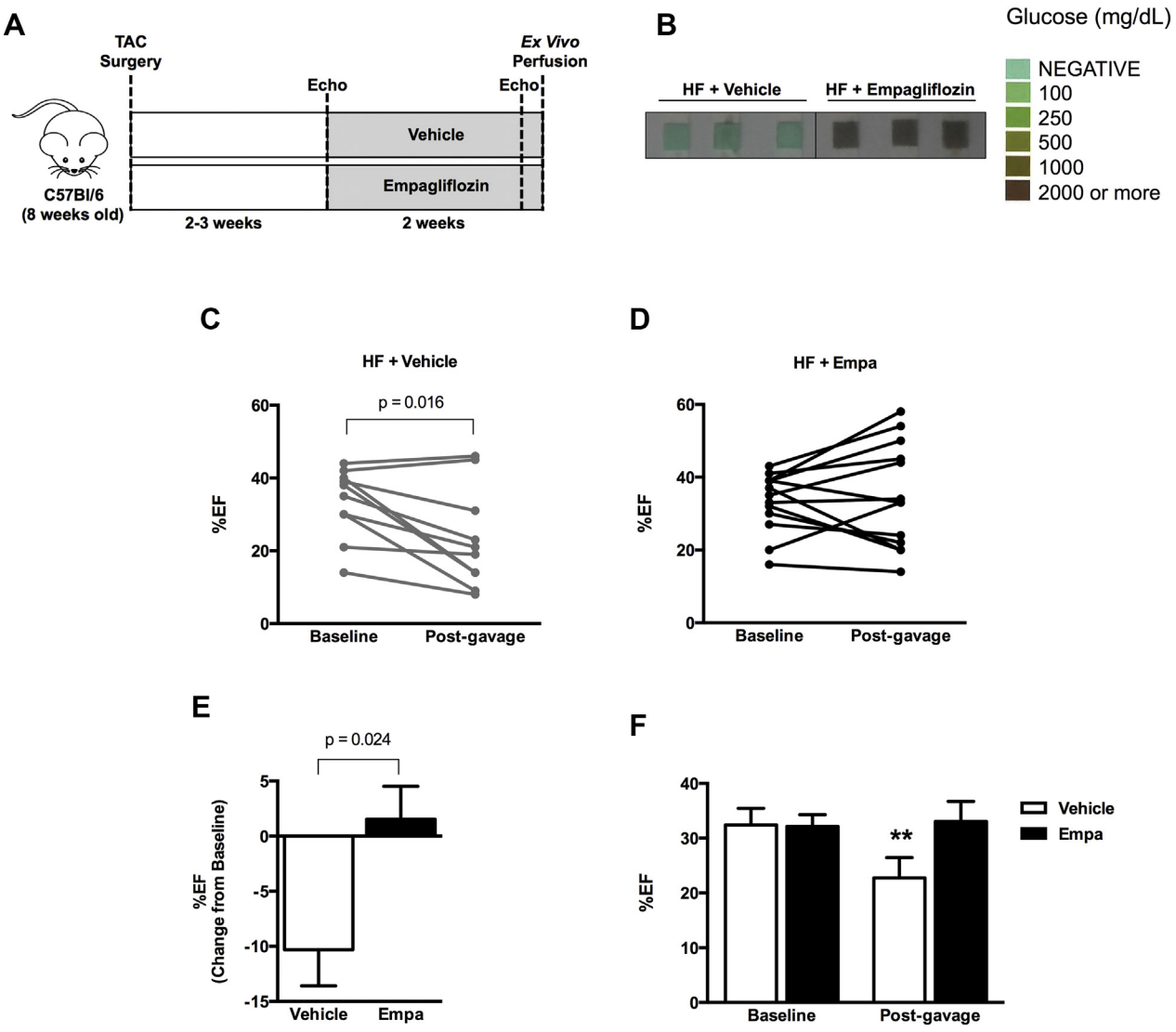
Despite the profound and precocious efficacy, the physiological and biomolecular mechanisms responsible for this ~40% reduction in cardiovascular mortality and heart failure are not known. Interestingly, intrinsic changes to the cardiac tissue itself have largely been ruled out because SGLT2 receptors are not known to be present in the heart (2). Based in part on this fact, the prevailing theories explaining how empagliflozin may exert its beneficial effects in heart failure involve either improved hemodynamics through osmotic diuresis and natriuresis (3) or by promoting enhanced ketone oxidation by the heart through increased ketone concentrations in the blood (3). However, these theories have not been fully investigated. In addition, because empagliflozin is used to treat diabetes, it is not known whether the drug is capable of eliciting equally beneficial effects on heart failure outcomes in nondiabetic patients.

METHODS

EXPERIMENTAL ANIMALS. All protocols involving mice were approved by the University of Alberta Institutional Animal Care and Use Committee and conform to the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (8th edition, revised 2011). The University of Alberta adheres to the principles for biomedical research involving animals developed by the Council for International Organizations of Medical Sciences and complies with the Canadian Council on Animal Care guidelines. Eight-week old male C57Bl/6 mice underwent transverse aortic constriction (TAC) surgery to induce pressure overload. At 2 to 3 weeks post-surgery, most of the mice transitioned into reduced ejection fraction (EF) heart failure. Mice considered to be in heart failure (%EF <45%) were randomly assigned to receive either vehicle (0.5% hydroxyethyl cellulose [Natrosol]) (Sigma-Aldrich, Ontario, Canada) or empagliflozin (MedChemExpress, Princeton, New Jersey) (10 mg/kg/day) for 2 weeks by oral gavage.

TAC SURGERY. Transverse aortic constriction surgery was performed as previously described (4,5). Briefly, male 8-week-old mice were anesthetized by intraperitoneal injection of a cocktail of ketamine (100 mg/kg) and xylazine (10 mg/kg), intubated, and

FIGURE 1 Empagliflozin Treatment Prevents Worsening of Cardiac Function in Mice With Heart Failure

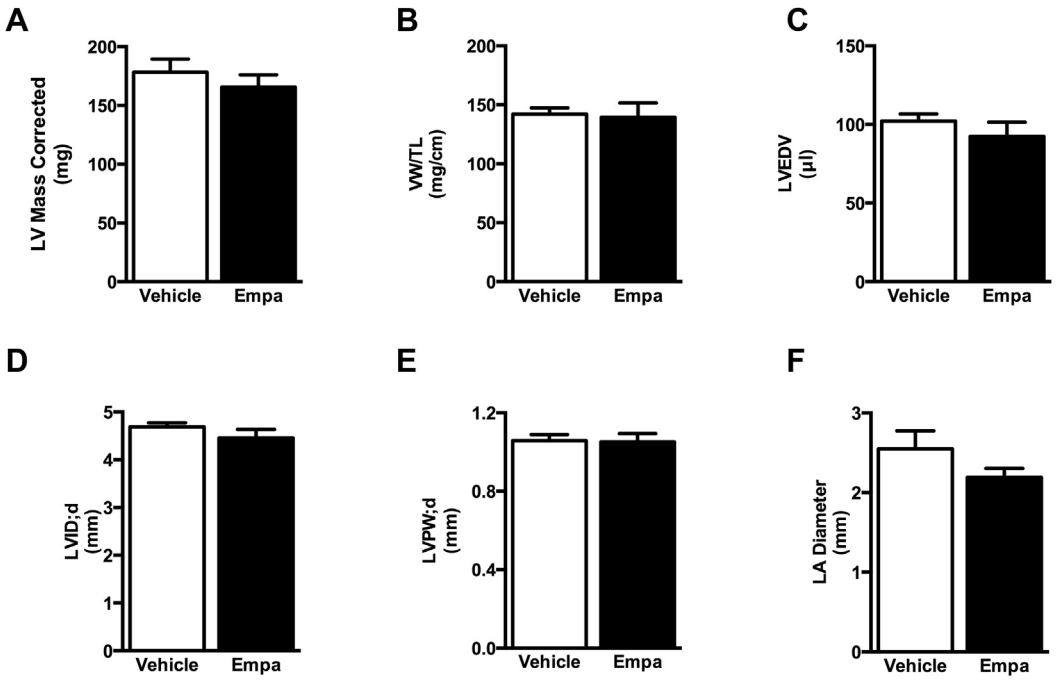


Experimental design of Empa treatment in mice in which HF was induced using TAC. **(A)** Urine glucose levels are shown for vehicle-treated and Empa-treated HF mice **(B)** (n = 3). Ejection fraction (%EF) of vehicle-treated **(C)** and Empa-treated **(D)** mice with HF and expressed as change from baseline **(E)** and pre and post gavage **(F)** based on echocardiographic assessment (n = 10 to 13). Results are mean ± SEM. Wilcoxon signed rank test was used to evaluate pre- versus post-gavage data **(C, D)**. Comparisons between groups were performed using Mann-Whitney *U* test **(E)** and repeated measures 2-way ANOVA followed by Sidak multiple comparisons tests **(F)**. **p < 0.01 versus baseline. Empa = empagliflozin; HF = heart failure; TAC = transverse aortic constriction.

connected to a mouse ventilator (MiniVent; Harvard Apparatus, Holliston, Massachusetts). Following midline sternotomy, a double-blunted 27-gauge needle was tied encircling the aorta between the innominate and left common carotid arteries using a 6/0 silk suture. The needle was then removed, and chest and skin were sutured and closed.

ECHOCARDIOGRAPHY. Mice were anesthetized with 1.0% to 1.5% isoflurane with 1 to 1.5 l/min 100% oxygen, and in vivo cardiac function was assessed by transthoracic echocardiography using a Vevo 3100 high-resolution imaging system equipped with a 30-MHz transducer (model RMV-707B, VisualSonics, Toronto, Ontario, Canada), as previously described (4,5). Pressure overload was confirmed in all mice at 2 weeks after TAC by measuring trans-stenotic gradient by pulsed-wave Doppler flow. Full systolic and diastolic parameters were measured prior to and

FIGURE 2 Cardiac Structural Remodeling Showed No Differences in Mice With HF Treated With Empagliflozin



Hearts from vehicle- and Empa-treated mice with HF displayed no significant changes in corrected LV mass **(A)**, wet VW-to-TL ratio **(B)**, LV volume **(C)**, LVID during diastole **(D)**, LV posterior wall thickness during diastole **(E)**, and LA diameter **(F)** (n = 6 to 10). Results are mean ± SEM. Comparisons between groups were performed using Mann-Whitney *U* test. LA = left atrial; LV = left ventricular; LVID;d = LV internal diameter during diastole; LVPW = left ventricular posterior wall thickness during diastole; VW/TL = ventricle weight-to-tibia length ratio; other abbreviations as in Figure 1.

following 2-week oral gavage of vehicle or empagliflozin.

HISTOLOGY. Masson's trichrome and hematoxylin-eosin stains of paraffin-embedded left ventricular heart sections taken mid-papillary were visualized using microscopy (DMLA microscope, Leica Microsystems, Wetzlar, Germany; equipped with a Retiga 1300i FAST 1394 charge-coupled device camera, OImaging, Surrey, British Columbia, Canada), as described previously (6). Three representative images were taken of each sample.

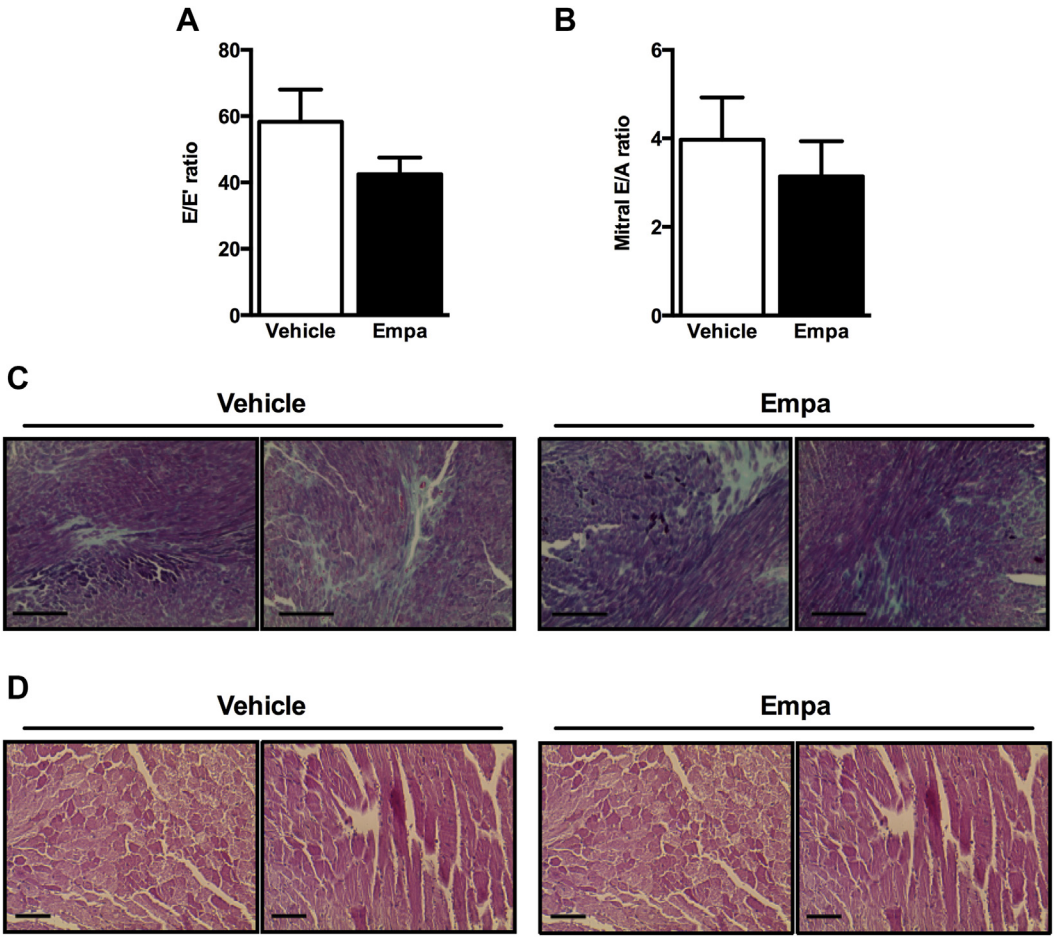
EX VIVO HEART PERFUSIONS. Hearts were perfused in the working heart mode at 11.5 mm Hg preload and 50 mm Hg afterload with Krebs-Henseleit buffer containing 0.8 mmol/l oleate prebound to 3% delipidated bovine serum albumin, 5 mmol/l glucose, and 50 μU/ml insulin, as described previously (4,7).

STATISTICS. Results are expressed as mean ± SEM. Statistical analyses were performed using Prism software (GraphPad Corp., La Jolla, California). Comparisons between groups were performed by Wilcoxon signed-rank test, Mann-Whitney *U* test, or repeated measures 2-way ANOVA, followed by Sidak multiple comparisons test where appropriate. Wilcoxon signed rank test was used to evaluate pre- versus post-gavage data (Figure 1). A p value of <0.05 was considered significant.

RESULTS

In order to help address the effects of empagliflozin in heart failure in the absence of diabetes, we subjected healthy C57Bl/6 mice to TAC surgery to induce heart failure as described previously (7). Following TAC surgery, mice were subjected to pulsed wave Doppler M-mode echocardiography. Mice demonstrating an EF of <45% were subsequently randomized and treated with either vehicle (0.5% hydroxyethyl cellulose) or empagliflozin (10 mg/kg) by daily oral gavage for a period of 2 weeks (Figure 1A). As expected, empagliflozin significantly increased glucose concentrations in the urine during the treatment period (Figure 1B). In addition, whereas vehicle-treated mice with heart failure continued to display a significant drop in %EF over the 2-week treatment

FIGURE 3 Diastolic Function, Cardiac Fibrosis, and Macrophage Infiltration Showed No Differences in Mice With Heart Failure Treated With Empagliflozin

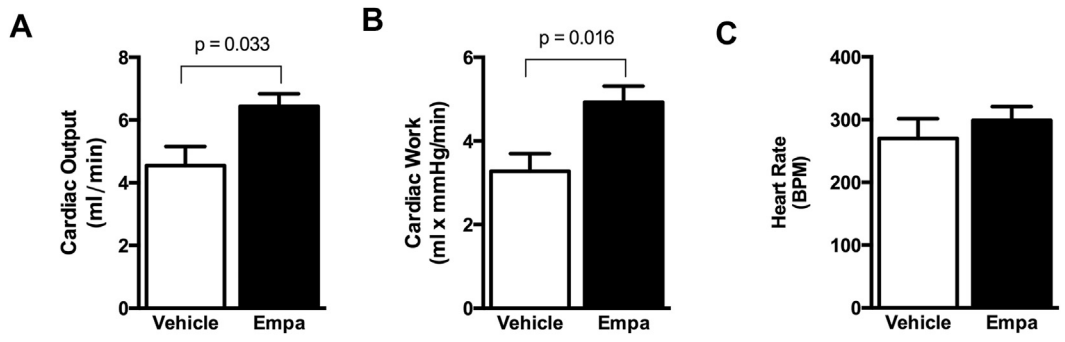


Hearts from vehicle- and Empa-treated mice with HF displayed no significant changes in indices of diastolic function such as the left ventricular filling ratio E/E′ **(A)** and mitral E/A ratio **(B)** (n = 6 to 10). Representative images of left ventricular heart sections taken mid-papillary and stained with Masson's trichrome at ×20 magnification **(C)** and hematoxylin-eosin stain (**[D]** n = 5 to 7). **Scale bars** = 100 μm. Results are mean ± SEM. Comparisons between groups were performed using Mann-Whitney *U* test. Abbreviations as in Figure 1.

period (Figures 1C, 1E, and 1F), empagliflozin-treated mice maintained stable cardiac function (Figures 1D to 1F). In order to account for dependent data, a repeated measures 2-way ANOVA was used to show that a statistically significant interaction existed between the effect of time and treatment on %EF ($p = 0.011$). There were no statistically significant differences in treatment ($p = 0.230$), but there were statistically significant differences between baseline and post-gavage times ($p = 0.033$). Interestingly, empagliflozin prevented the time-dependent decline in systolic function without significantly affecting body weight (23.79 ± 0.45 g vs. 24.41 ± 0.54 g for vehicle and empagliflozin-treated mice with heart failure, respectively), or without changing measurements of corrected left ventricular (LV) mass (Figure 2A) or wet ventricle weight-to-tibia length (VW/TL) ratio (Figure 2B). Furthermore, other indices of structural remodeling, such as LV end-diastolic volume (LVEDV) (Figure 2C), LV internal diameter during diastole (LVID;d) (Figure 2D), LV posterior wall thickness (LVPW) during diastole (Figure 2E), and left atrial (LA) diameter (Figure 2F), were also similar between groups.

In addition to development of cardiac hypertrophy, we also measured indices of diastolic function such as LV filling pressure (E/E′ ratio) (Figure 3A) and the mitral E/A ratio (Figure 3B), which were also not

FIGURE 4 Improved In Vivo Cardiac Function in Empagliflozin-Treated Mice With Heart Failure Is Preserved Ex Vivo



Hearts from vehicle- and Empa-treated mice with HF were perfused ex vivo to measure cardiac function. Cardiac output **(A)**, cardiac work **(B)**, and heart rate **(C)** were measured using ex vivo perfused working hearts (n = 9 to 10). Results are mean ± SEM. Abbreviations as in Figure 1.

significantly changed between groups. Furthermore, because excessive myocardial fibrosis is a major result of pressure overload-induced heart failure (8,9), we used Masson trichrome staining to investigate whether treatment with empagliflozin reduced cardiac collagen content. Consistent with our observation that empagliflozin did not reduce myocardial stiffness, there was no obvious reduction in cardiac fibrosis compared to vehicle-treated mice with heart failure (Figure 3C). Furthermore, we also investigated the presence of macrophage infiltration in hearts from vehicle- and empagliflozin-treated mice with heart failure to assess the effect of empagliflozin on the inflammatory response to pressure overload. Interestingly, there was no obvious presence of macrophage infiltration in either group (Figure 3D), suggesting that the inflammatory response to pressure overload had likely resolved prior to when the mice were euthanized and hearts used for histology.

To ascertain whether or not the protective effects observed in the empagliflozin-treated mice with heart failure were related to extrinsic factors that controlled cardiac function (such as hemodynamics or ketone oxidation), we subjected vehicle- and empagliflozin-treated mice to ex vivo functional assessment using an isolated perfused working heart system (7). In the presence of matching pre-load and after-load pressures, identical concentrations of insulin, fatty acids, and glucose, as well as in the absence of ketones (7), ex vivo perfused hearts still demonstrated significantly improved ex vivo cardiac output (Figure 4A) and cardiac work (Figure 4B), without any differences in heart rate (Figure 4C). This ability of empagliflozin to provide a sustained benefit in isolated hearts suggests that the empagliflozin-mediated prevention of worsening cardiac function in mice with heart failure may be due to an intrinsic and sustained cardiac effect and is not based on potential hemodynamic changes or a potentially confounding blood-based environmental milieu associated with heart failure and other factors related to empagliflozin treatment.

DISCUSSION

Together, our data show for the first time that empagliflozin treatment of nondiabetic mice with reduced EF heart failure blunts the progressive decline in cardiac function both in vivo and ex vivo. Interestingly, although SGLT1 mRNA is abundantly expressed in the human heart as well as in other tissues, SGLT2, the selective target of empagliflozin, has been identified in skeletal muscle and kidney but not in heart (10,11). Thus, our findings introduce a completely novel concept that empagliflozin can directly influence cardiac function despite no definitive evidence of molecular targets in cardiac tissue.

Based on the results of EMPA-REG OUTCOMES and other studies that have primarily shown a lower incidence of hospitalization for heart failure and death in patients with type 2 diabetes treated with empagliflozin (1) and other SGLT2 inhibitors (12,13), several theories have been put forward to explain the beneficial effects of SGLT2 inhibition. Theories include natriuresis/diuresis, improved myocardial energetics through increases in ketone oxidation (14,15), and more recently, through a direct effect that inhibits sodium-hydrogen exchange in cardiomyocytes (16). Interestingly, numerous studies propose that empagliflozin may reduce cardiometabolic risk in diabetic

patients by significantly reducing body weight and adiposity (17,18); however, no changes in body weight were found in our study. Although it has also been suggested that the glucose-lowering effects of empagliflozin may reduce the cardiac effects of glucotoxicity (19,20), our data suggest that empagliflozin has cardioprotective benefits even in the absence of elevated blood glucose. Furthermore, despite previous findings that empagliflozin mildly reduces cardiac hypertrophy, improves diastolic function, and reduces collagen deposition in female mice with diabetes and obesity (21), these improvements were not apparent in our study using a mouse model of heart failure without impaired glucose handling.

STUDY LIMITATIONS. Although it was not measured in the current study, the effect of empagliflozin on the abnormal ventricular electrophysiological profile exists in cardiac hypertrophy and failure (22–25) may offer insight into the mechanism of action and would therefore be an area to explore in future studies. Furthermore, although we observed increased cardiac function both in vivo and ex vivo, another limitation of our study is that we do not know whether empagliflozin improves cardiomyocyte contractility, which could provide additional insight into potential mechanism of action.

CONCLUSIONS

Although there are no data for biomarkers in patients with heart failure treated with SGLT2 inhibitors, preliminary data suggest that SGLT2 inhibition can reduce atrial natriuretic peptide and B-type natriuretic peptide in zebrafish models of heart failure (26) and improve measurements of diastolic function in humans with diabetes and clinical cardiovascular disease (27). Based on these results, clinical trials have been initiated to investigate the role of SGLT2 inhibitors in the treatment of patients with established heart failure where diabetes is not an inclusion criterion per se (i.e., EMPEROR-Reduced [Empagliflozin Outcome Trial in Patients With Chronic Heart Failure With Reduced Ejection Fraction]; NCT03057977; EMPEROR-Preserved [EMPagliflozin outcomE tRial in Patients With chrOnic heaRt Failure With Preserved Ejection Fraction]; NCT03057951, and Dapa-HF [Study to Evaluate the Effect of Dapagliflozin on the Incidence of Worsening Heart Failure or Cardiovascular Death in Patients With Chronic Heart Failure]; NCT03036124). However, the scientific community has been waiting for translational and mechanistic studies to elucidate if this strategy is associated with a change in LV mass, remodeling, and cardiac function. Although human cardiac magnetic resonance studies are also currently underway (EMPA-HEART [Effects of Empagliflozin on Cardiac Structure in Patients With Type 2 Diabetes]; NCT02998970), these studies also are being carried out in subjects with diabetes and previous myocardial infarction. Therefore, the novelty of our work underscores a potential application of this therapy in established heart failure without diabetes.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Despite various pharmacological and nonpharmacological advances, heart failure remains a growing cause of global morbidity and mortality. Inhibitors of SGLT2 are a class of oral antihyperglycemic agents that were found to surprisingly reduce rates of heart failure and CV mortality in 2 large outcome studies (EMPA-REG OUTCOMES and CANVAS [CANagliflozin cardioVascular Assessment Study]) by approximately one-third. Very little is known about how SGLT2 inhibitors affect the structure and function of the heart and particularly whether this effect is restricted to diabetes. Large-scale studies to evaluate SGLT2 inhibitors in cardiac failure are currently underway.

TRANSLATIONAL OUTLOOK: SGLT2 inhibitors may be effective agents with which to treat heart failure in patients with and without diabetes, and ongoing studies to examine this thesis are currently underway. Further research into the cellular and molecular mechanisms is warranted. Whether or not heart failure with preserved ejection fraction or reduced ejection fraction or both is responsive to this treatment also requires further research.

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KEY WORDS empagliflozin, heart failure, SGLT2, sodium/glucose cotransporter 2 inhibitor