Ketone pharmacokinetic study in HFrEF

Senthil Selvaraj, MD, MS, MA
Duke University School of Medicine
NCT05757193

Version Date: 7.24.23, version 1.8

1 CLINICAL SIGNIFICANCE

Despite advances in the treatment of patients with heart failure (HF), there remains substantial residual morbidity and mortality. Even with insights into diverse mechanisms of myocardial dysfunction, virtually all current medical treatment is focused on modulating the "neurohormonal axis". Our project extends these principles to an emerging frontier, the ketone "metabolic axis", as a means of ameliorating symptoms through exogenous delivery of a ketone ester. In this pharmacokinetic study, we first seek to find the optimal dose of a ketone ester product. We will subsequently use this information to inform dosing for a randomized trial of ketone therapy in HF.

2 SPECIFIC AIMS

HF is a major public health problem: it affects 6.2 million people in the U.S.[1] The lifetime risk for developing HF after the age of 40 years approaches 20%,[2] and five-year survival after HF hospitalization is a dismal 35%.[3] These statistics highlight the urgent need to identify novel therapies to reduce the substantial morbidity and mortality of the disease process. Current HF therapies focus on ameliorating pathophysiologic adaptations, including antagonism of the activated renin-angiotensin-aldosterone and sympathetic nervous systems, reducing excess vasoconstriction, and mitigating fluid retention. At its most basic level, however, HF is a metabolic imbalance - an inability of the heart to generate sufficient energy to adequately supply blood to the body, or only to do so at elevated filling pressures. The heart is a very metabolically demanding organ, which requires nearly 12 times its own body weight in ATP daily.[4] Abnormal myocardial energetics have been described in both HFrEF.[5] Targeting the metabolic axis in HF therefore may hold great therapeutic promise.

Under physiologic conditions, fatty acids are the predominant energetic substrate for the heart, providing 50-70% of the ATP need. Glucose serves as a secondary source, necessary for flexibility and adaptive fuel utilization shifts during embryologic development and during a diverse array of nutritional and physiologic conditions.[6, 7] However, two important studies lead by our team have demonstrated that free fatty acid oxidation is impaired in advanced HF due to mitochondrial substrate reprogramming, and the hypertrophied and failing heart utilizes ketone bodies as a metabolic substrate, in both animals and humans.[4, 8] It was previously unknown whether this shift was adaptive or maladaptive, but recent small and large animal data demonstrates that ketone body oxidation is an adaptive feature of the failing heart. Importantly, delivery of exogenous ketones improves pathologic cardiac remodeling and dysfunction while enhancing mitochondrial bioenergetics.[7]

We now have a unique opportunity to study a ketone ester (KE), (R)-3-hydroxybutyl (R)-3-hydroxybutyrate (commercially known as "DeltaG"), that provides systemic ketosis in a deep phenotyping study of HF patients.[9] Initial studies of DeltaG demonstrated significant improvement in exercise capacity among endurance athletes.[10] In this first study, we attempt to find the optimal dose of the ketone product. As patients may be taking minimally ketogenic medications (SGLT2 inhibitors), we will study the pharmacokinetic effects of ketone ester with patients on, and not on, these medications.

Our Specific Aims are as follows:

Aim 1. To assess the pharmacokinetics and safety of ketone ester in patients on, and not on, SGLT2 inhibitors. We will perform serial peripheral blood sampling to understand the effects of acute ketone ester on pH, glucose, systolic blood pressure, and ketone levels to find an optimal dose of the product.

Aim 2. To assess the effects of KE on metabolomics, proteomics, and systemic biomarkers (pending sufficient funding).

3 BACKGROUND AND SIGNIFICANCE

HF affects 6.2 million Americans and is associated with substantial morbidity and mortality in the contemporary era.[1] By 2030, the burden of HF is anticipated to surpass 8 million with an associated economic toll of a staggering $69.8 billion.[1] The cardinal clinical feature of patients with HF is reduced exercise capacity, which is associated with a substantial reduction in quality of life.[11] Despite significant advancements in therapy for patients with HF, particularly among those with reduced ejection fraction (EF), many still suffer from limitations in exercise capacity and a poor prognosis. As such, exploring other avenues to reduce morbidity from HF is of vital interest from societal, economic, clinical, and patient-centered perspectives.

Recently, several alterations in the cardiac metabolism of patients with HF have been described, setting the stage for randomized trials of novel therapies that modulate energy fuel metabolism.[4, 7, 8] Among healthy individuals, the heart, a very metabolically active organ, uses a combination of free fatty acids and glucose (to a lesser extent) as the primary sources of adenosine triphosphate (ATP) production. However, free fatty acid oxidation is impaired in the failing heart, and the heart increases its use of ketone bodies for fuel.[7]

Ketone bodies (acetoacetate, β-hydroxybutyrate [BHB], and acetone) are water-soluble molecules produced from acetyl-CoA following breakdown of fatty acids (and amino acids) mainly in hepatic cells. Ketone bodies are typically absent from the circulation during the normal fed state, but are produced continuously during metabolic stress conditions and/or periods of carbohydrate deprivation such as acute medical stress, prolonged exercise, or starvation, and become a key source of energy to vital organs including the brain and heart under these circumstances.[12] Ketone production has long been teleologically considered as a means to provide the brain with an efficient, alternative fuel source during starvation, sparing carbohydrate and protein stores, since the brain cannot effectively metabolize free fatty acids (which represent 99% of the body's energy stores).[12, 13] When completely oxidized in the citric acid cycle, ketones have a respiratory quotient similar to glucose (acetoacetate = 1.0, BHB = 0.89) and produce greater amounts of ATP, releasing approximately 30% more energy per molecule than pyruvate.[14, 15] The liver produces ketone bodies from mobilized fat stores that then can be utilized by neural tissue to produce ATP. It is therefore not surprising that several neurologic diseases have been treated, or are under study, with ketone therapy, such as epilepsy, Parkinson's disease, and Alzheimer's disease.[16]

Macronutrient resources (low glucose, high free fatty acids) and hormonal signaling (low insulin, high glucagon, high cortisol) are the primary methods of regulating ketone production, and thus dietary modification has been studied extensively to treat these conditions (the "ketogenic diet"). Adherence to this diet, however, can be challenging, and may also be accompanied by dyslipidemia (due to increased flux of free fatty acids), impaired cardiac metabolism, or gastro-intestinal upset, leading to interest in alternative exogenous modes of delivery.[17, 18] Exogenous delivery of ketones holds several advantages over dietary modification such as increased compliance, lack of alteration on other fuel stores (such as intramuscular glycogen), and its unique effect of lowering both glucose and free fatty acids due to negative feedback on lipolysis.[19]

Different formulations of oral ketone therapy have been studied, including ketone salts and KE. Oral ketone salts result in a high amount of salt intake (which may be problematic for patients with HF) and results in half the plasma concentration of D-betahydroxybutyrate (D-BHB).[17] A novel KE compound, I-3-hydroxybutI(R)-3-hydroxybutyrate (DeltaG), has been extensively studied for the purposes of therapeutic exogenous ketosis and is approved as a nutraceutical by the Food and Drug Administration (FDA).[9, 10, 17, 20-23] Advantages of DeltaG consumption include a rapid rise of blood levels of BHB in humans to ~3 mmol/L within 60 minutes of consumption with moderate doses of KE, equivalent to a week of fasting, which is markedly increased from the levels of detectable ketones in normal populations (<0.5 mmol/L).[9, 17, 21] By way of comparison, a carbohydrate-

restricted (high-fat) diet typically results in mild hyperketonemia, with BHB levels of ≥ 0.5 mmol/L, which is currently considered as an accepted definition of ketosis.[24]

Benefits of KE therapy among athletes have already been demonstrated. In a blinded, crossover study of endurance athletes, DeltaG increased exercise distance by an average of 411 meters over 30 minutes cycling duration, demonstrating that ketone therapy improves exercise capacity among highly trained athletes.[10] Importantly, it has been suggested that pathologic conditions that cause metabolic dysregulation, and where incremental improvements in energy transduction may translate to significant increases in exercise capacity, may afford the greatest clinical benefit, such as in HF.[10]

Further data has emerged regarding potential benefits of intravenous infusions of sodium-3-hydroxybutyrate.[25] In a crossover study of 16 patients with HF and reduced EF (HFrEF) randomized to 6 hours of ketone infusion vs. isotonic saline (placebo arm), infusion of the ketone increased cardiac output by 2 L/min with an absolute improvement in left ventricular EF of 8%. Notably, the hemodynamic effects of ketones were dose-dependent and detectable even in the physiological concentration range. While important, this trial was limited by use of an intravenous infusion (impractical for the outpatient management) and concerns related to salt infusion, such as pH disturbances and sodium loading. In addition, this trial did not assess patient-centered metrics, such as exercise capacity, which is an important determinant of quality of life in HF.[26] However, the fact that intravenous ketone therapy increased cardiac output to this extent supports the hypothesis that there is a fundamental limitation of fuel utilization in HF that can be therapeutically targeted. In addition, recent data shows important improvements in cardiac output and function without significant safety concerns in patients with cardiogenic shock at 500 mg/kg of the KE.[27]

In this first study, we seek to determine whether the optimal dose of a liquid ketone ester drink in patients on, and not on, SGLT2 inhibitors.

4 Published Data on Ketone Ester

DeltaG is a ketone ester (KE) that undergoes complete enzymatic hydrolysis to form BHB (and R-1,3-butanediol) by carboxylesterases expressed widely, including within the gastrointestinal tract, liver, and blood. R-1,3-butanediol, itself, is further metabolized in the liver by first-pass metabolism and blood to produce BHB by alcohol dehydrogenase and aldehydye dehydrogenase. BHB undergoes further metabolism to acetoacetate and acetone, though only BHB and acetoacetate are taken up by extra hepatic tissues as energy sources.

KE is eliminated through at least first order kinetics and capacity limited elimination. Elimination is accomplished via 3 predominant means, including (1) conversion to acetone and lung exhalation, (2) renal elimination (of BHB and acetoacetate, though this contribution is negligible particularly during exercise), and (3) uptake by tissues using monocarboxylate transporters for conversion to ATP.[10, 21]

Concentrations of the KE compound of roughly 3-5 mmol/L are reached within 60 minutes at doses comparable to what is studied here.[9, 10] The half-life of the compound is 0.8-3.1 hours.[9] The KE compound has a very favorable safety profile with adverse effects limited to dose-dependent, gastrointestinal distress that typically occur only at the highest levels of administration (714 mg/kg).[9, 28]

Request for IND exemption: We believe this study qualifies under the criteria for IND exemption. DeltaG has been granted **GRAS** status through the FDA as a food, and the GRAS documentation will be separately submitted. We believe this study is exempt from the IND requirements because it meets the conditions of FDA guidance (sections VI.D.1 & VI.D.2). Specifically, DeltaG, as used in this study, is not intended for use "in the diagnosis, cure, mitigation, treatment, or prevention of disease". As DeltaG is only received on *one* study date as an acute study, and endpoints assessed here are for safety, we believe the use of DeltaG fulfills these critiera here. Participants will be notified in the ICF specifically that they will not benefit as a result of participating in the study. The intent of the study is to describe the acute effect of DeltaG (a food) on venous pH, glucose, and blood pressure as a safety mechanism. In addition, the results of this study are not intended to affect the marketing or labeling of DeltaG.

We have previously performed research with this compound at another institution in patients with heart failure, who have given IND exemption for the use of ketone ester. This letter has also been submitted.

5 Research Design and Methods

Overview: We first begin with a pharmacokinetic study to establish dosing among 20 HF participants.

5.1 Study population

Study population (Inclusion criteria)
Participants aged at least 18 years of age with a diagnosis of HFrEF and with systolic blood pressure ≥90 mmHg will be included for this study. We will recruit 20 HFrEF (LVEF <=45%) participants. Participants will be stratified by use of prescribed SGLT2i (taken as part of their clinical care and not provided through this research study). Participants will be recruited from within the Duke Health System.

Exclusion Criteria

1. Intentional ketogenic (high fat, low carbohydrate) diet (must be off ketogenic diet for ≥7 days prior to visit)
2. Significant liver disease (cirrhosis) or alcohol abuse disorder (>14 drinks/week)
3. Unique cardiomyopathies: infiltrative/hypertrophic cardiomyopathy, pericardial disease, or other unique cardiomyopathies that in the opinion of the investigator would be less likely to respond to ketone therapy
4. Estimated glomerular filtration rate<25 mL/min/1.73 m^2 as the most recent value in the last year.
5. Type 1 diabetes mellitus
6. Use of ventricular assist device, history of heart transplant, or use of continuous inotropes
7. Pregnant women. Due to unknown affects of nutritional ketosis in pregnant women, pregnancy will be an exclusion. Accordingly, women of childbearing age with a menstrual cycle within the past year will be asked to submit a urine specimen for pregnancy testing.

5.2 Participant clinical data collection

We will assess the following clinical data parameters on study intake, which may include but are not limited to:

1. Age, date of birth, gender, race
2. New York Heart Association class
3. Smoking status, alcohol status.
4. Comorbidities (supplemented by chart view of the problem list): previous HF hospitalization in last 6 months, myocardial infarction, stroke, coronary artery bypass graft surgery, percutaneous coronary intervention, chronic obstructive pulmonary disorder, asthma, hypertension, peripheral artery disease,

atrial fibrillation or flutter, dyslipidemia, implanted cardioverter-defibrillator, pacemaker, and diabetes mellitus, and others.

5. Vital signs: height, weight, heart rate, systolic blood pressure, diastolic blood pressure, oxygen saturation, and others
6. Medications will be obtained from the medical chart and confirmed with the participant.

5.3 Pharmacokinetic study:

SGLT2i are standard-of-care ketogenic medications in HFrEF that minimally raise ketone levels though still well within normal limits (0.10mM to 0.13mM, normal <~0.5mM) through reducing insulin:glucagon ratio.[29, 30] Since exogenous ketones *increase* this ratio as shown in our previous work[31] and *reduce* endogenous ketosis, the combination of exogenous ketones and SGLT2i is not expected to perpetuate ketoacidosis risk by SGLT2i (which has not been a significant concern in clinical trials of HFrEF).[32] Further, recent work has supported the safety of ketogenic interventions in patients taking SGLT2 inhibitors.[33] Additionally, pH changes with robust ketosis by KE are noted (1 study showed a pH decrease of 0.10 to an arterial pH of 7.31 after the ketone drink as an expected change).[17] Nevertheless, we will first perform a dose-finding study in 10 HFrEF participants on SGLT2i and 10 not on SGLT2i to assess safety (**Table 1**). We will measure venous pH, glucose, blood pressure, and BHB every 60 minutes for 4 hours. After a standardized meal containing carbohydrate (to limit endogenous ketosis), we will assess 5 participants in each stratum using 250 mg/kg to initially target BHB levels ~1-2 mmol/L, and then dose escalate to 500 mg/kg for 5 participants[27] which is predicted to achieve BHB levels ~3 mmol/L (safely achieved in HF patients previously with KE[34] without inducing significant side effects including mild acidosis).[9, 28, 35, 36] This is also the dose used in a recent trial in cardiogenic shock without significant safety concerns but with significant efficacy.[27] We will not escalate past 500 mg/kg given increasing risk for gastrointestinal symptoms.[9, 37]

Table 1. Pharmacokinetic Study Details and Dose Escalation/De-escalation Plan

Strata	Dose	Timepoints assessed	Endpoints assessed
SGLT2i arm and no SGLT2i arm	250 mg/kg KE for 5 participants, then 500 mg/kg for 5 participants	Every 60 minutes for 4 hours	Venous pH, systolic blood pressure, glucose

Food may be provided to participants if they have hypoglycemia (glucose <55 mg/dl) but otherwise we will ask participants to refrain from consuming food after the ketone drink is administered until the 4 hour blood draw.

Prior to the ketone drink, we will collect blood –twice - in the fasted status (>8 hours of fasting) and then after a light breakfast. We will collect blood 6 times after ketone drink. There will be at most 8 blood draws as below.

Blood draw number	Approximate time of blood draw	Blood draw for endpoint assessment	Blood draw for storage
1	Fasting, prior to ketone drink		x
2	After light meal, prior to ketone drink		x
3	30 minutes after ketone drink		x
4	60 minutes after ketone drink	x	x

5	90 minutes after ketone drink		x
6	120 minutes after ketone drink	x	x
7	180 minutes after ketone drink	x	x
8	240 minutes after ketone drink	x	x

Timepoints delinated here are approximate. We aim to draw blood as close to the timeline outlined here, but +/- 15 minutes from the timepoint is considered acceptable without deviation. Likewise, lack of blood draws at times not used for endpoint assessment will not be considered a deviation.

We preferentially enroll unique patients in this study (i.e. 20 different patients). However, in the event of difficult enrollment in the "no SGLT2i arm", participants currently taking an SGLT2i may enroll and hold the SGLT2i for at least 4 days (with the permission of their treating physician). This amount of time is similar to what is advised prior to some procedures (i.e. surgery). Therefore, participants could enroll twice in this study (in each arm).

A total of ~100 mL of blood will be obtained at this visit.

Blood samples will be stored (either within the Duke Heart Center or the Duke Molecular Physiology Institute) at -80C for later analysis. We may perform routine blood testing (metabolic panels, blood counts), comprehensive metabolomic/proteomic profiling from this peripheral blood, as well as other biomarkers, in addition to other testing, pending sufficient funding.

6 Risks and Safety Assessment

Ketone Ester: This proposal includes a ketone ester which is commercially available and has been granted "Generally Regarded as Safe" (GRAS) status from the FDA. This KE undergoes complete enzymatic hydrolysis to form BHB (and R-1,3-butanediol) by carboxylesterases expressed widely, including the gastrointestinal tract, liver and blood. R-1,3-butanediol, itself, is further metabolized in the liver and blood to produce BHB. The TMax ranges from ~1-3 hours. Elimination is accomplished via conversion to acetone and lung exhalation, renal elimination, and uptake by tissues using for conversion to ATP (including the brain, heart, kidney, and skeletal muscles).[10, 21] We exclude participants both with a history of significant renal impairment (eGFR <25 ml/min/m^2) or hepatic impairment (history of cirrhosis, alcohol abuse). Peak concentrations of KE are reached around 60 minutes at doses anticipated to be administered here.[9, 10] The half-life of BHB from the compound is 0.8-3.1 hours.[9] Adverse effects are infrequent and dose-dependent; gastrointestinal distress occurs at the highest levels of administration (714 mg/kg), consistent with our own experience.[9, 28] No clinically significant changes in vital signs, lipid levels, blood counts, renal function, and urinalysis parameters were observed at doses similar to those studied here. Participants may be on SGLT2i which are minimally ketogenic medications in themselves with minimal risk for ketoacidosis in HF.[32] As outlined in this section, it is not anticipated that the KE will amplify risk of ketoacidosis with SGLT2i given the differing biologies of endogenous versus exogenous ketosis. However, this potential risk will be outlined to participants who enroll in this study. **Dose:** In this proposal, we will perform a pharmacokinetic study to determine optimal dose without significant safety concerns which will be explored in our pharmacokinetic study. Our product is purchased commercially as "DeltaG" (TdeltaS, Orlando, FL). We have administered these doses proposed here to 15 HFpEF patients in an ongoing study without significant adverse events related to the nutraceutical. We will monitor for any adverse events throughout the study visit. The compound half-life is short as indicated above. To aid intolerability, participants are served a light meal. In addition, water (which may be naturally flavored and/or carbonated) is freely permitted.

Phlebotomy and blood draws: We leverage healthcare professionals who have experience in blood draws and maintaining venous catheters in our study. Risks from the peripheral (e.g. antecubital fossa) venous catheterization include minor discomfort, minor bruising, bleeding, hematoma and/or fainting associated with the drawing of blood. There is also a very small chance (less than 1%) of infection at the blood draw site. The amount of blood to be drawn during each study is modest (100 mL).

7 Statistical analysis plan

Primary outcomes

1. Venous pH at 1 hour
2. Systolic blood pressure at 1 hour
3. Glucose at 1 hour
4. Beta-hydroxybutyrate level at 1 hour

Participant characteristics will first be summarized using means (standard deviations), medians (interquartile range), and counts (percentages) as appropriate for the type of variable and its distribution. We will plot summary estimates of venous pH, glucose, ketone levels, and systolic blood pressure over time, overall and stratified by SGLT2i use and ketone ester dose. We will compare whether achieved venous pH, glucose, ketone levels, and systolic blood pressure vary by background SGLT2i use, accounting for related baseline measurements. Sensitivity analysis will adjust for age, sex, ketone ester dose, eGFR, and hemoglobin a1c (if available). Repeated measures techniques (accounting for clustering at the level of the participants) may be used to assess overall changes in pH, ketone levels, systolic blood pressure, and glucose. We will describe peak ketone concentrations achieved by arm. Similarly, we will assess the relationship between peak ketone levels (dependent variable) and dose of KE (independent variable). $P<0.05$ will be used to indicate statistical significance.

References

1. Benjamin EJ, Muntner P, Alonso A, Bittencourt MS, Callaway CW, Carson AP, Chamberlain AM, Chang AR, Cheng S, Das SR, et al. Heart disease and stroke statistics-2019 update: A report from the american heart association. *Circulation*. 2019;139:e56-e66
2. Lloyd-Jones DM, Larson MG, Leip EP, Beiser A, D'Agostino RB, Kannel WB, Murabito JM, Vasan RS, Benjamin EJ, Levy D. Lifetime risk for developing congestive heart failure: The framingham heart study. *Circulation*. 2002;106:3068-3072
3. Owan TE, Hodge DO, Herges RM, Jacobsen SJ, Roger VL, Redfield MM. Trends in prevalence and outcome of heart failure with preserved ejection fraction. *The New England journal of medicine*. 2006;355:251-259
4. Bedi KC, Jr., Snyder NW, Brandimarto J, Aziz M, Mesaros C, Worth AJ, Wang LL, Javaheri A, Blair IA, Margulies KB, et al. Evidence for intramyocardial disruption of lipid metabolism and increased myocardial ketone utilization in advanced human heart failure. *Circulation*. 2016;133:706-716
5. Neubauer S, Krahe T, Schindler R, Horn M, Hillenbrand H, Entzeroth C, Mader H, Kromer EP, Riegger GA, Lackner K, et al. 31p magnetic resonance spectroscopy in dilated cardiomyopathy and coronary artery disease. Altered cardiac high-energy phosphate metabolism in heart failure. *Circulation*. 1992;86:1810-1818
6. Lopaschuk GD, Ussher JR, Folmes CD, Jaswal JS, Stanley WC. Myocardial fatty acid metabolism in health and disease. *Physiol Rev*. 2010;90:207-258
7. Horton JL, Davidson MT, Kurishima C, Vega RB, Powers JC, Matsuura TR, Petucci C, Lewandowski ED, Crawford PA, Muoio DM, et al. The failing heart utilizes 3-hydroxybutyrate as a metabolic stress defense. *JCI Insight*. 2019;4
8. Aubert G, Martin OJ, Horton JL, Lai L, Vega RB, Leone TC, Koves T, Gardell SJ, Kruger M, Hoppel CL, et al. The failing heart relies on ketone bodies as a fuel. *Circulation*. 2016;133:698-705
9. Clarke K, Tchabanenko K, Pawlosky R, Carter E, Todd King M, Musa-Veloso K, Ho M, Roberts A, Robertson J, Vanitallie TB, et al. Kinetics, safety and tolerability of (r)-3-hydroxybutyl (r)-3-hydroxybutyrate in healthy adult subjects. *Regul Toxicol Pharmacol*. 2012;63:401-408
10. Cox PJ, Kirk T, Ashmore T, Willerton K, Evans R, Smith A, Murray AJ, Stubbs B, West J, McLure SW, et al. Nutritional ketosis alters fuel preference and thereby endurance performance in athletes. *Cell Metab*. 2016;24:256-268
11. Lewis EF, Lamas GA, O'Meara E, Granger CB, Dunlap ME, McKelvie RS, Probstfield JL, Young JB, Michelson EL, Halling K, et al. Characterization of health-related quality of life in heart failure patients with preserved versus low ejection fraction in charm. *European journal of heart failure*. 2007;9:83-91
12. Cahill GF, Jr. Fuel metabolism in starvation. *Annu Rev Nutr*. 2006;26:1-22
13. Owen OE, Morgan AP, Kemp HG, Sullivan JM, Herrera MG, Cahill GF, Jr. Brain metabolism during fasting. *J Clin Invest*. 1967;46:1589-1595
14. Sato K, Kashiwaya Y, Keon CA, Tsuchiya N, King MT, Radda GK, Chance B, Clarke K, Veech RL. Insulin, ketone bodies, and mitochondrial energy transduction. *FASEB J*. 1995;9:651-658
15. Veech RL. The therapeutic implications of ketone bodies: The effects of ketone bodies in pathological conditions: Ketosis, ketogenic diet, redox states, insulin resistance, and mitochondrial metabolism. *Prostaglandins Leukot Essent Fatty Acids*. 2004;70:309-319

16. Veech RL, Chance B, Kashiwaya Y, Lardy HA, Cahill GF, Jr. Ketone bodies, potential therapeutic uses. *IUBMB Life*. 2001;51:241-247
17. Stubbs BJ, Cox PJ, Evans RD, Santer P, Miller JJ, Faull OK, Magor-Elliott S, Hiyama S, Stirling M, Clarke K. On the metabolism of exogenous ketones in humans. *Front Physiol*. 2017;8:848
18. Holloway CJ, Cochlin LE, Emmanuel Y, Murray A, Codreanu I, Edwards LM, Szmigielski C, Tyler DJ, Knight NS, Saxby BK, et al. A high-fat diet impairs cardiac high-energy phosphate metabolism and cognitive function in healthy human subjects. *Am J Clin Nutr*. 2011;93:748-755
19. Mikkelsen KH, Seifert T, Secher NH, Grondal T, van Hall G. Systemic, cerebral and skeletal muscle ketone body and energy metabolism during acute hyper-d-beta-hydroxybutyratemia in post-absorptive healthy males. *J Clin Endocrinol Metab*. 2015;100:636-643
20. Cox PJ, Clarke K. Acute nutritional ketosis: Implications for exercise performance and metabolism. *Extrem Physiol Med*. 2014;3:17
21. Shivva V, Cox PJ, Clarke K, Veech RL, Tucker IG, Duffull SB. The population pharmacokinetics of d-beta-hydroxybutyrate following administration of (r)-3-hydroxybutyl (r)-3-hydroxybutyrate. *AAPS J*. 2016;18:678-688
22. Holdsworth DA, Cox PJ, Kirk T, Stradling H, Impey SG, Clarke K. A ketone ester drink increases postexercise muscle glycogen synthesis in humans. *Medicine and science in sports and exercise*. 2017;49:1789-1795
23. Stubbs BJ, Cox PJ, Evans RD, Cyranka M, Clarke K, de Wet H. A ketone ester drink lowers human ghrelin and appetite. *Obesity (Silver Spring)*. 2018;26:269-273
24. Gibson AA, Seimon RV, Lee CMY, Ayre J, Franklin J, Markovic TP, Caterson ID, Sainsbury A. Do ketogenic diets really suppress appetite? A systematic review and meta-analysis. *Obes Rev*. 2015;16:64-76
25. Nielsen R, Moller N, Gormsen LC, Tolbod LP, Hansson NH, Sorensen J, Harms HJ, Frokiaer J, Eiskjaer H, Jespersen NR, et al. Cardiovascular effects of treatment with the ketone body 3-hydroxybutyrate in chronic heart failure patients. *Circulation*. 2019;139:2129-2141
26. Lewis EF, Claggett BL, McMurray JJV, Packer M, Lefkowitz MP, Rouleau JL, Liu J, Shi VC, Zile MR, Desai AS, et al. Health-related quality of life outcomes in paradigm-hf. *Circ Heart Fail*. 2017;10
27. Berg-Hansen K, Christensen KH, Gopalasingam N, Nielsen R, Eiskjaer H, Moller N, Birkelund T, Christensen S, Wiggers H. Beneficial effects of ketone ester in patients with cardiogenic shock: A randomized, controlled, double-blind trial. *JACC Heart Fail*. 2023
28. Stubbs BJ, Cox PJ, Kirk T, Evans RD, Clarke K. Gastrointestinal effects of exogenous ketone drinks are infrequent, mild and vary according to ketone compound and dose. *Int J Sport Nutr Exerc Metab*. 2019:1-23
29. Ferrannini E, Baldi S, Frascerra S, Astiarraga B, Heise T, Bizzotto R, Mari A, Pieber TR, Muscelli E. Shift to fatty substrate utilization in response to sodium-glucose cotransporter 2 inhibition in subjects without diabetes and patients with type 2 diabetes. *Diabetes*. 2016;65:1190-1195
30. Selvaraj S, Fu Z, Jones P, Kwee LC, Windsor SL, Ilkayeva O, Newgard CB, Margulies KB, Husain M, Inzucchi SE, et al. Metabolomic profiling of the effects of dapagliflozin in heart failure with reduced ejection fraction: Define-hf. *Circulation*. 2022
31. Selvaraj S, Margulies KB, Dugyala S, Schubert E, Tierney A, Arany Z, Pryma DA, Shah SH, Rame JE, Kelly DP, et al. Comparison of exogenous ketone administration versus dietary carbohydrate restriction on myocardial glucose suppression: A crossover clinical trial. *J Nucl Med*. 2022;63:770-776

32. Zannad F, Ferreira JP, Pocock SJ, Anker SD, Butler J, Filippatos G, Brueckmann M, Ofstad AP, Pfarr E, Jamal W, et al. Sglt2 inhibitors in patients with heart failure with reduced ejection fraction: A meta-analysis of the emperor-reduced and dapa-hf trials. *Lancet*. 2020;396:819-829
33. Hanson P, Randeva H, Cuthbertson DJ, O'Hare PJ, Parsons N, Chatha K, Reidy G, Weickert MO, Barber TM. The dapa-diet study: Metabolic response to dapagliflozin combined with dietary carbohydrate restriction in patients with type 2 diabetes mellitus and obesity-a longitudinal cohort study. *Endocrinol Diabetes Metab*. 2022:e381
34. Monzo L, Sedlacek K, Hromanikova K, Tomanova L, Borlaug BA, Jabor A, Kautzner J, Melenovsky V. Myocardial ketone body utilization in patients with heart failure: The impact of oral ketone ester. *Metabolism*. 2021;115:154452
35. Dearlove DJ, Harrison OK, Hodson L, Jefferson A, Clarke K, Cox PJ. The effect of blood ketone concentration and exercise intensity on exogenous ketone oxidation rates in athletes. *Med Sci Sports Exerc*. 2020
36. Faull OK, Dearlove DJ, Clarke K, Cox PJ. Beyond rpe: The perception of exercise under normal and ketotic conditions. *Front Physiol*. 2019;10:229
37. Stubbs BJ, Cox PJ, Kirk T, Evans RD, Clarke K. Gastrointestinal effects of exogenous ketone drinks are infrequent, mild, and vary according to ketone compound and dose. *Int J Sport Nutr Exerc Metab*. 2019;29:596-603