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Low-dose empagliflozin does not modify myocardial energetics and function in a large-animal model of hibernating myocardium

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Authors contribution

- Danielle Medina-Hernández: experimental work, draft manuscript
- Carlos Galán-Arriola: experimental work, critical revision to manuscript draft
- Pedro Luis Cepas: experimental work, critical revision to manuscript draft
- Daniel Pérez-Camargo: experimental work, critical revision to manuscript draft
- Javier Sánchez-González: imaging studies set up and analysis, critical revision to manuscript draft
- Laura Cádiz: mitochondrial respiration evaluations, critical revision to manuscript draft
- Daniel Pereda: experimental work, critical revision to manuscript draft
- Ana García-Alvarez: conceptualization, supervision, critical revision to manuscript draft.
- Borja Ibanez: conceptualization, supervision, critical revision to manuscript draft, approved final draft. Takes full responsibility of the study.

Ischemic heart disease (IHD) is the second leading cause of morbidity and mortality globally.¹ Hibernating myocardium, a form of IHD, is characterized by its reduced contractility while maintaining viable myocardium, this phenomenon is known as hibernating myocardium.² Hibernating myocardium undergoes metabolic changes and energy supply remodeling.³ SGLT2 inhibitors may offer therapeutic benefits as they have been known to modulate cardiac energetics in the setting of post-acute myocardial ischemia and anthracycline-induced cardiotoxicity (AIC), conditions that experience energetic remodeling.^{4,5}

Female large-white pigs (n= 66, 2 mo. old) underwent surgical ameroid implant procedure as performed by Martínez-Milla et al., where a casein ameroid was surgically placed on the proximal left anterior descending (LAD) coronary artery, leading to progressive stenosis of the vessel.³ Three months following the procedure, the pigs underwent cardiac magnetic resonance (CMR) imaging and a coronary angiography to confirm the phenotype of hibernating myocardium defined by severe coronary stenosis and hypokinetic yet still viable LAD territory supplied by collaterals and segmental anterior LV dysfunction with <50% transmural LGE. Of the 66 pigs, 13 met the criteria for hibernating myocardium, the remaining animals either died postoperatively or did not develop the phenotype. A total of 13 pigs with the confirmed hibernating myocardium were then randomized 1:1 to no empagliflozin (n= 6) or 10 mg oral empagliflozin daily (n= 7), with the first dose given upon confirming the phenotype. The selected 10 mg dose was based on prior porcine studies demonstrating cardioprotective benefit at this dosing regimen.^{4,5} All pigs were followed for two additional months and CMR and phosphorus magnetic resonance spectroscopy (31P-MRS) were performed to assess cardiac function and myocardial energetics, respectively, prior to sacrificing the animals. Afterwards, the left ventricle (LV) was collected for high-resolution

mitochondria respirometry to assess mitochondrial respiration and to measure ketone body concentration. Plasma was collected to evaluate levels of circulating ketones.

On the first CMR, performed three months after undergoing surgery, the pigs randomized to control had a median LVEF of 52% (51.3, 54.3) compared to 45% (38, 58.5; $p = 0.52$ vs control) in the pigs with hibernating myocardium randomized to receive a daily treatment of 10 mg of empagliflozin. On the final CMR performed two months after randomization (month 5), the control group had a final LVEF of 58.5% (56.5, 60.5) with a 4.5-point change in LVEF from baseline. The pigs treated with empagliflozin 10 mg had a final LVEF of 51% (44.5, 59), a 1.0-point increase compared to baseline. There was no significant difference in final LVEF between the hibernating myocardium control and empagliflozin groups (58.5% [56.5, 60.5]) vs. 51% [44.5, 59]; $p = 0.3$; $n = 7$ treated, $n = 6$ untreated) (Figure 1A). There were no significant differences in ATP/PCr ratio between pigs receiving 10 mg empagliflozin and untreated pigs (0.628 (IQR=0.489, 0.825) vs. 0.488 (IQR= 0.406, 0.688); $p = 0.54$; $n = 6$ treated, $n = 5$ untreated) (Figure 1B). One pig from each group was excluded from the analysis due to poor acquisition. Mitochondria isolated from the LV were studied using a high-resolution Oxygraph-O2K respirometer. Analysis of isolated mitochondria revealed that there were no significant differences in oxygen consumption in the OXPHOS_{CI} state between pigs receiving 10 mg empagliflozin and untreated pigs (6420 pmol·s⁻¹·mg⁻¹ (IQR= 5330, 7090) vs. 5390 pmol·s⁻¹·mg⁻¹ (IQR= 5020, 7300); $p = 0.93$; $n = 6$ treated, $n = 5$ untreated) (Figure 1C). LV tissue samples revealed no differences in myocardial tissue concentration of ketone bodies between controls and pigs treated with 10 mg of empagliflozin (106 µg/g (IQR= 69.8, 148) vs. 49.9 µg/g (IQR= 29.5, 67.3); $p = 0.27$; $n = 7$ treated, $n = 5$ untreated) (Figure 1D). Similarly, there were no differences in circulating plasma of ketone bodies between the untreated and treated groups (5.71 µg (IQR= 2.97, 6.65) vs. 1.50 µg (IQR= 1.26, 6.09); $p =$

0.31; n= 5 treated, n= 5 untreated (Figure 1E). Ketone analyses were limited as remaining samples were improperly stored and not suitable for analysis.

In our large-animal model of hibernating myocardium, treatment with 10 mg of empagliflozin did not lead to significantly improved cardiac function, myocardial energetics, or mitochondrial respiration. A significant limitation of our study is that animals in the treatment group had lower LVEFs at baseline, which may have influenced the observed final LVEFs. Another limitation is the absence of male pigs in our study which may not consider the potential ischemic and metabolic adaptations, as well as response to empagliflozin, thereby limiting the generalizability of our findings. The lack of significant overall improvement may also be attributed to an insufficient dose of empagliflozin to fully exert its cardioprotective effects. A similar dose-dependent effect has been observed in a large animal model of AIC. Specifically, in the context of AIC, 10 mg of empagliflozin was not sufficient to ameliorate cardiotoxicity, whereas increasing the dose to 20 mg demonstrated significantly preserved LVEF, improved myocardial energetics, as reflected by the ATP/PCr ratio and maintained mitochondrial function, in association with enhanced ketone body utilization.⁵ Although the insult is distinct, both are chronic progressive models that exhibit metabolic reprogramming characterized by a myocardium shift from predominately fatty acid oxidation to increase in glucose uptake accompanied by impaired mitochondrial respiration, as an adaptive response.^{3,5} These changes develop over weeks to months, in contrast to the acute myocardial infarction model, where the injury occurs abruptly.⁴ Increasing the dose in future hibernating myocardium studies could potentially potentiate the cardioprotective effects, leading to more robust restoration of cardiac function, myocardial energetics, and mitochondrial activity.

References

1. Khan, M. A. *et al.* Global Epidemiology of Ischemic Heart Disease: Results from the Global Burden of Disease Study. *Cureus* **12**, e9349 (2020).
2. Kloner, R. A. Stunned and Hibernating Myocardium: Where Are We Nearly 4 Decades Later? *J. Am. Heart Assoc.* **9**, e015502 (2020).
3. Martínez-Milla, J. *et al.* Translational large animal model of hibernating myocardium: characterization by serial multimodal imaging. *Basic Res. Cardiol.* **115**, 33 (2020).
4. Santos-Gallego, C. G. *et al.* Cardioprotective Effect of Empagliflozin and Circulating Ketone Bodies During Acute Myocardial Infarction. *Circ. Cardiovasc. Imaging* **16**, e015298 (2023).
5. Medina-Hernández, D. *et al.* SGLT2i Therapy Prevents Anthracycline-Induced Cardiotoxicity in a Large Animal Model by Preserving Myocardial Energetics. *JACC CardioOncology* <https://doi.org/10.1016/j.jaccao.2024.12.004> (2025) doi:10.1016/j.jaccao.2024.12.004.