

Alterations of myocardial ketone metabolism in heart failure with preserved ejection fraction (HFpEF)

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Abstract

Introduction Cardiac energy metabolism is disrupted in heart failure with preserved ejection fraction (HFpEF), as characterized by a switch from glucose oxidation towards fatty acid oxidation. However, although oxidation of ketones is an important source of ATP it remains unclear how the heart oxidizes ketones in HFpEF. It is also unclear whether elevating ketone supply to the heart can improve cardiac energetics and/or provide functional benefit for the hearts in HFpEF.

Aims The present study investigated the effects of increasing ketone supply to the heart via ketone supplementation or SGLT2 inhibitor treatment in a mouse model of HFpEF.

Methods HFpEF was induced in 13-month-old C57BL/6N female mice with 60% high-fat diet and L-NAME (0.5 g/L/day in the drinking water) for 6 weeks. In parallel, two other groups of mice were maintained on the HFpEF protocol while also receiving either a ketone ester supplement (1–3 butanediol 1 g/kg/day) or SGLT2 inhibitor (empagliflozin 10 mg/kg/day) for 6 weeks. Control mice were fed with regular low-fat diet and regular drinking water. Hearts of the mice were excised and perfused in the isolated working mode aerobically with 5-mM glucose, 0.8-mM palmitate, 100-μU/mL insulin, with either low (0.6 mM) or high (1 mM) levels of β-hydroxybutyrate. Metabolic rates of the hearts were measured with radiolabelled [^{14} C] glucose, [9,10- ^{3} H] palmitate and [3- ^{14} C] β-hydroxybutyrate.

Results In HFpEF mouse hearts, glucose oxidation was significantly decreased with a parallel increase in fatty acid oxidation. Increasing β-hydroxybutyrate levels from 0.6 to 1 mM in the perfusate resulted in a rise in ketone oxidation rates in control hearts (from 861 ± 63 to 1377 ± 94 nmol g dry wt⁻¹ min⁻¹), which was muted in HFpEF hearts (from 737 ± 68 to 897 ± 134 nmol g dry wt⁻¹ min⁻¹). Following ketone ester supplement or SGLT2 inhibitor treatment, HFpEF mice presented with restored ketone oxidation rates (from 674 ± 36 to 1181 ± 115 nmol g dry wt⁻¹ min⁻¹ with ketone ester supplement and from 797 ± 121 to 1240 ± 120 nmol g dry wt⁻¹ min⁻¹ with SGLT2i). Yet, this was not associated with improvement in cardiac function.

Conclusions In HFpEF mice, the heart switches from glucose oxidation to fatty acid oxidation, with ketone oxidation being impaired. Increasing ketone supply to the heart via ketone ester supplementation or SGLT2 inhibitor treatment increases myocardial ketone oxidation rates but was not associated with functional improvements. Unlike HFrEF, ketone supplementation strategies may be less effective in HFpEF due to an impairment of myocardial ketone oxidation in HFpEF.

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[†]Dr. G.D. Lopaschuk passed away and Dr. John Seubert was nominated by all authors to be corresponding author. Due to Dr. Lopaschuk's passing, his signed agreement to change the author list could not be obtained.

Introduction

Heart failure with preserved ejection fraction (HFpEF) is a cardiometabolic disease that accounts for half of all heart failure cases.¹ Previous research has shown that both myocardial glucose and fatty acid metabolism is dysregulated in the set-

ting of HFpEF.² However, it is not clear what happens to myocardial ketone oxidation, another crucial metabolic pathway, in HFpEF. Ketone oxidation is upregulated in heart failure with reduced ejection fraction (HFrEF),³ and exogenous ketone infusion has promising cardioprotective effects in HFrEF.⁴ Whether similar ketone metabolic changes occur

in HFpEF is not well understood. The aims of the study were to directly assess myocardial ketone oxidation rates in a mouse model of HFpEF and to evaluate whether increasing ketone supply to the heart via ketone supplement therapy can mitigate disease progression.

The experimental set-up is illustrated in *Figure 1A,i*. In short, 13-month-old female C57BL/6N mice were randomized to total four experimental groups over the span of 6 weeks: (1) control, mice received regular chow diet and drinking water; (2) HFpEF, mice received 60% high-fat diet (HFD) combined with L-NAME, an endothelial nitric oxide inhibitor, (0.5 g/L/day in the drinking water)⁵; (3) HFpEF + EMPA, mice treated with sodium glucose co-transporter 2 inhibitor (SGLT2) inhibitor (empagliflozin 10 mg/kg/day) in addition to HFD + L-NAME; (4) HFpEF + KS, mice treated with a ketone ester supplement (R-1,3-butanediol 1 g/kg/day) in addition to HFD + L-NAME. Aged female mice were chosen based on clinical observation that female sex was associated with the presence of diastolic dysfunction and worse clinical outcomes particularly in elderly patients.^{6,7} The '2-Hit' HFpEF mouse model was chosen based on the assumption that HFpEF disease progression commonly involves metabolic stressors, such as obesity, and haemodynamic stressors, such as hypertension. The '2-Hit' model was chosen for the present study as it centres around a metabolic form of heart failure. However, it is important to recognize that this model only represents the obesity + hypertension comorbidities phenotype of HFpEF and that HFpEF is a heterogeneous syndrome with diverse aetiologies that can be classified into different subgroups based on respective phenotypes.⁸ Future studies are required to further investigate the other subgroups of HFpEF with diverse pathophysiology.

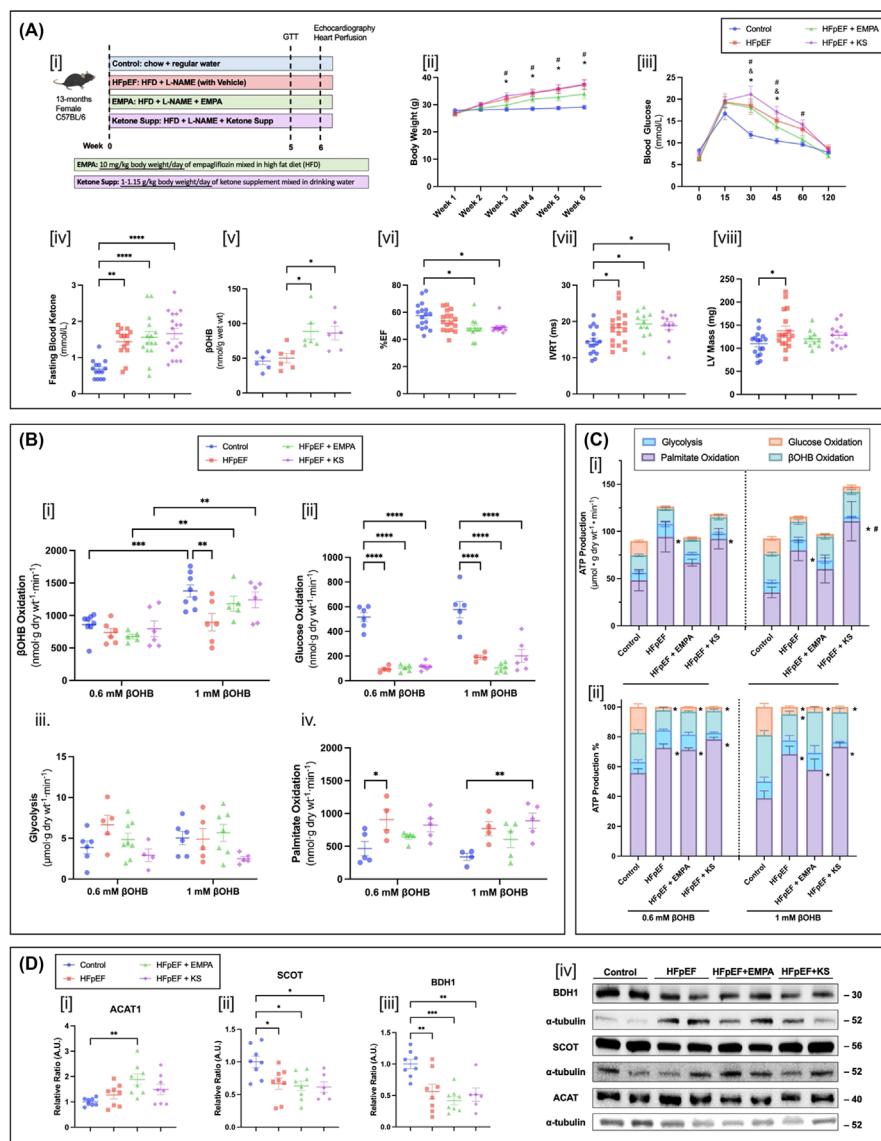
We show that all HFpEF groups, including those receiving vehicle, EMPA or KS, exhibited accelerated weight gain and impaired glucose tolerance compared with control mice (*Figure 1A,ii–iii*), although EMPA-treated mice showed slightly attenuated weight gain relative to the other HFpEF groups (*Figure 1A,iii*). Fasting blood ketone levels were elevated across all HFpEF groups, with no significant further increase in fasting ketone levels observed with EMPA or KS treatment (*Figure 1A,iv*). Nonfasting blood ketone levels were also assessed but exhibit significant variability, as it varies depending on the feeding status of the mice (data not shown). Importantly, cardiac tissues levels of β OHB were elevated with both EMPA and KS treatments (*Figure 1A,v*), supporting an increase in ketone supply to the heart via the two interventions. The underlying mechanism that leads to this increase in cardiac tissue levels of β OHB is unclear and warrants further investigation. Potentially it could be a combinational effect of elevated ketone uptake and impaired ketone oxidation by the heart. Surprisingly, transthoracic echocardiography revealed no cardioprotective effects of either EMPA or KS treatments, with a decrease in ejection fraction (%EF) (*Figure 1A,vi*) and persistently high isovolumic relaxation time (IVRT) values (*Figure 1A,vii*), indicating impaired systolic and diastolic dys-

function respectively. Notably, both EMPA and KS showed some efficacy in reducing cardiac hypertrophy in HFpEF, as evidenced by lower left ventricular (LV) mass (*Figure 1A,viii*). Recent clinical study shows that 2 weeks of ketone ester treatment increased cardiac output in patients with T2D and HFpEF.⁹ This discrepancy from preclinical findings may reflect interspecies differences, as murine models, while valuable for mechanistic studies, cannot fully recapitulate the complex and heterogeneous pathophysiology of clinical HFpEF.

To better understand this lack of cardioprotective effects with exogenous ketone supplementation to HFpEF mice, we directly assessed cardiac energy metabolism by perfusing the hearts with radiolabeled energy substrates under two conditions: low (0.6 mM) and high (1 mM) β OHB concentrations. The results demonstrates that healthy control hearts exhibited a 1.6-fold increase in ketone oxidation rates in response to higher ketone availability (0.6 mM vs. 1 mM β OHB) (*Figure 1B,i*). Conversely, HFpEF hearts showed impaired ketone oxidation, as indicated by a reduced response in myocardial β OHB oxidation rates to higher ketone availability (*Figure 1B,i*). Importantly, this attenuated ketone metabolism was restored in HFpEF hearts treated with EMPA or KS, as evidenced by elevated ketone oxidation rates under 1 mM β OHB conditions (*Figure 1B,i*). Consistent with previous findings,² we observed a decrease in insulin-stimulated glucose oxidation and an increase in fatty acid oxidation in HFpEF hearts under both the 0.6-mM and 1-mM β OHB perfusion conditions (*Figure 1B,ii–iii*). This suggests that acutely increasing ketone concentration from 0.6 to 1 mM did not further aggravate the alterations with glucose and fatty acid oxidation in HFpEF hearts. Likewise, hearts from EMPA and KS-treated HFpEF mice exhibited similar patterns in glucose and fatty acid oxidation as HFpEF hearts (*Figure 1B,ii–iii*). Neither acute nor chronic increases in ketone supply, at the concentrations achieved in this study, led to significant changes in glucose or fatty acid oxidation. Additionally, no significant changes in glycolytic rates were observed among the groups (*Figure 1B,iv*).

Actual ATP production rates and the %ATP derived from the four major metabolic pathways is shown in *Figure 1C,i–ii*. We found that overall myocardial ATP production rates were not impaired in HFpEF hearts compared with controls hearts, primarily due to an elevated ATP derived from fatty acid oxidation (*Figure 1C,i*). In healthy hearts, the majority of ATP production was derived from glucose and fatty acid oxidation, accounting for 17% and 55% of total ATP production, respectively. Increasing ketone concentration from 0.6 to 1 mM led to a significant increase in ATP production from ketone oxidation (from 20% to 31%). In contrast, HFpEF hearts displayed a distinct metabolic profile, with a pronounced reliance on fatty acids as the major contributor to myocardial ATP production (70%) at the expense of glucose oxidation (2%). Although increasing ketone concentration from 0.6 to 1 mM slightly increased the

Figure 1 Myocardial ketone oxidation is impaired in heart failure with preserved ejection fraction (HFpEF). (A) HFpEF protocol and empagliflozin (EMPA) and ketone supplement (KS) protocol (i), body weight changes over the HFpEF protocol (ii), blood glucose changes over the 2-h course of intraperitoneal glucose-tolerance test (GTT), which were performed by injection of glucose (2 g kg^{-1} in saline) after 16-h fasting (iii), fasting blood ketone levels assessed by sampling blood from mice tail and read with FreeStyle precision β -Ketone test strips paired with its analyser ($n = 14$ –17) (iv), cardiac tissue levels of β OHB assessed with a colorimetric assay kit from Abcam (ab83390) ($n = 6$) (v), transthoracic echocardiographic analysis of LV ejection fraction (%EF) ($n = 11$ –17) (vi), isovolumic relaxation time (IVRT) ($n = 11$ –17) (vii) and LV mass (LV mass) ($n = 11$ –17) (viii). (B) β OHB/ketone oxidation rates ($n = 5$ –8) (i), glucose oxidation rates ($n = 4$ –6) (ii), glycolysis ($n = 4$ –8) (iii) and palmitate/fatty acid oxidation rates ($n = 4$ –5) (iv) in isolated working hearts perfused with appropriately radiolabelled 5-mM glucose, 0.8-mM palmitate, 3% bovine serum albumin (BSA), 100 $\mu\text{U/mL}$ insulin and either 0.6- or 1-mM β OHB. (C) ATP production rates were calculated based on the oxidation rates from glucose (31 moles of ATP from glucose oxidation), fatty acid (104 moles of ATP from palmitate oxidation) and β OHB (21.25 moles of ATP from β OHB oxidation) (i), %ATP production is calculated by matching the ATP production rates attributed to each of the four metabolic pathways normalized to the sum of ATP production rates (ii). (D) Quantification of western blotting showing expression of enzymes involved in myocardial ketone oxidation (ACAT1 [i], BDH1 [ii] and SCOT [iii]), and representative blots ($n = 6$ –8) (iv). Data are mean $\pm$ SEM. Statistics were performed in GraphPad Prism 10. Significant differences were determined by using a one-way or two-way analysis of variance (ANOVA) followed by a Tukey's multiple comparisons *post hoc* test. For Panel C, $*P < 0.05$ compared with control group under the same condition, $\#P < 0.05$ compared with HFpEF under the same condition. For all other panels, $*P < 0.05$ compared with corresponding group with bracket. Mouse studies were approved by the University of Alberta Animal Care and Use Committee and comply with Animal Research: Reporting of In Vivo Experiments (ARRIVE) and National Institutes of Health guidelines. Abbreviations: ACAT1: acetyl-CoA Acetyltransferase 1; ATP, adenosine triphosphate; β OHB, β -hydroxybutyrate; BDH1, β -hydroxybutyrate dehydrogenase 1; EMPA, empagliflozin; EF, ejection fraction; HFD, high-fat diet; HFpEF, heart failure with preserved ejection fraction; IVRT, isovolumetric relaxation time; KS, ketone supplement; L-NAME, L-NG-nitro-arginine methyl ester, N(G)-nitro-L-arginine methyl ester; LV, left ventricle; SCOT, succinyl-CoA:3-ketoacid CoA transferase; Supp, supplement.



percentage of ATP from ketone oxidation in HFpEF hearts (from 13% to 17%), this increase was less pronounced compared with control hearts (from 20% to 31%). While both EMPA and KS treatments partially restored ketone oxidation in HFpEF hearts, the metabolic profile remained similar to that of untreated HFpEF hearts, characterized by an increased proportion of ATP production from fatty acid oxidation and a minimal contribution from glucose oxidation. Mechanistically, the impaired myocardial ketone metabolism in HFpEF heart is supported by a decreased expression of enzymes involved in ketone oxidation, as we observed that both beta-hydroxybutyrate dehydrogenase (BDH1) and succinyl-CoA-3-oxaloacid CoA transferase (SCOT) expression were reduced in HFpEF hearts (Figure 1D,i–iv), with no effect of EMPA or KS on expression of these enzymes. As such, the elevated myocardial ketone oxidation rates in hearts from HFpEF mice treated with EMPA and KS is not due to changes of ketone oxidative enzyme expression, but more likely attributed to the increase of ketone supply. Previous studies showed that BDH1 expression is upregulated in rat with HFrEF, but absent in failing human hearts.¹⁰ As such, whether the changes in BDH1 and SCOT expression would be parallelly observed in hearts samples from patients with HFpEF is unknown and warrants further investigation.

In summary, by directly measuring energy metabolic flux we show that myocardial ketone oxidation rates are impaired in HFpEF, which is in contrast to the upregulated ketone metabolism observed in HFrEF. Increasing ketone supply to the heart via EMPA or KS did not result in cardiac functional improvement in HFpEF, possibly due to decreased ketone metabolic enzyme expression and ketone oxidation rates. In

addition, both acutely and chronically increasing ketone supply to the heart elevated the proportion of myocardial ATP production originating from ketone oxidation. However, this did not alter the predominant metabolic signature in HFpEF hearts, characterized by high fatty acid oxidation and low glucose oxidation rates. These findings indicate that while targeting myocardial energetics is a promising approach, its direct translation into HFpEF therapy remains uncertain. Given that our study is based on preclinical animal models, caution must be taken in extrapolating these results to clinical settings. Unlike HFrEF, ketone supplementation strategies may be less effective in HFpEF due to an impairment of ketone oxidation in HFpEF. While metabolic modulation may be a component of future therapeutic strategies, additional investigations are needed to assess its potential clinical relevance. It is also likely that targeting a single metabolic pathway might not be sufficient to mitigate HFpEF, whereas multifaceted approach at reversing different aspects of cardiac energy metabolism should be considered and is worth further investigation. Additionally, the interventions with EMPA and KS did not exert comprehensive cardioprotective effects, which showcases the limited efficacy of these treatments for HFpEF, at least with the ‘2-Hit’ murine model. Yet, others have shown that EMPA and KS can rescue HFpEF phenotype, but with a different murine model.¹¹ Clinical studies have also shown promising cardioprotective effects of EMPA in patients with HFpEF.¹² Given the controversies, further studies are needed to clarify whether metabolic interventions can be broadly effective across the spectrum of diverse HFpEF phenotypes in human or are limited to specific experimental conditions.

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