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Empagliflozin Ameliorates Adverse Left Ventricular Remodeling in Nondiabetic Heart Failure by Enhancing Myocardial Energetics

Carlos G. Santos-Gallego, MD,[a] Juan Antonio Requena-Ibanez, MD,[a,b] Rodolfo San Antonio, MD,[a,c] Kiyotake Ishikawa, MD,[d] Shin Watanabe, MD,[d] Belen Picatoste, PhD,[e] Eduardo Flores, MD,[a,c] Alvaro Garcia-Ropero, MD,[a] Javier Sanz, MD,[d] Roger J. Hajjar, MD,[d] Valentin Fuster, MD, PhD,[d] Juan J. Badimon, PhD[a]

ABSTRACT

BACKGROUND Empagliflozin cardiac benefits in the EMPA-REG OUTCOME (Empagliflozin Cardiovascular Outcome Event Trial in Type 2 Diabetes Mellitus Patients) trial cannot be explained exclusively by its antihyperglycemic activity.

OBJECTIVES The hypothesis was that empagliflozin's cardiac benefits are mediated by switching myocardial fuel metabolism away from glucose toward ketone bodies (KB), which improves myocardial energy production.

METHODS Heart failure was induced in nondiabetic pigs (n = 14) by 2-h balloon occlusion of the proximal left anterior descending artery. Animals were randomized to empagliflozin or placebo for 2 months. Animals were evaluated with cardiac magnetic resonance imaging and 3-dimensional echocardiography. Myocardial metabolite consumption was analyzed by simultaneous blood sampling from coronary artery and coronary sinus. Myocardial samples were obtained for molecular evaluation. Nonmyocardial infarction animals served as comparison.

RESULTS Despite similar initial ischemic myocardial injury in both groups, the empagliflozin group showed amelioration of adverse remodeling at 2 months (lower left ventricular [LV] mass, reduced LV dilatation, less LV sphericity) versus the control group. LV systolic function (LV ejection fraction and echocardiography-derived strains) was improved, as was neurohormonal activation. Compared with nonmyocardial infarction, control animals increased myocardial glucose consumption mainly through anaerobic glycolysis while reducing utilization of free fatty acid (FFA) and branched-chain amino acid (BCAA). Empagliflozin-treated pigs did not consume glucose (reduction in myocardial glucose uptake, and glucose-related enzymes) but instead switched toward utilization of KB, FFA, and BCAA (increased myocardial uptake of these 3 metabolites, and enhanced expression/activity of the enzymes implicated in the metabolism of KB/FFA/BCAA). Empagliflozin increased myocardial ATP content and enhanced myocardial work efficiency.

CONCLUSIONS Empagliflozin ameliorates adverse cardiac remodeling and heart failure in a nondiabetic porcine model. Empagliflozin switches myocardial fuel utilization away from glucose toward KB, FFA, and BCAA, thereby improving myocardial energetics, enhancing LV systolic function, and ameliorating adverse LV remodeling.
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The sodium glucose cotransporter type 2 (SGLT2) inhibitor empagliflozin has demonstrated to reduce cardiovascular mortality by 38% and heart failure (HF) hospitalizations by 35% in patients with type 2 diabetes mellitus (T2DM) in the EMPA-REG OUTCOMES (Empagliflozin Cardiovascular Outcome Event Trial in Type 2 Diabetes Mellitus Patients) clinical trial (1). Of note, the

From the [a]AtheroThrombosis Research Unit, Cardiovascular Institute, Icahn School of Medicine at Mount Sinai, New York, New York; [b]Cardiology Department, University Hospital of Ciudad Real, Ciudad Real, Spain; [c]Cardiology Department, Hospital Clinic de Barcelona, Barcelona, Spain; [d]Cardiovascular Institute, Icahn School of Medicine at Mount Sinai, New York, New York; and the [e]Biochemistry Department, Weill Cornell Medical College, New York, New York. This research was supported by an independent grant from Boehringer Ingelheim Pharmaceuticals, which provided both drug and financial support for the study. The authors have reported that they have no relationships relevant to the contents of this paper to disclose. Nikolaus Marx, MD, served as Guest Associate Editor for this paper.

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ABBREVIATIONS AND ACRONYMS

ATP = adenosine triphosphate

BCAA = branched-chain amino acid

BDH1 = β-hydroxybutyrate dehydrogenase

CMR = cardiac magnetic resonance

FFA = free fatty acid

KB = ketone bodies

LDH = lactate dehydrogenase

PDH = pyruvate dehydrogenase

SCOT = succinyl-CoA,3-oxoacid CoA transferase

SGLT2 = sodium glucose cotransporter type 2

T2DM = type 2 diabetes mellitus

incidence of myocardial infarction (MI) or stroke was not different between both groups. However, the mechanism(s) for these cardiac benefits are not well understood yet.

The glucose- and/or blood pressure-lowering effects of empagliflozin seem unlikely to explain these benefits, because differences in glycemic control were (by design) minimal and diabetic/blood pressure improvements should translate into reduced incidence of MI/stroke, which did not change in the trial (1); moreover, it takes years to show the benefits of controlling glycemia/blood pressure (2), while the curves in EMPA-REG OUTCOMES trial separated in the first months (1).

Myocardial metabolic remodeling is integral to HF development (3), with a shift from free fatty acids (FFA) utilization (which produce many ATP (adenosine triphosphate) molecules but also requires many oxygen molecules) in healthy myocardium toward glucose consumption (which produce less ATP but is more oxygen-efficient [4,5]) in failing hearts. Despite past research focus exclusively on FFA and glucose, the heart possesses metabolic flexibility and is an omnivore capable of oxidizing other substrates such as ketone bodies (KB), lactate, and branched-chain amino acid (BCAA). Importantly, KB are the most energetically efficient fuel because they produce many ATP molecules while showing the lowest oxygen requirements (4,5). Of note, empagliflozin-induced glycosuria reduces both plasma glucose and insulin levels and increases both lipolysis and plasma glucagon concentration (thus resembling fasting state), which causes ketogenesis and hyperketonemia (6,7).

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We hypothesized that the cardiac benefits of empagliflozin are due to improvement in myocardial energetics via switching myocardial fuel metabolism away from glucose to ketone bodies, which ameliorates adverse LV remodeling.

METHODS

STUDY DESIGN. The study design is presented in Online Figure 1. Proximal left anterior descending artery (LAD) was occluded with a percutaneous intracoronary balloon catheter for 2 h to induce MI as previously reported by our group (8). One day post-MI induction, all surviving animals underwent 3-dimensional (3D) echocardiography and cardiac magnetic resonance (CMR) to evaluate the extent of myocardial damage. Thereafter, animals were randomized to receive empagliflozin (10 mg daily orally, n = 7) or placebo (n = 7) for 2 months. At the end of the treatment period, 3D echocardiography and CMR procedures were repeated. Immediately after, cardiac catheterization was performed to assess myocardial metabolite consumption (simultaneous blood sampling from coronary arteries and coronary sinus). Animals were subsequently sacrificed, and tissue samples were collected for assessment of myocardial energetics and molecular markers of cardiac metabolism. A third group consisting of naïve pigs (non-MI, thus healthy myocardium, n = 6) was also investigated to evaluate metabolism of normal myocardium. All analyses were performed by investigators blinded to the treatment arm. Myocardial uptake was defined as differences in metabolite concentration between coronary artery and coronary sinus adjusted by blood flow and LV mass. Myocardial oxygen consumption and myocardial work efficiency were also calculated.

ONLINE APPENDIX. The protocols for MI induction, drug administration, CMR, echocardiography, metabolism evaluation, and Western blot are outlined in detail in the Online Appendix.

STATISTICAL ANALYSIS. Results are presented as mean ± SD. Values were compared between groups using the nonparametric Mann-Whitney *U* test when 2 groups were compared or Kruskal Wallis test when more than 2 groups were compared; when variances looked different (ratio >2), Welch's *t* test or analysis of variance was used instead, respectively. Post hoc analysis with the Dunn-Sidak procedure was performed when the overall analysis of a multiple group comparison was significant. Two-way repeated-measures analysis of variance was employed to compare values between 2 groups at different time points. All statistical calculations were performed using Prism 6.0. Differences were considered statistically significant if $p < 0.05$.

RESULTS

ANIMAL MODEL OF HF. Female Yorkshire pigs (n = 20; weight 20 ± 1 kg) underwent MI induction; 6 animals died from refractory ventricular arrhythmias during LAD occlusion. The remaining animals were randomized to empagliflozin or placebo (n = 7/group) on day 1 post-MI. No animal died after randomization during the 2-month follow-up period.

The animals in the treatment group showed marked glycosuria (**Table 1**), indicative of effective SGLT2 inhibition. Body weight, blood pressure,

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glycemia, and concentrations of uric acid were numerically lower while hemoglobin levels were numerically higher in the empagliflozin group but overall were not statistically significant from the control group (**Table 1**, other biochemical parameters are listed in Online Table 1).

There were no differences post-MI between both groups in MI size (41.9 ± 6.7%) for empagliflozin and 41.7 ± 2.8% for control; p = 0.8 or LV volumes or function (**Figures 1 and 2, Table 2**).

EMPAGLIFLOZIN AMELIORATES ANATOMICAL POST-MI LV REMODELING. Reduced LV end-systolic and end-diastolic volumes were noted both with CMR (**Figures 1A and 1B**) and 3D echocardiography (**Figures 1C and 1D**) in the treatment group, which proves mitigated LV dilatation in the treated pigs. LV mass was significantly lower in empagliflozin-treated animals, both using CMR and direct weight immediately after necropsy (**Figure 1E**). Furthermore, the heart of the empagliflozin-treated animals was less spherical than control animals, as demonstrated by lower 3D-LV sphericity index (**Figure 1F**), thus demonstrating decreased architectural changes in the treatment arm.

EMPAGLIFLOZIN IMPROVES LV SYSTOLIC FUNCTION POST-MI. Our CMR results show that the amelioration of LV remodeling in the empagliflozin group was associated with an increase in left ventricular ejection fraction (LVEF) 2 months post-treatment (**Figure 2A**). Contractile reserve, determined by dobutamine CMR, was also improved in the treated versus the control group (**Figure 2C**).

Quantification of LVEF by 3D echocardiography showed results comparable to those attained by CMR. There were no differences at baseline (either pre-MI or 1-day post-MI) but significantly better LVEF values were observed in the empagliflozin group 2 months post-MI (**Figure 2B**). Furthermore, LV mechanics, assessed by 3D speckle-tracking echocardiography, also demonstrated better preservation of longitudinal, radial, and circumferential strains in empagliflozin-treated animals versus controls two months post-MI (**Figures 2D to 2F**).

EMPAGLIFLOZIN AMELIORATES NEUROHORMONAL ACTIVATION AND CARDIAC INJURY. The improved anatomic changes in the empagliflozin group were paralleled by a mitigation of sympathetic overdrive as demonstrated by lower plasma levels of normetanephrine (catabolite of norepinephrine) (**Figure 3A**) in the treated group. The empagliflozin group additionally exhibited lower concentrations of B-type natriuretic peptide (BNP) (**Figure 3B**) (which confirms less cardiac stretch/dilatation) and ST2 (**Figure 3C**, which endorses less cardiac remodeling/fibrosis). Finally, there was also less cardiac injury in the treated groups as confirmed by lower levels of TnI (**Figure 3D**).

TABLE 1 Hemodynamic and Analytic Data of the Experiment Animals

Values at 2 Months	Empagliflozin Pigs	Control Pigs	p Value
Weight, kg	27.4 (26.6–29.5)	28.9 (26.8–34.3)	0.20
Systolic blood pressure, mm Hg	123 (118.8–132.5)	128 (116.5–129.7)	0.72
Diastolic blood pressure, mm Hg	79 (72.5–81.5)	83 (76–86.7)	0.95
Mean arterial pressure, mm Hg	97 (94–110)	102 (94.7–105.2)	0.66
Hemoglobin, g/dl	8.2 (8.1–9.1)	7.8 (7.2–8.6)	0.19
Hematocrit, %	24 (21.2–25.7)	23 (22.2–23.8)	0.22
Glycemia, mmol/l	3.89 (3.23–4.2)	4.1 (3.46–4.89)	0.33
Ketonemia, μmol/l	225.3 (120.7–260.8)	57.5 (51.7–81.3)	<0.001
Glycosuria, mmol/l	50.7 (41.4–71.4)	0.3 (0.05–0.39)	<0.001
Uric acid, mg/dl	5.8 (5.3–6.1)	6.1 (5.6–6.7)	0.18

Values are median (interquartile range).

EMPAGLIFLOZIN SWITCHES MYOCARDIAL METABOLISM AWAY FROM GLUCOSE OXIDATION TOWARD KETONE AND FREE FATTY ACID METABOLISM. Healthy myocardium in naïve, non-MI pigs predominantly consumed FFA (**Figure 4A**, Online Table 2) with low utilization of glucose (**Figure 4B**); also, healthy myocardium consumed (instead of produced) lactate (**Figure 4C**), a sign of oxidative instead of anaerobic metabolism. Non-MI myocardium also consumed BCAA (**Figure 4D**) but low KB rates (**Figure 4E**).

At 2 months post-MI, the remodeled failing myocardium of control animals showed a metabolic switch characterized by marked reduction on FFA and enhanced glucose consumption compared with the non-MI group. Control animals showed enhanced myocardial glucose uptake (**Figure 4B**) that was mainly metabolized through anaerobic metabolism as confirmed by net myocardial lactate production (high lactate levels in the coronary sinus indicating net myocardial lactate production) (**Figure 4C**) and marked increase in both activity and expression of lactate dehydrogenase (LDH) (the key enzyme in glycolysis) (**Figures 5A and 5B**). Glucose oxidation is also somewhat raised as the activity of pyruvate dehydrogenase (PDH) (the rate-limiting enzyme in glucose oxidation) is slightly increased (**Figure 5C**). The reduction in FFA consumption was confirmed by a decrease in myocardial FFA uptake (**Figure 4A**) and the down-regulated expression of both CD36 (which regulates cardiomyocyte FFA uptake) (**Figure 5K**) and carnitine palmitoyltransferase I (the rate-limiting enzyme in FFA metabolism, which transports acylcarnitine—the intermediate metabolite of FFA—into the mitochondria) (**Figure 5J**). Reduced cardiac BCAA consumption in control subjects was demonstrated

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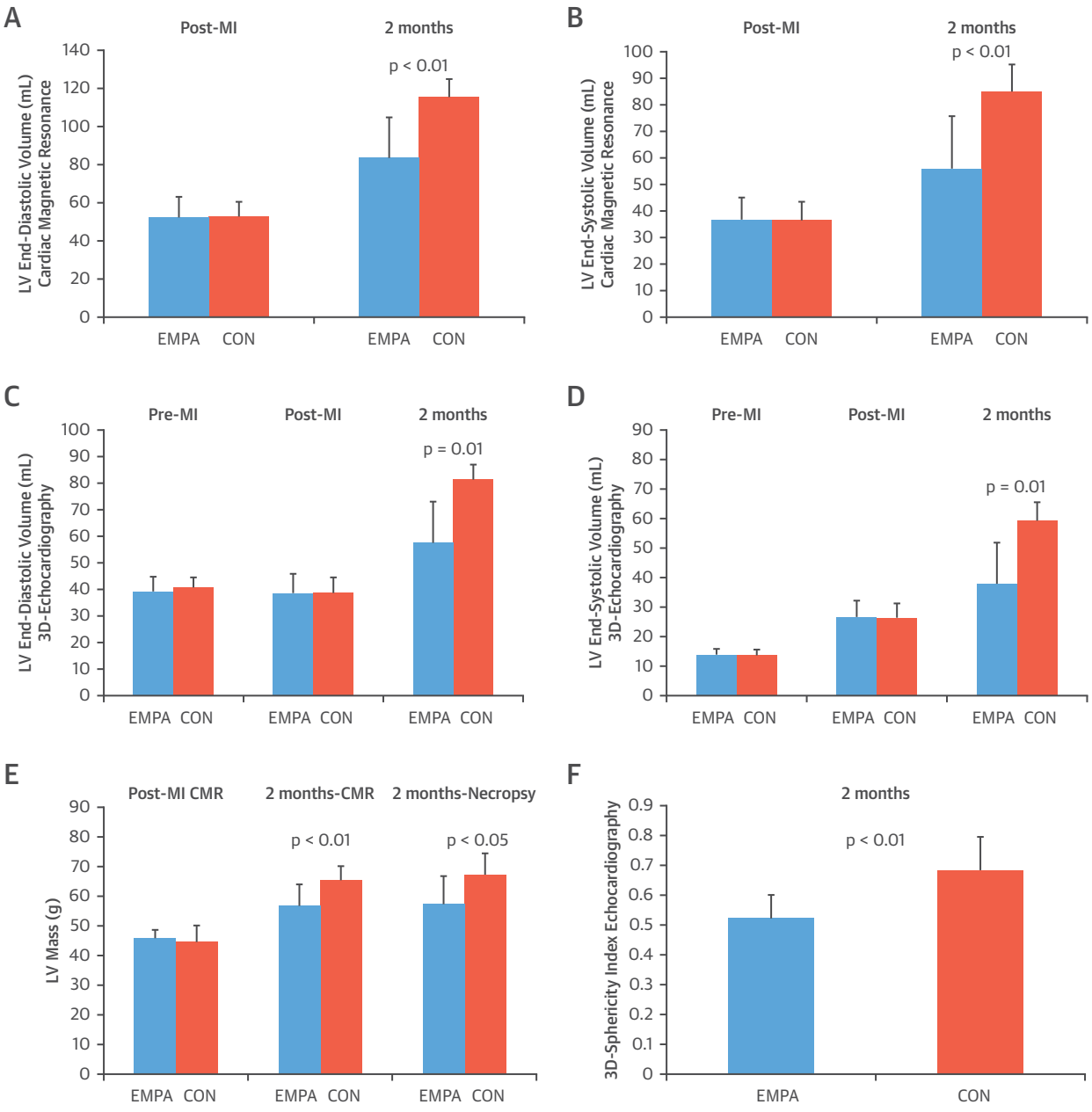
FIGURE 1 Empagliflozin Ameliorates Anatomical LV Remodeling



Despite similar LV volumes 1-day post-MI, empagliflozin-treated (EMPA) pigs exhibited smaller LVEDV **(A)** and smaller LVESV **(B)** by CMR at 2 months post-MI. Three-dimensional (3D) echocardiography confirmed smaller LVEDV **(C)** and LVESV **(D)** in treated animals. Empagliflozin treatment also resulted in lower LV mass **(E,** both using CMR and necropsy) and reduced 3D-sphericity index **(F)**. CON = control; LVEDV = left ventricular end-diastolic volume. LVESV = left ventricular end-systolic volume; MI = myocardial infarction.

by lower myocardial BCAA uptake (Figure 4D), decreased activity of branched-chain α-keto acid dehydrogenase complex (BCKD) (the key enzyme in BCAA catabolism) (Figure 5H) and increased phosphorylation of the BCKD-E1α subunit (Figure 5I) (which is inversely proportional to BCKD activity [9]). Myocardial KB uptake was borderline increased in HF controls (Figure 4E), as already known.

Of the utmost importance, empagliflozin induced a myocardial metabolic switch away from glucose

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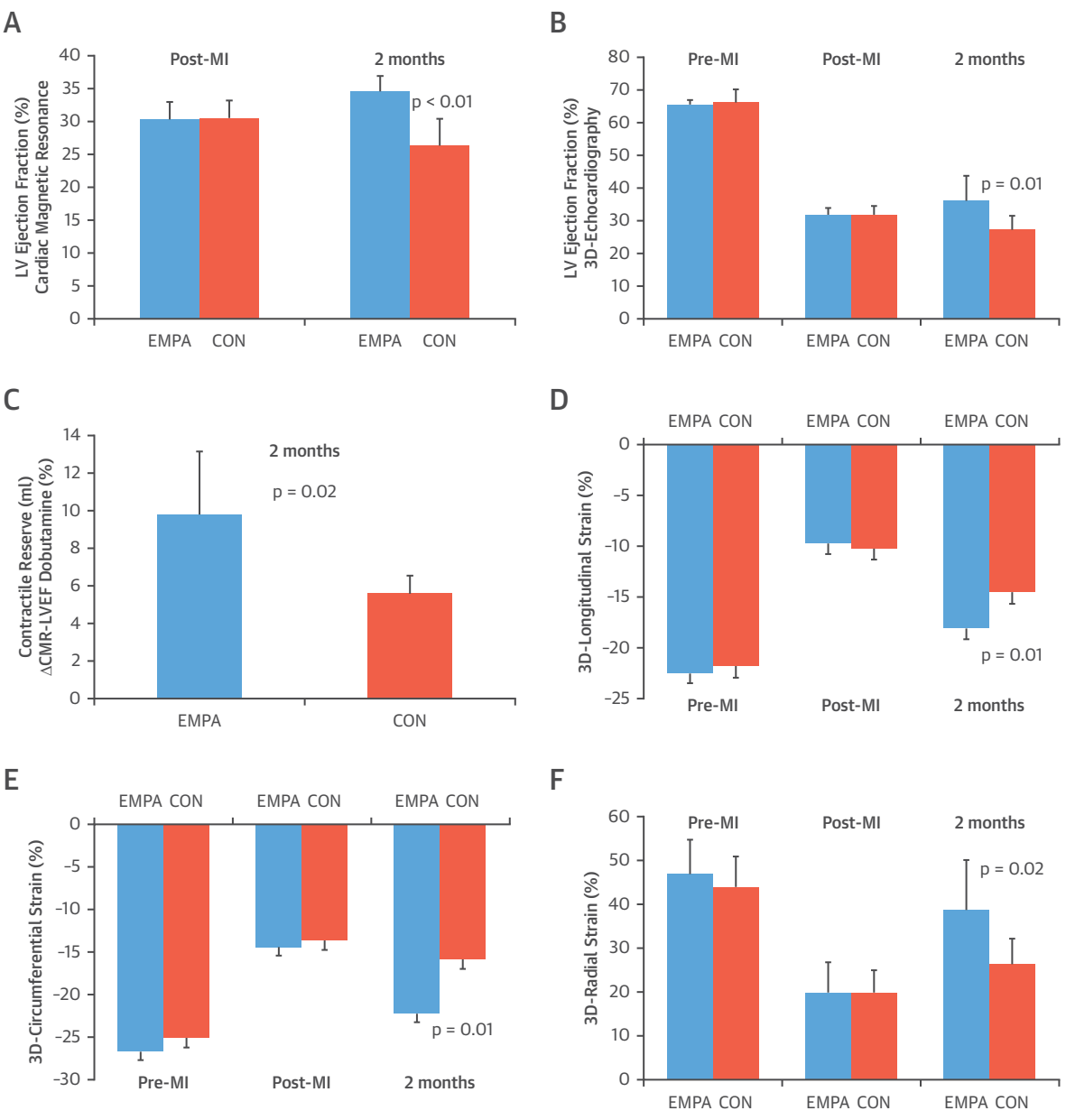
FIGURE 2 Empagliflozin Improves LV Systolic Function Post-MI



At the end of the 2 months of study duration, the empagliflozin group exhibited higher LVEF by both CMR **(A)** and 3D-echocardiography **(B)**, better contractile reserve by dobutamine CMR **(C)**, as well as more preserved 3D-longitudinal strain **(D)**, 3D-circumferential strain **(E)**, and 3D-radial strain **(F)**. CMR = cardiac magnetic resonance; LVEF = left ventricular ejection fraction; other abbreviations as in Figure 1.

into myocardial consumption of KB, FFA, and BCAA. Empagliflozin-treated pigs showed reduced myocardial glucose metabolism demonstrated by lower myocardial glucose uptake (Figure 4B), no net lactate production (Figure 4C) and reduced LDH levels and activity (Figures 5A and 5B) (hence demonstrating absence of glucose anaerobic metabolism) (Figure 5) versus control subjects. The myocardial reliance on KB metabolism in the empagliflozin group is clearly demonstrated by augmented myocardial KB uptake (8-fold increase vs. non-MI pigs and 4-fold increase vs. control animals) (Figure 4E); increased activity (Figure 5D) and expression (Figure 5E) of succinyl-CoA:3-oxoacid CoA-transferase (SCOT), the

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TABLE 2 Cardiac Magnetic Resonance and 3D Echocardiography Results

	Pre-MI			1 Day			2 Months		
	Control	Empagliflozin	p Value	Control	Empagliflozin	p Value	Control	Empagliflozin	p Value
CMR results									
LV mass, g	NA	NA	NA	42.8 (39.6 to 49.3)	44.5 (42.8 to 48.3)	0.76	65.2 (62.1 to 69.7)	56.5 (52.1 to 58.9)	<0.01
LVEDV, ml	NA	NA	NA	51.3 (48.8 to 55.8)	54.6 (43.2 to 60.4)	0.87	115.0 (108.5 to 121.7)	77.4 (57.2 to 61.7)	<0.001
LVESV, ml	NA	NA	NA	35.1 (33.6 to 38.3)	37.6 (29.4 to 42.4)	0.94	86.9 (76.6 to 90.4)	48.6 (36.9 to 73.8)	<0.001
LVEF, %	NA	NA	NA	31.1 (30.8 to 32.4)	31.1 (27.9 to 32.1)	0.76	25.3 (24.0 to 28.7)	37.2 (27.0 to 40.3)	0.01
3D echocardiography									
LVEDV, ml	41.8 (38.9 to 43.3)	39.1 (33.3 to 43.3)	0.44	36.9 (34.1 to 44.1)	40.8 (31.1 to 44.1)	0.93	78.9 (77.5 to 85.3)	48.5 (42.9 to 74.0)	<0.001
LVESV, ml	14.8 (12.0 to 15.1)	13.3 (11.5 to 15.6)	0.82	24.2 (22.9 to 31.2)	28.1 (20.9 to 31.5)	0.98	59.0 (54.4 to 62.5)	29.7 (25.3 to 51.1)	<0.001
LVEF, %	65.5 (63.6 to 69.7)	65.6 (64.8 to 66.6)	0.47	32.3 (31.1 to 33.8)	31.8 (31.0 to 33.2)	0.74	25.3 (25.0 to 29.6)	38.7 (28.4 to 42.1)	0.01
Peak GLS, %	−21.9 (−23.1 to −20.6)	−23.0 (−24.0 to −21.1)	0.58	−9.3 (−11.9 to −7.4)	−9.3 (−11.0 to −9.2)	0.72	−14.9 (−15.8 to −12.8)	−19.1 (−19.5 to −17.5)	0.01
Peak GCS, %	−25.1 (−26.5 to −23.9)	−27.9 (−29.5 to −24.1)	0.34	−13.7 (−15.3 to −10.1)	−15.7 (−17.0 to −13.2)	0.76	−16.0 (−16.7 to −14.9)	−22.9 (−27.1 to −18.2)	0.01
Peak GRS, %	43.6 (41.4 to 50.1)	42.7 (40.1 to 54.0)	0.52	20.3 (15.6 to 24.3)	18.5 (14.2 to 23.7)	0.94	24.6 (22.8 to 28.7)	34.6 (32.6 to 49.8)	0.02

Values are median (interquartile range).

CMR = cardiac magnetic resonance; GCS = global circumferential strain; GLS = global longitudinal strain; GRS = global radial strain; LV = left ventricular; LVEDV = left ventricular end-diastolic volume; LVEF = left ventricular ejection fraction; LVESV = left ventricular end-systolic volume.

rate-limiting enzymes in KB oxidation/metabolism; higher expression of β-hydroxybutyrate dehydrogenase (BDH1) (**Figure 5F**; for liver expression of SCOT and BDH1, see Online Figure 2), another key enzyme in KB metabolism; and higher ratio of serum-to-myocardial KB (an index of KB utilization in the heart [10]) (**Figure 5G**). Empagliflozin normalized BCAA uptake (**Figure 4D**), increased activity of BCKD (**Figure 5H**), and lowered phosphorylation of BCKD-E1α subunit (**Figure 5I**) (which is inverse to BCKD activity), thus confirming enhanced myocardial BCAA utilization. Finally, empagliflozin increased myocardial FFA uptake without normalizing it (**Figure 4A**) and the expression of carnitine palmitoyltransferase I (**Figure 5J**) with also a trend toward higher CD36 expression (**Figure 5K**), which suggests boosted FFA myocardial utilization.

Of note, the enhanced myocardial energetics induced by empagliflozin treatment is supported by higher myocardial ATP content in the treated animals (**Figure 6A**) and increased activation of AMPK (adenosine 5′ monophosphate-activated protein kinase) and PGC-1α (the key enzymes in cellular energy metabolism) in the treated compared with the placebo animals (**Figures 6B to 6D**). Finally, myocardial work efficiency is increased in the empagliflozin-treated pigs versus controls (**Figure 4F**).

DISCUSSION

This study examined the mechanism of action of the cardiac benefits induced by empagliflozin (**Central Illustration**). The first important finding of our study is that empagliflozin administration in our *nondiabetic* porcine model of HF significantly ameliorated adverse anatomical LV remodeling, enhanced LV systolic function, and decreased neurohormonal activation. Therefore, it seems reasonable to conclude that the benefits of empagliflozin are, at least in part, mediated via a mechanism independent of its glucose-lowering activity. The second important finding is that these cardiac benefits of empagliflozin are mediated by a switch in myocardial fuel metabolism away from the low-yield energy-producing glucose metabolism toward KB, FFA, and BCAA, which improves myocardial energy production. This enhanced myocardial energetics ameliorates maladaptive LV remodeling and boosts LV systolic function.

The cardiac benefits (reduced death and HF hospitalization) in the EMPA-REG OUTCOME trial (1) are robust, but the responsible mechanism(s) remain elusive. Improved glycemic control also seems unlikely given that differences in glycemic control were (by design) minimal, it would also have reduced MI/strokes, the benefits would have taken years, and

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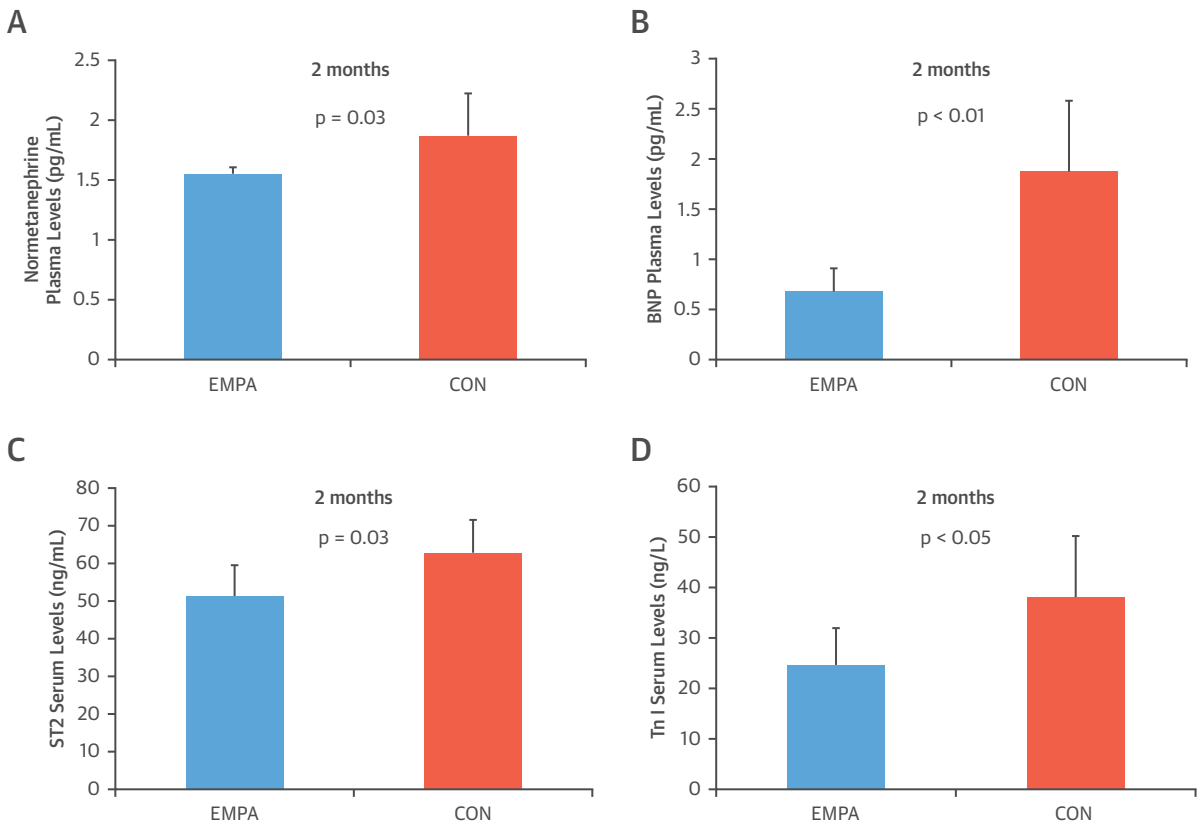
FIGURE 3 Empagliflozin Ameliorates Neurohormonal Activation



Empagliflozin-treated animals at 2 months post-MI showed lower levels of normetanephrine (**A**, which demonstrates less sympathetic overdrive), B-type natriuretic peptide (BNP) (**B**, which confirms less cardiac dilatation/stretch), ST2 (**C**, which endorses less cardiac remodeling/fibrosis), and troponin I (**D**, which corroborates less cardiac injury).

tight glycemic control has previously failed to reduce either mortality or HF (11). Empagliflozin hypotensive effect seems unlikely because blood pressure-lowering would also reduce strokes (which remained similar in both groups) and requires years for the curves to separate (while the event curves in EMPA-REG actually separate in 2 months). Empagliflozin's diuretic effect also seems unlikely because greater decreases in intravascular volume and net sodium balance are obtained by loop diuretics or thiazides, but these diuretic drugs do not reduce cardiovascular death, and their effects of HF hospitalizations are much more modest (12). An inhibition in the Na/H exchanger has been demonstrated (13), but it is unclear how this in vitro mechanism translates in an in vivo situation in patients; moreover, the Na/H exchanger inhibitor cariporide previously failed to show benefits in human patients (14). The empagliflozin-associated increase in hematocrit (either due to diuresis-mediated hemoconcentration or secondary to an increase in erythropoietin) has been postulated to improve tisular oxygenation (4), but the fact that hematocrit in our study was similar among the groups rules this hypothesis out. Therefore, the mechanism of the cardiac benefits of EMPA remains obscure. Most importantly, as there were no statistically significant differences in blood pressure, glycemia, or hematocrit in our animals, the differences in cardiac remodeling reported in this study are unlikely to be completely explained by the former hypotheses.

EMPAGLIFLOZIN AMELIORATES CARDIAC REMODELING AND HF. Adverse anatomical remodeling occurs at several levels, including anatomical, metabolic, and neurohormonal. Moreover, remodeling is an important determinant of patient morbidity and long-term outcomes (15,16). Our data are the first to demonstrate that empagliflozin treatment ameliorates

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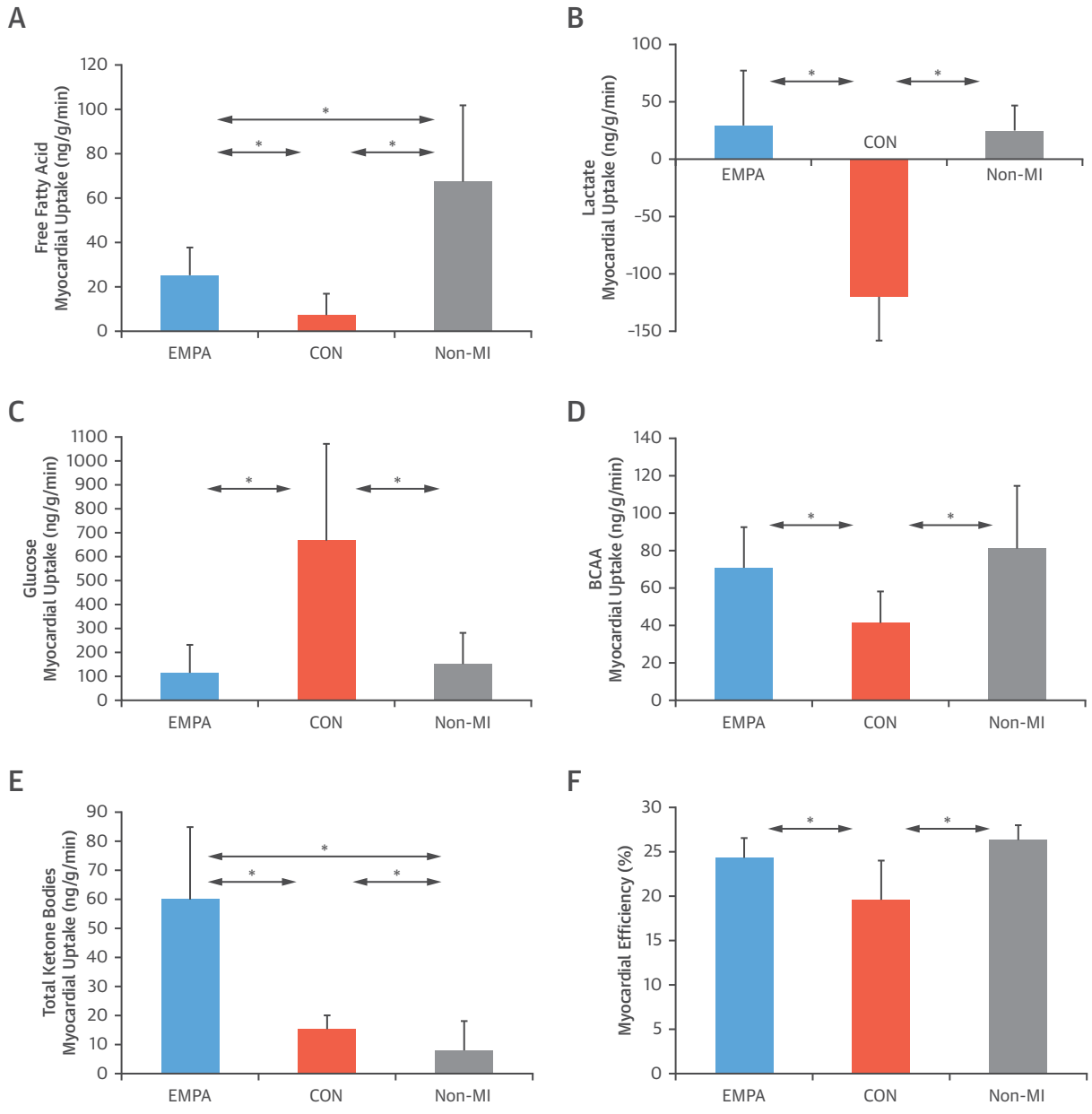
FIGURE 4 Empagliflozin Modifies Myocardial Metabolite/Fuel Uptake



HF control animals showed reduced myocardial FFA uptake versus non-MI animals, while empagliflozin increases myocardial fatty acid uptake **(A)**. Myocardial glucose uptake is augmented in control animals versus non-MI pigs, and this boosted glucose uptake is abolished by empagliflozin **(B)**. Control animals exhibit net myocardial lactate production (a sign of anaerobic metabolism) as compared with cardiac lactate consumption in the non-MI group; empagliflozin normalizes myocardial lactate consumption **(C)**. Myocardial uptake of BCAA is decreased in control animals compared with non-MI pigs, while empagliflozin increases cardiac BCAA uptake **(D)**. Empagliflozin treatment increases myocardial KB uptake compared with both non-MI and control groups **(E)**. Myocardial efficiency is impaired in control pigs versus non-MI animals, but is improved in the empagliflozin group **(F)**. *$p < 0.05$. BCAA = branched-chain amino acid; FFA = free fatty acid; other abbreviations as in Figure 1.

adverse cardiac remodeling at each of those levels and improves HF.

Anatomical remodeling is characterized by LV dilatation, hypertrophy, and geometrical remodeling (the heart becomes more spherical). In our study, the hearts of empagliflozin-treated pigs dilated less than those of control animals. Specifically, both LV end-diastolic and end-systolic volumes, strong predictors of

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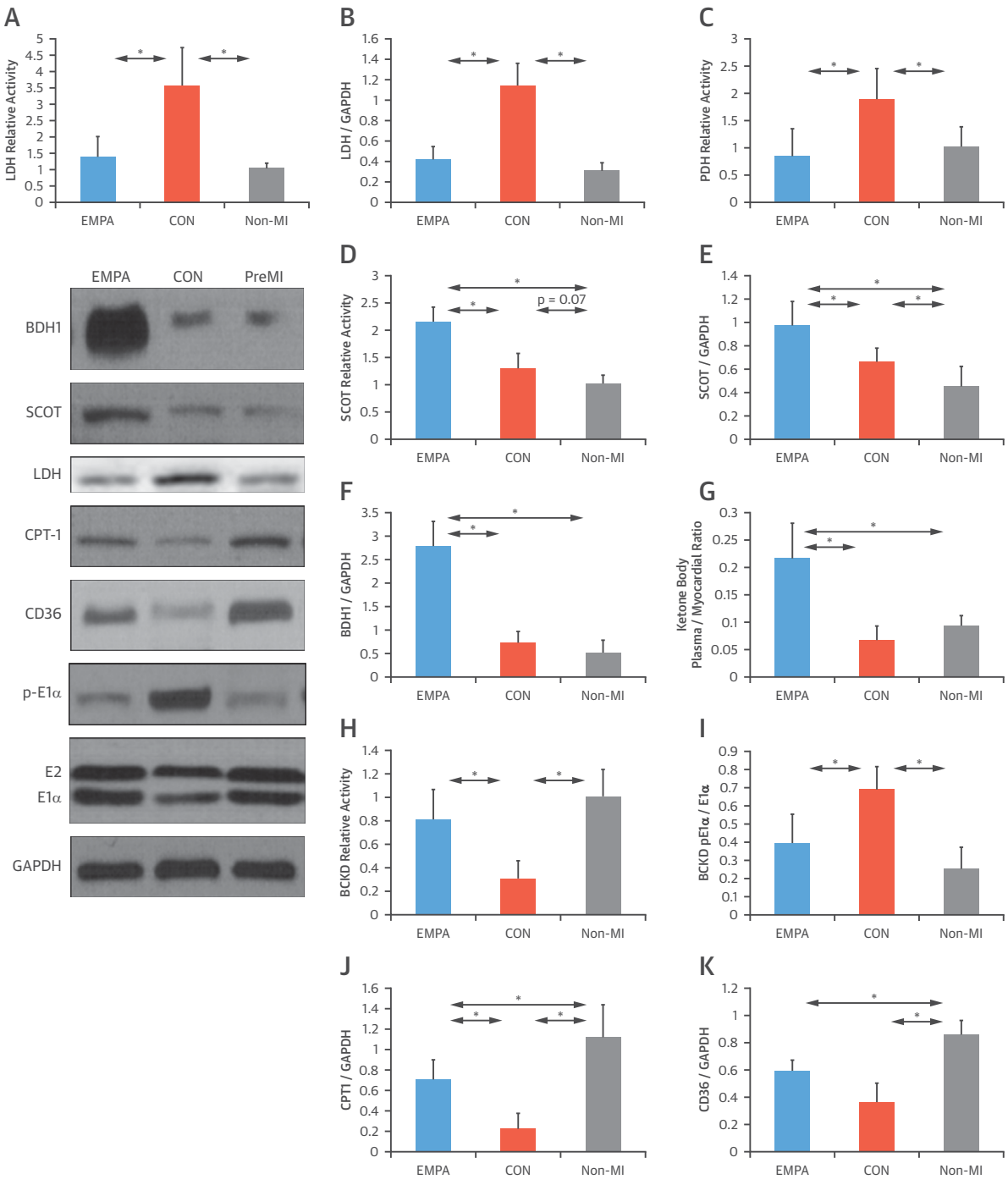
FIGURE 5 **Empagliflozin Shifts Myocardial Metabolism Away From Glucose Toward KB, FFA and BCAA Utilization**



Direct evidence in the myocardium. Control animals showed increased activity **(A)** and expression **(B)** of LDH and raised activity of PDH **(C)**, suggestive of increased glucose consumption that was normalized by empagliflozin. Empagliflozin-treated animals exhibit a higher cardiac KB utilization, evidenced by increased activity **(D)** and expression **(E)** of SCOT (the rate-limiting enzyme in KB metabolism), augmented expression of BDH1 **(F)** than control animals, and higher values of KB plasma/myocardium ratio **(G)**. Control animals exhibited lower activity of BCKD **(H)** and increased phosphorylation of BCKD E1α **(I)** that normalized by empagliflozin. Control animals show markedly reduced expression of CPT1 **(J)** and CD36 **(K)** versus non-MI that is increased with empagliflozin. *$p < 0.05$. BCKD = branched-chain α-keto acid dehydrogenase complex; BDH1 = β-hydroxybutyrate dehydrogenase; CPT1 = carnitine palmitoyltransferase 1; KB = ketone bodies; LDH = lactate dehydrogenase; PDH = pyruvate dehydrogenase; SCOT = succinyl-CoA:3-oxoacid CoA transferase.

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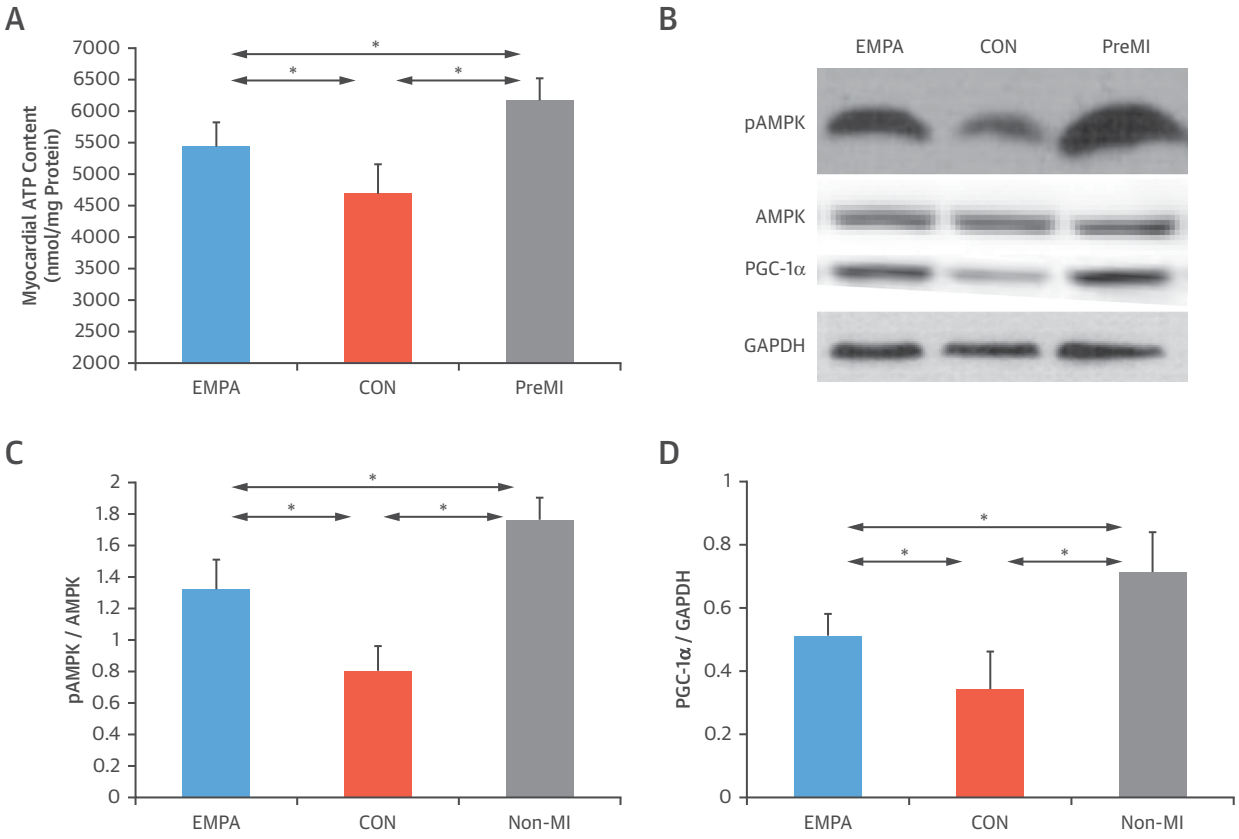
FIGURE 6 Empagliflozin Ameliorates Myocardial Energetic Metabolism



Empagliflozin-treated animals exhibited higher myocardial ATP (adenosine triphosphate) content **(A)**. The empagliflozin group also showed increased activation of AMPK (adenosine 5′ monophosphate-activated protein kinase) and PGC-1α (peroxisome proliferator-activated receptor gamma coactivator 1-alpha), two master regulators in cardiomyocyte energetics (Western blot images in **B**, quantification in **C and D**). *$p < 0.05$.

adverse outcomes in HF even after adjusting for LVEF and MI size (17,18), were significantly smaller in empagliflozin-treated animals. Hypertrophy of the remote noninfarcted myocardium is initially a compensatory mechanism, but chronic cardiac hypertrophy leads to worse outcomes (19). Treatment with empagliflozin was associated with significantly lower LV mass post-MI, suggesting a reduced need for this compensatory mechanism. Finally, remodeled hearts become more spherical (architectural remodeling); in fact, a large sphericity index predicts adverse outcomes (20). Of note, the treated animals showed a smaller sphericity index (i.e., less spherical and thus less remodeled heart) than control animals, which supports the beneficial effects of empagliflozin.

Chronic activation of the neurohormonal response, especially of the sympathetic nervous system and natriuretic peptides, is a major hallmark of adverse LV remodeling. Plasma levels of catecholamines (21) and BNP (22) are clinically used as predictors for cardiovascular mortality in HF patients. We focused on metanephrines, the degradation products of plasma catecholamines, because they offer higher stability and better reflect long-term neurohormonal activity (8). Interestingly, empagliflozin reduced plasma levels of normetanephrine and BNP, suggesting an interruption of the pathological neurohormonal cycle. Whether this reduced sympathetic overdrive is secondary to empagliflozin-induced ameliorated cardiac remodeling or to direct sympathetic antagonism via KB-induced inhibition of GPR41 (23) needs to be ascertained. Empagliflozin significantly reduced several markers of cardiomyocyte injury (TnI) and cardiac stress and fibrosis (ST2), confirming the mitigated cardiac remodeling/injury in the treated group.

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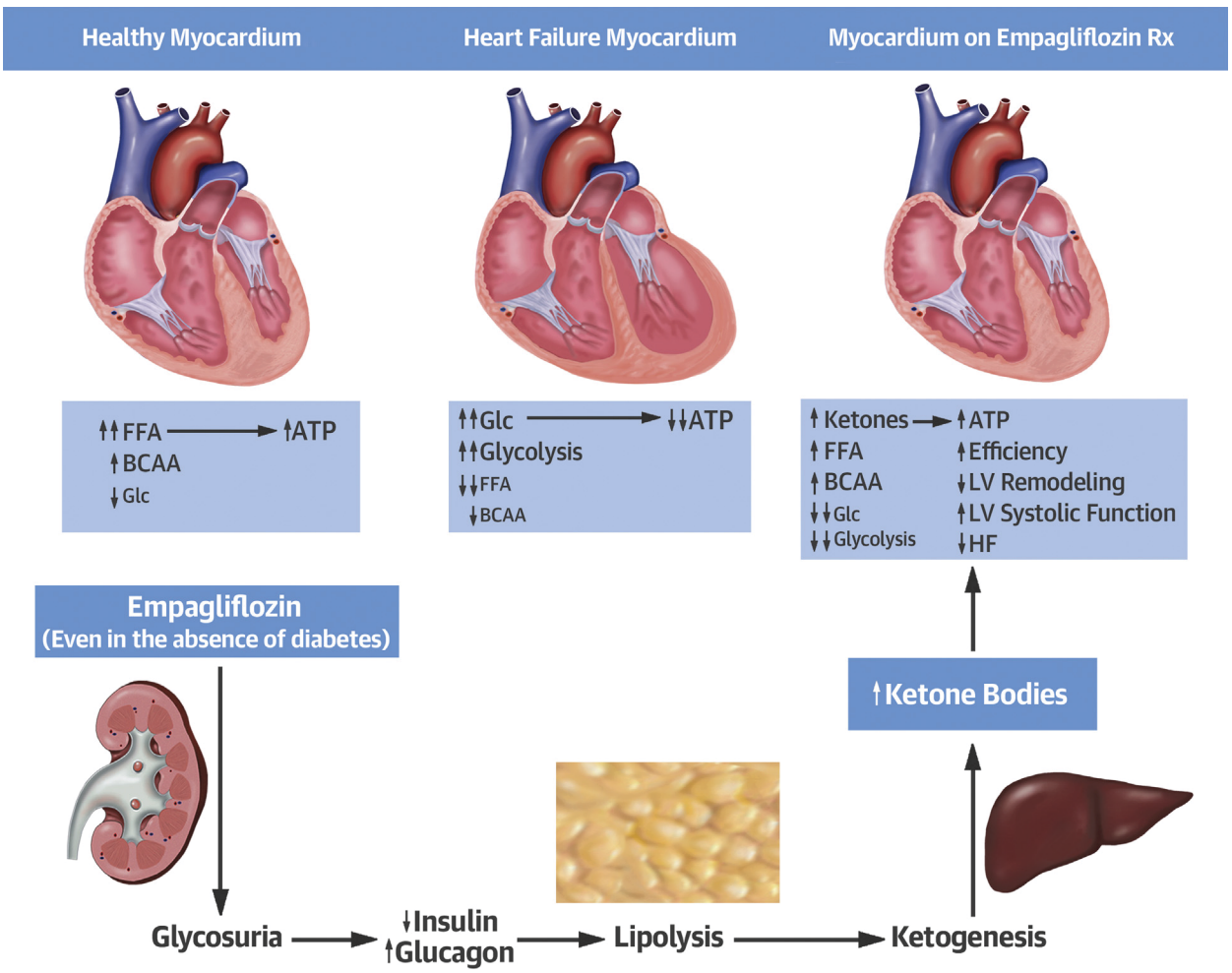
CENTRAL ILLUSTRATION Postulated Effect of Empagliflozin on Heart Failure



Santos-Gallego, C.G. et al. J Am Coll Cardiol. 2019;73(15):1931–44.

Empagliflozin improves cardiac function by switching away from glucose towards ketone bodies and free fatty acids as source of energy. AMPK = adenosine 5′ monophosphate-activated protein kinase; ATP = adenosine triphosphate; BCAA = branched-chain amino acid; FFA = free fatty acid; Glc = glucose; LV = left ventricular; Rx = treatment.

The mitigation in LV remodeling was paralleled by improvements in LV systolic performance. Empagliflozin-treated pigs showed higher LVEF and significantly greater contractile reserve than control animals, parameters associated with improved outcomes (24). LVEF is commonly used in clinical routine but is hampered by several caveats, such as load dependency; thus, we measured strains using echocardiography, which allows a more accurate evaluation of myocardial mechanics and clinical outcomes than LVEF (25,26). Our data clearly indicate that empagliflozin treatment resulted in significantly improved 3D-longitudinal, 3D-circumferential, and 3D-radial strains versus control animals.

EMPAGLIFLOZIN AMELIORATES MYOCARDIAL METABOLIC REMODELING IN HF. Abnormalities in myocardial energy metabolism precede and contribute to HF (3,27). The high energy demands of healthy myocardium are primarily met by the mitochondrial oxidation of FFA; however, the heart possesses metabolic flexibility, which allows utilization of different substrates (including glucose, KB, BCAA, and lactate) according to workload and substrate availability. FFA are the preferred myocardial fuel for

oxidative metabolism because complete oxidation of 1 palmitate molecule generates more ATP (energy liberated 298 kcal/mol), but this is at the expense of high oxygen requirements (the P/O ratio—meaning the number of ATP molecules produced per oxygen molecule reduced by the mitochondria—for palmitate is 2.33) (4,5). Under hypoxic conditions such as the increased workload found in HF, myocardial substrate oxidation switches from fat to carbohydrate oxidation (fetal-like metabolism); glucose becomes the preferred substrate because it is more oxygen-efficient (P/O ratio 2.58) than FFA oxidation (4,5), but this is at the expense of lower energy produced (energy liberated 224 kcal/mol) (4,5). This shift from in myocardial metabolism from FFA to glucose consumption creates an energy deficit (27) that impairs cardiac efficiency and aggravates HF. This energy deficiency is magnified because glucose in our control animals is predominantly metabolized through anaerobic glycolysis that produces only 2 ATP molecules per glucose molecule compared with glucose oxidation or palmitate oxidation, which produce 34 and 106 ATP molecules per metabolite molecule, respectively.

Critically, KB are more energetically efficient fuels, producing ATP in a more oxygen-efficient manner than FFA (P/O 2.5 for KB vs. 2.33 for palmitate) and with more energy liberated in KB oxidation than glucose (244 kcal/mol of KB vs. 224 kcal/mol for glucose) (4,5). In fact, in the isolated working rat heart model, the addition of physiological concentrations of KB to buffer with glucose increased myocardial efficiency (hydraulic work/energy from O_2 consumed) of the working rat heart by 25% (28). Therefore, consumption of KB instead of glucose produces enhanced energetics and boosts work efficiency in the myocardium, which may explain the cardiac benefits of empagliflozin in the EMPA-REG trial. Our study supports this hypothesis, as empagliflozin-treated animals exhibited higher myocardial ATP levels and improved myocardial work efficiency.

Importantly, plasma KB concentrations are elevated with SGLT2 inhibitor treatment. Empagliflozin-induced glycosuria lowers portal insulin-to-glucagon ratio, which causes lipolysis and increased FFA delivery to the liver, thus resembling prolonged fasting and stimulating ketogenesis (29). This explains the hyperketonemia in our empagliflozin-treated animals; in fact, raised KB levels have been reported both in diabetic and nondiabetic patients with empagliflozin (4,5,7) but importantly, despite initial concerns, without increasing the risk of diabetic ketoacidosis (30). Furthermore, the myocardium is the most avid organ consumer of KB per unit mass and oxidizes KB in a concentration-dependent manner (4,5,31); that is, KB will contribute significantly to myocardial energetics only when KB serum levels are increased (e.g., starvation or during SGLT2 inhibition). Finally, myocardial reliance on KB metabolism has been recently demonstrated in animals and humans both in HF (10,32,33) and T2DM (34); of note, greater myocardial BDH1 expression has been recently demonstrated both in humans (10) and animal models (32,35). Based on these facts (empagliflozin-induced hyperketonemia, myocardial utilization of KB, and the energy efficiency of KB), we hypothesized that empagliflozin causes a shift in myocardial metabolism from glucose toward KB utilization.

Empagliflozin caused a metabolic switch away from glucose into KB utilization. Empagliflozin-treated pigs exhibited high myocardial uptake of KB (4-fold higher than control animals), higher levels of SCOT and BHB1, and an increased ratio of serum-to-myocardial KB, which confirms increased reliance on KB metabolism. Conversely, glucose metabolism was decreased in the treated group given the low myocardial glucose uptake (6-fold lower than controls), no lactate production (lack of anerobic glucose metabolism), and lower activity/levels of LDH and PDH. Moreover, the treated group demonstrated improved myocardial energetics, as shown both by higher ATP content and by enhanced activation of AMPK and PGC1-α (master regulators of cellular energy metabolism).

Recent data in vivo support our hypothesis that a shift toward myocardial KB metabolism (specifically induced by empagliflozin in our study) maintains adequate fuel supply for ATP oxidative production in the absence of FFA metabolism. In murine models of transverse aortic constriction, increased myocardial KB metabolism (obtained by cardiac-specific BDH1 overexpression) ameliorated HF and improved cardiac contractility (35) while, conversely, reduced myocardial KB consumption (obtained by cardiomyocyte-specific SCOT knock-out) worsened HF phenotype (36). Furthermore, genetic inhibition of FFA transfer into cardiomyocytes (thus recapitulating HF myocardial metabolism) impaired cardiac energetics, increased myocardial glucose uptake, and caused cardiac remodeling; ketogenic diet ameliorates this HF phenotype (37), which confirms the benefits of boosting myocardial KB metabolism.

Defects in BCAA catabolism also contribute to HF. Previous studies revealed impaired BCAA catabolism in HF both in animals and humans (9). Furthermore, mice with BCKD genetic deficiency

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(thus suppressed BCAA catabolism) developed HF (9), while conversely, pharmacological enhancement of BCKD function blunted HF after aortic constriction (9). The fact that empagliflozin improves myocardial BCAA consumption seems therefore beneficial. A recent metabolomics study reports increased levels of short-chain acylcarnitines (BCAA metabolites) in the plasma of empagliflozin-treated patients (38); this indirect evidence in T2DM (38) supports our direct demonstration of enhanced myocardial BCAA consumption in HF. Finally, empagliflozin increases myocardial FFA utilization, thus improving cardiac energy production, which explains higher myocardial ATP content, ameliorated LV remodeling, and augmented LV systolic function.

STUDY STRENGTHS AND LIMITATIONS. There are several strengths of this paper. First, the use of a large animal model with high translational value (8). Second, the normoglycemic nature of our model extends the benefits of empagliflozin also to nondiabetic patients. Building up on this hypothesis, we have initiated the EMPA-TROPISM (Are the "Cardiac Benefits" of Empagliflozin Independent of Its Hypoglycemic Activity?) (NCT03485222) clinical trial to investigate the effects of empagliflozin specifically in nondiabetic HF (39), while other event-driven large clinical trials such as EMPEROR-REDUCED (EMPagliflozin outcomE tRial in Patients With chrOnic heaRt Failure With Reduced Ejection Fraction) (NCT03057977) or EMPEROR-PRESERVED (EMPagliflozin outcomE tRial in Patients With chrOnic heaRt Failure With Preserved Ejection Fraction) (NCT03057951) also include HF patients independently of diabetic status.

One of the major questions not answered by our study is whether our observations are a class effect or just specific to empagliflozin. The sample size may be considered a limitation, but the fact of attaining statistical significances with this number of animals might also be seen as indicative of the magnitude of the treatment effects. We used prepuberal female pigs, but a potential effect of menstrual cycle in our data cannot be ruled out.

CONCLUSIONS

Our data demonstrate that chronic SGLT2 inhibition with empagliflozin ameliorates adverse anatomical LV remodeling, enhances LV systolic function, and decreases neurohormonal activation in a nondiabetic porcine model of HF. These cardiac benefits of empagliflozin seem to be mediated by a shift in myocardial fuel metabolism away from glucose towards cardiac utilization of KB, FFA, and BCAA, which improves myocardial energetics and cardiac function. Our findings highlight the therapeutic potential of empagliflozin for HF even in nondiabetic patients and warrant further investigation.

ADDRESS FOR CORRESPONDENCE: Dr. Juan J. Badimon, Mount Sinai School of Medicine, Department of Cardiology, 1 Gustave L. Levy Place, Box 1030, New York, New York 10029-0310. E-mail: juan.badimon@mssm.edu. Twitter: @MountSinaiNYC. OR Dr. Carlos G. Santos-Gallego, Atherothrombosis Research Unit, Cardiovascular Institute, Icahn School of Medicine at Mount Sinai, 1 Gustave L. Levy Place, New York, New York 10029. E-mail: carlos.santos-gallego@mssm.edu OR carlosgsantos.gallego@gmail.com. Twitter: @IcahnMountSinai, @SantosGallegoMD.

PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: In a nondiabetic model of heart failure, empagliflozin ameliorates adverse left ventricular remodeling and improves systolic function switching cardiac metabolism away from glucose consumption toward utilization of more energy-producing ketone bodies, free fatty acids, and branch-chain amino acids, resulting in improved myocardial energetics and efficiency.

TRANSLATIONAL OUTLOOK: The efficacy of empagliflozin and other SGLT2 inhibitors should be studied in both diabetic and nondiabetic patients with heart failure.

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KEY WORDS animal models, cardiac remodeling, diabetes, heart failure, myocardial metabolism, SGLT2 inhibition

APPENDIX For an expanded Methods section as well as supplemental tables and figures, please see the online version of this paper.