Ketone Therapy Prevents Semaglutide-induced Loss of Cardiac Mass

Schmidt & Abuetabh et al. Ketone Therapy Preserves Cardiac Mass

Mya A. Schmidt, MSc[a,b,c,d,†]; Yasser Abuetabh, PhD[a,b,c,d,†]; Masaaki Naganuma, MD, PhD[a,b,c,d]; Ramana Vaka, PhD[a,b,c,d]; Matthieu C.P. Zolondek, BSc[a,b,c,d]; Jody L. Levasseur, BSc[a,b,c,d]; Daniela Y. Morales-Llamas[a,b,c,d]; Mahmoud A. El-Ghiaty, PhD[a,b,c,d]; Shelly Braun, BSc[e]; Darius Sahid[a,b,c,d]; Laibah Khan, BSc[a,b,c,d]; Ethan Kwan, BSc[a,b,c,d]; Danica K. Roth[a,b,c,d]; Mourad Ferdaoussi, PhD[b,f]; Richard Fahlman, PhD[e]; and Jason R.B. Dyck, PhD[a,b,c,d,*].

[a]Cardiovascular Research Centre, [b]Alberta Diabetes Institute, [c]Women and Children's Health Research Institute, [d]Department of Pediatrics, [e]Department of Biochemistry, Faculty of Medicine & Dentistry, University of Alberta, Edmonton, Canada.

[f]Faculty Saint-Jean, University of Alberta, Edmonton, AB, Canada

† authors contributed equally to this work

***Correspondence**

Dr. Jason R. B. Dyck, 458 Heritage Medical Research Centre, University of Alberta, Edmonton, Alberta, Canada, T6G 2S2. Telephone: +1 (780) 492-0314. Fax: (780) 492-9753. Email: jason.dyck@ualberta.ca. Twitter @JasonRBDyck & @DyckLab

Word Count: 1,000
Abstract: NA
Figures: 1**; Tables:** 0

Glucagon-like peptide-1 receptor agonists (GLP-1RAs), including semaglutide, are effective treatments for obesity and type 2 diabetes (T2D). Large-scale clinical trials have shown that semaglutide confers substantial and clinically meaningful short-term cardiovascular benefits in both individuals with and without T2D (1). In addition, semaglutide improves adverse cardiac remodeling in obese patients with heart failure with preserved ejection fraction (HFpEF) (2), likely secondary to reversing obesity-related pathological changes. Moreover, we recently discovered that treatment with semaglutide reduces cardiac mass in both obese and normoweight mice even in the absence of pre-existing cardiac enlargement or dysfunction (3). Thus, while semaglutide has beneficial effects in the diseased or injured myocardium (2; 4), it may also reduce cardiac mass in otherwise healthy hearts. If this also occurs in humans, it suggests that there may be patients who would benefit from this reduction in cardiac mass and some patients

where semaglutide therapy could have detrimental effects. For the latter, preserving cardiac mass while maintaining adipose loss could maximize the therapeutic benefits of GLP-1RA therapy.

Previous work has shown that ketones play a major role in maintaining heart and skeletal muscle mass in normal physiology (5). Ketones are molecules that are produced by the liver from fatty acids and can be readily oxidized in the mitochondria of extrahepatic organs as a source of ATP. Recent studies have demonstrated that ketones play a major role in maintaining energy stability by reducing mitochondrial stress during decreased caloric intake, and that the loss of a key ketolytic enzyme, β-hydroxybutyrate dehydrogenase 1 (BDH1) in muscle contributes to skeletal muscle loss (6). Consistent with this, we show that transcript and protein levels of BDH1 are reduced in hearts from diet-indued obese mice following 3 weeks of semaglutide treatment (Figure 1A). Based on these findings, we speculated that the semaglutide-induced reduction in cardiac BDH1 decreased ketone utilization in the heart and contributed to the loss of cardiac mass. Consequently, we also surmised that promoting ketolytic flux via ketone therapy could prevent these effects.

To investigate the potential of ketone therapy to mitigate semaglutide-induced cardiac muscle loss, we fed mice a 60% high-fat diet (HFD) for 17 weeks to induce obesity (Figure 1A). Following this, mice were switched back to a regular chow diet to mimic the lifestyle intervention employed in the STEP1 clinical trial (7). Mice were then randomized to receive either vehicle (phospho-buffered saline), semaglutide (30 nM/kg/day, intraperitoneally), or semaglutide and ketone therapy. To establish an appropriate dosing regimen for the palatable ketone supplement bis-octanoyl (R)-1,3-butanediol (BO-BD; Qitone), we conducted a pilot study in normoweight mice and found that 0.114 g BO-BD/mL in drinking water produced sustained elevations in the primary ketone, β-hydroxybutyrate (Figure 1B). The HFD protocol resulted in an average bodyweight (BW) of 49.9 ± 3.8 g at the start of semaglutide treatment, which decreased by ~24% in the vehicle control group (due to the switch to regular chow diet), ~55% in the semaglutide-treated mice, and ~36% in mice given both semaglutide and BO-BD (Figure 1B). While semaglutide-induced loss of BW was blunted with ketone co-therapy, whole body composition analysis using EchoMRI revealed that actual fat mass loss was not different between semaglutide treatment alone (Figure 1B). Instead, we show that the BW differences between these two groups could largely be accounted for by loss of lean mass (Figure 1B). Specifically, semaglutide-treated mice lost significantly more lean mass than vehicle-treated controls (-9.0 ± 0.3 g vs. -2.3 ± 1.3 g), and this effect was attenuated in semaglutide-treated mice also receiving ketone co-therapy (-3.0 ± 0.9 g) (Figure 1B). Consistent with this preservation of lean mass, we also show that ketone therapy effectively prevents excessive loss of cardiac mass (Figure 1C), without compromising any of the functional benefits of semaglutide as assessed by echocardiography. In agreement with our previous finding that in vivo semaglutide treatment reduces cardiomyocyte area (3), we also show that this reduction is significantly prevented by ketone co-therapy (Figure 1C).

Given that semaglutide reduces the expression of BDH1, we speculated that decreases in other mitochondrial proteins may also occur and that ketone therapy could prevent this. We first examined the transcript levels of *Bdh1* and show that the semaglutide-induced decrease in *Bdh1* was prevented by ketone supplementation (Figure 1D). Additionally, we examined a panel of five genes encoding for proteins involved in the electron transport chain (ETC) that have

previously been shown to be upregulated with ketone treatment in muscle cells (Figure 1D) (8). We found that transcript levels of all five of these genes were significantly decreased in hearts from semaglutide-treated mice compared to vehicle-treated mice (dashed line) and restored in hearts from mice co-treated with semaglutide and ketones (Figure 1D). We also show that expression of *Cox7a1*, which encodes a mitochondrial protein essential for complex IV dimer stabilization and oxidative phosphorylation during muscle maturation (9), and is known to decline with age (10), is partially restored with ketone therapy (Figure 1D). In light of these findings, we performed transcriptomic analysis to gain a broader view of the mitochondrial genes differentially regulated by semaglutide and ketone co-treatment. Consistent with our targeted approach, unbiased transcriptomic analysis revealed a robust and consistent downregulation of numerous ETC-encoding genes spanning all four complexes following semaglutide treatment, an effect that was notably prevented with ketone co-administration (Figure 1D). Together, while multiple pathways are likely involved, these findings suggest that the loss of mitochondrial function may be one mechanism that contributes to the loss of cardiac mass with semaglutide treatment and that can be prevented by ketone co-therapy.

Overall, we show that ketone co-therapy during semaglutide-treatment allows for maintained fat loss, helps preserve lean and cardiac mass, and does not interfere with the beneficial cardiac remodeling necessary for maintaining cardiac function. While we further provide evidence that semaglutide may negatively impact cardiac mitochondrial function and that ketone co-therapy can prevent this from occurring, further research is needed to establish this conclusively. Nevertheless, we show that ketone therapy is a safe, efficacious, and highly feasible strategy for preserving cardiac and lean mass, making it a strong candidate for rapid deployment in clinical trials.

SOURCES OF FUNDING

This work was supported by grants from Canadian Institutes of Health Research (CIHR) to Dr. Dyck. Dr. Dyck is a Canada Research Chair in Molecular Medicine. Ms. Schmidt was supported by the Canada Graduate Scholarship – Master's Program, Women and Children's Health Research Institute (WCHRI) Graduate Studentship, and Alberta Diabetes Institute (ADI) Graduate Studentship. Dr. Abuetabh was supported by a Fellowship from the WCHRI of Alberta.

DISCLOSURES

All authors have reported that they have no relationships relevant to the contents of this paper to disclose.

AUTHORSHIP

JRBD, YA, and MAS contributed to the conception and design of the work. JRBD, YA, MAS, MN, RV, MCPZ, JLL, DYML, MAE, SB, DS, LK, EK, DKR, MF, and RF contributed to the acquisition, analysis, and interpretation of data for the work. MA, JRBD, and YA drafted the manuscript. JRBD, MA, and YA critically revised the manuscript. All authors gave final

approval and agreed to be accountable for all aspects of the work ensuring integrity and accuracy.

DATA AVAILABILITY

The data underlying this article are available in the article and in its online supplementary material. Any specific data will be shared on reasonable request to the corresponding author.

REFERENCES

1. Spagnolo M, Capodanno D. 2025. Effect of Semaglutide on Mortality and Cardiovascular Events in Patient at High Cardiovascular Risk: An Updated Systematic Review and Meta-analysis. *Eur J Prev Cardiol doi: 10.1093/eurjpc/zwaf358. Online ahead of print*
2. Solomon SD, Ostrominski JW, Wang X, Shah SJ, Borlaug BA, et al. 2024. Effect of Semaglutide on Cardiac Structure and Function in Patients With Obesity-Related Heart Failure. *J Am Coll Cardiol* 84:1587-602
3. Martens MD, Abuetabh Y, Schmidt MA, Zolondek MCP, Silver HL, et al. 2024. Semaglutide Reduces Cardiomyocyte Size and Cardiac Mass in Lean and Obese Mice. *JACC Basic Transl Sci* 9:1429-31
4. Withaar C, Meems LMG, Nollet EE, Schouten EM, Schroeder MA, et al. 2023. The Cardioprotective Effects of Semaglutide Exceed Those of Dietary Weight Loss in Mice With HFpEF. *JACC Basic Transl Sci* 8:1298-314
5. Soni S, Skow RJ, Foulkes S, Haykowsky MJ, Dyck JRB. 2025. Therapeutic potential of ketone bodies on exercise intolerance in heart failure: looking beyond the heart. *Cardiovasc Res* 121:230-40
6. Williams AS, Crown SB, Lyons SP, Koves TR, Wilson RJ, et al. 2024. Ketone flux through BDH1 supports metabolic remodeling of skeletal and cardiac muscles in response to intermittent time-restricted feeding. *Cell Metab* 36:422-37.e8
7. Wilding JPH, Batterham RL, Calanna S, Davies M, Van Gaal LF, et al. 2021. Once-Weekly Semaglutide in Adults with Overweight or Obesity. *N Engl J Med* 384:989-1002
8. Wang Q, Lan X, Ke H, Xu S, Huang C, et al. 2024. Histone β-hydroxybutyrylation is critical in reversal of sarcopenia. *Aging Cell* 23:e14284
9. García-Poyatos C, Arora P, Calvo E, Marques IJ, Kirschke N, et al. 2024. Cox7a1 controls skeletal muscle physiology and heart regeneration through complex IV dimerization. *Dev Cell* 59:1824-41.e10
10. Rönn T, Poulsen P, Hansson O, Holmkvist J, Almgren P, et al. 2008-05-17. Age influences DNA methylation and gene expression of COX7A1 in human skeletal muscle. *Diabetologia 2008 51:7* 51

FIGURE LEGEND

Figure 1. The Effects of Ketone Co-therapy on Semaglutide-treated Obese Mice. (A) Cardiac transcript levels of *Bdh1* (n = 6-7) in vehicle (V) and semaglutide-treated (S) obese mice; representative immunoblot of cardiac BDH1 expression and total protein stain (n = 4) in vehicle (-) and semaglutide-treated (+) obese mice; and study design. (B) Circulating β-hydroxybutyrate levels of 8-week-old C57BL/6 normoweight male mice following addition of 0.114 g/mL BO-BD to drinking water twice daily (n = 10); and changes in bodyweight, fat mass, and lean mass following initiation of vehicle (V), semaglutide (S) and semaglutide + ketone ester (S+K) (n = 5-7). (C) Relative heart weight (n = 5-7); left ventricular posterior wall thickness in diastole (n = 5-7); ejection fraction (n = 5-7); isovolumic relaxation time (n = 5-7); representative images of left ventricle sections stained with wheat germ agglutinin; and quantification of cardiomyocyte area (n = 865-2116) from all groups of mice. (D) Cardiac transcript expression of *Bdh1*, *Atp5e*, *Uqcrq*, *Cox20*, *Smdt1*, *Uqcr11*, and *Cox7a1* relative to vehicle-treated mice (n = 5-7); and RNA-seq heatmap of cardiac transcripts (n = 5). Data are shown as mean ± SEM. Statistical comparisons between 2 groups were performed using Mann-Whitney U test while comparisons between 3 groups were performed using one-way ANOVA followed by Tukey multiple comparisons test in GraphPad Prism 10. Mouse studies were approved by the University of Alberta Institutional Animal Care and Use Committee and conform to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health. The University of Alberta adheres to the principles for biomedical research involving animals developed by the Council for International Organizations of Medical Sciences and complies with the Canadian Council on Animal Care guidelines. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. S+K = semaglutide and ketone-treated mice; S = semaglutide-treated mice; V = vehicle-treated mice.

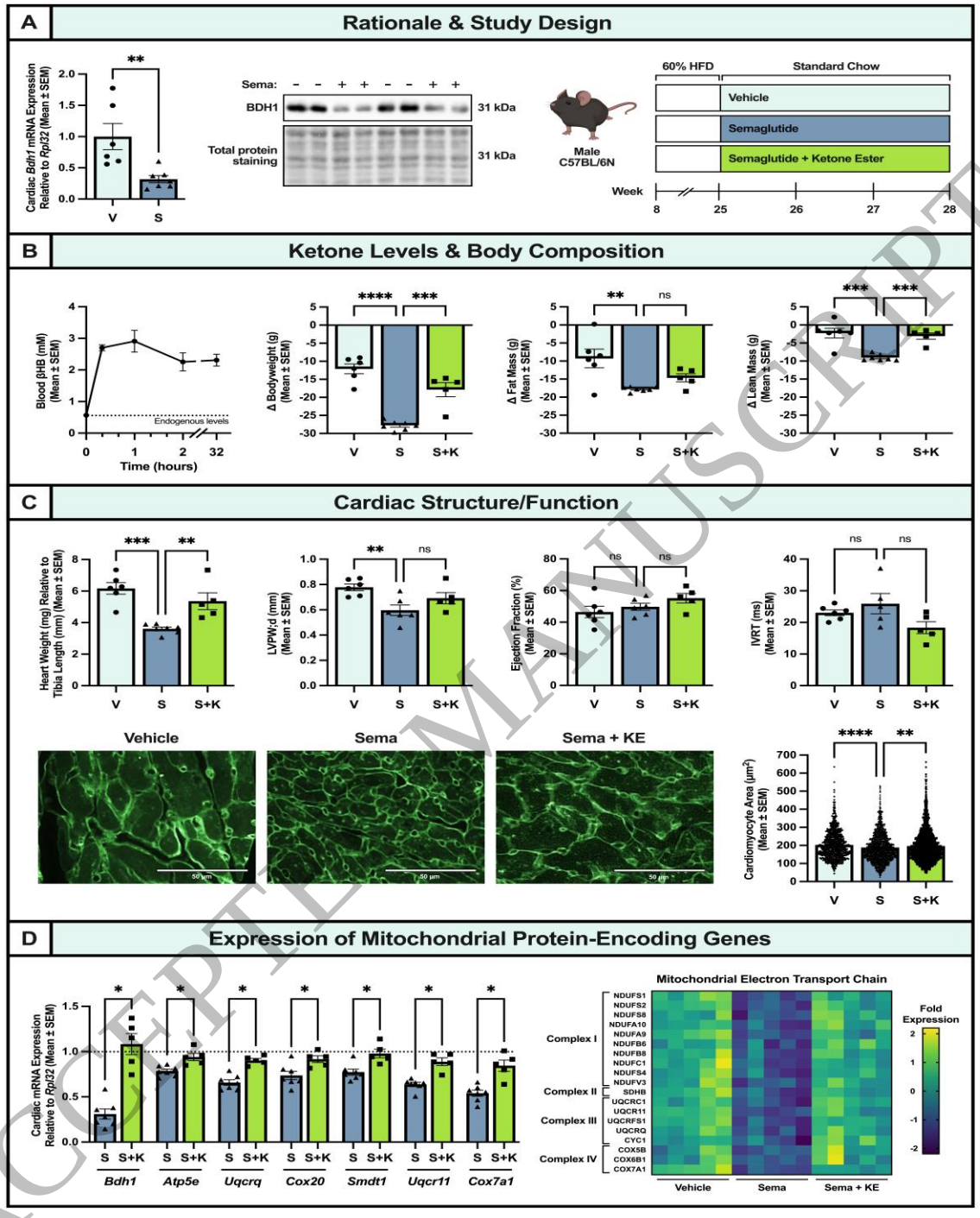

Figure 1

168x243 mm (x DPI)