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

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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.
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.

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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

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

**A** 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 = empagli

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Byrne et al. AUGUST 2017:347–54
Empagliflozin Prevents Worsening of Cardiac Function

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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
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 ($23.79 \pm 0.45$ g vs. $24.41 \pm 0.54$ 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 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 $\times 20$ 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.
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.

## REFERENCES

1. Zinnman B, Wanner C, Lachin JM, et al. Empagliflozin, cardiovascualr outcomes, and mortality in type 2 diabetes. N Engl J Med 2015;373:217–28.
2. von Lewinski D, Rainer PP, Gasser R, et al. Glucose-transporter-mediated positive inotropic effects in human myocardium of diabetic and nondiabetic patients. Metabolism 2010;59:1020–8.
3. Sattar N, McLaren J, Kristensen SI, Preiss D, McMurray JJ. SGLT2 Inhibition and cardiovascular events: why did EMPA-REG Outcomes surprise and what were the likely mechanisms? Diabetologia 2016;59:1333–9.
4. Byrne NJ, Levassier J, Sung MM, et al. Normalization of cardiac substrate utilization and
Empagliflozin Prevents Worsening of Cardiac Function

left ventricular hypertrophy precede functional recovery in heart failure regression. Cardiovasc Res 2016;110:249-57.

5. Sung MM, Byrne NJ, Robertson IM, et al. Resveratrol improves exercise performance and skeletal muscle oxidative capacity in heart failure. Am J Physiol Heart Circ Physiol 2017;312:H842-53.

6. Kienesberger PC, Pulivillunni T, Sung MM, et al. Myocardial ATGL overexpression decreases the reliance on fatty acid oxidation and protects against pressure overload-induced cardiac dysfunction. Mol Cell Biol 2012;32:740-50.

7. Sung MM, Das SK, Levasseur J, et al. Resveratrol treatment of mice with pressure-overload-induced heart failure improves diastolic function and cardiac energy metabolism. Circ Heart Fail 2015;8:128-37.

8. Segura AM, Frazier OH, Buja LM. Fibrosis and heart failure. Heart Fail Rev 2014;19:173-85.

9. Creemers EE, Pinto YM. Molecular mechanisms that control interstitial fibrosis in the pressure-overloaded heart. Cardiovasc Res 2011;89:265-72.

10. Wright EM, Loo DD, Hirayama BA. Biology of human sodium glucose transporters. Physiol Rev 2011;91:733-94.

11. Zhou L, Cryan EV, D’Andrea MR, Belkowski S, Conway BR, Demarest KT. Human cardiomyocytes express high level of Na+/glucose cotransporter 1 (SGLT1). J Cell Biochem 2003;90:339-46.

12. Kosiborod M, Cavender MA, Fu AZ, et al. Lower Risk of Heart Failure and Death in Patients Initiated on SGLT-2 Inhibitors Versus Other Glucose-Lowering Drugs: the CVD-REAL study. Circulation 2017;136:249-59.

13. Neal B, Perkovic V, Mahaffey KW, et al. Empagliflozin and cardiovascular and renal events in type 2 diabetes. N Engl J Med 2017 June 12 [E-pub ahead of print].

14. Lopaschuk GD, Verma S. Empagliflozin’s fuel hypothesis: not so soon. Cell Metab 2016;24:200-2.

15. Ferrannini E, Mark M, Mayoux E. CV protection in the EMPA-REG OUTCOME trial: a “thirsty substrate” hypothesis. Diabetes Care 2016;39:1108-14.

16. Baartscheer A, Schumacher CA, Wust RC, et al. Empagliflozin decreases myocardial cytoplasmic Na⁺ through inhibition of the cardiac Na⁺/H⁺ exchanger in rats and rabbits. Diabetologia 2017;60:568-73.

17. Yanai H, Hakoshima M, Adachi H, et al. Effects of six kinds of sodium-glucose cotransporter 2 inhibitors on metabolic parameters, and summarized effect and its correlations with baseline data. J Clin Med Res 2017;9:605-12.

18. Neeland IJ, McGuire DK, Chilton R, et al. Empagliflozin reduces body weight and indices of adipose distribution in patients with type 2 diabetes mellitus. Diab Vasc Dis Res 2016;13:119-26.

19. Ferrannini E, Muscelli E, Frascerra S, et al. Metabolic response to sodium-glucose cotransporter 2 inhibition in type 2 diabetic patients. J Clin Invest 2014;124:499-508.

20. Abdul-Ghani M, Al Jobori H, Daniele G, et al. Inhibition of renal sodium-glucose co-transport with empagliflozin lowers fasting plasma glucose and improves beta cell function in subjects with impaired fasting glucose. Diabetes 2017 June 13 [E-pub ahead of print].

21. Habibi J, Aroor AR, Sowers JR, et al. Sodium glucose cotransporter 2 (SGLT2) inhibition with empagliflozin improves cardiac diastolic function in a female rodent model of diabetes. Cardiovasc Diabetol 2017;16:9.

22. Boulaksil M, Noorman M, Engelen MA, et al. Longitudinal arrhythmogenic remodelling in a mouse model of longstanding pressure overload. Neth Heart J 2010;18:509-15.

23. Boulaksil M, Winckels SK, Engelen MA, et al. Heterogenous Connexin43 distribution in heart failure is associated with dispersed conduction and enhanced susceptibility to ventricular arrhythmias. Eur J Heart Fail 2010;12:931-21.

24. Anastasiou-Nana MI, Nanas JN, Karagounis LA, et al. Relation of dispersion of QRS and QT in patients with advanced congestive heart failure to cardiac and sudden death mortality. Am J Cardiol 2000;85:1212-7.

25. Iuliano S, Fisher SG, Karasik PE, Fletcher RD, Singh SN. Department of Veterans Affairs Survival Trial of Antiarrhythmic Therapy in Congestive Heart F. QRS duration and mortality in patients with congestive heart failure. Am Heart J 2002;143:1085-91.

26. Shi X, Verma S, Yun J, et al. Effect of empagliflozin on cardiac biomarkers in a zebrafish model of heart failure: clues to the EMPA-REG OUTCOME trial? Mol Cell Biochem 2017 April 8 [E-pub ahead of print].

27. Verma S, Garg A, Yan AT, et al. Effect of empagliflozin on left ventricular mass and diastolic function in individuals with diabetes: an important clue to the EMPA-REG OUTCOME trial? Diabetes Care 2016;39:e212-3.

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