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Letter

RESEARCH LETTER

Metabolomic Signatures of Myocardial Glucose Uptake on Fluorine-18 Fluorodeoxyglucose Positron Emission Tomography

Fluorine-18 fluorodeoxyglucose positron emission tomography (FDG-PET) plays a critical role in evaluating myocardial inflammation. However, to highlight pathologic myocardial glucose uptake (MGU), physiological MGU must be suppressed, often accomplished through a ketogenic diet (KD).[1] Failure to suppress physiological MGU occurs in ~20% of subjects even following strict, highly supervised dietary interventions,[1,2] potentially leading to misdiagnosis, repeat scans, and unnecessary costs.

In a crossover trial (KEETO-CROSS [Ketogenic Endogenous versus Exogenous Therapies for Myocardial Glucose Suppression]; NCT04275453), we showed that ketone ester (KE) drink (exogenous ketosis) was inferior to the KD (endogenous ketosis) for suppressing MGU.[2] However, circulating ketone (beta-hydroxybutyrate [BHB]) levels strongly predicted myocardial glucose suppression (MGS) within each arm and could be used upstream of FDG-PET to assess myocardial preparation. Furthermore, the ketosis required to suppress the myocardium varied by technique (with significantly higher levels required for KE). To understand whether a common metabolite signature might underlie MGS irrespective of technique, we performed targeted metabolomic plasma profiling paired with 57 FDG-PET scans from 20 participants in KEETO-CROSS. We hypothesized that ketone and fatty acid oxidative markers would associate with MGS, suggesting a mechanism of substrate competition for successful suppression.

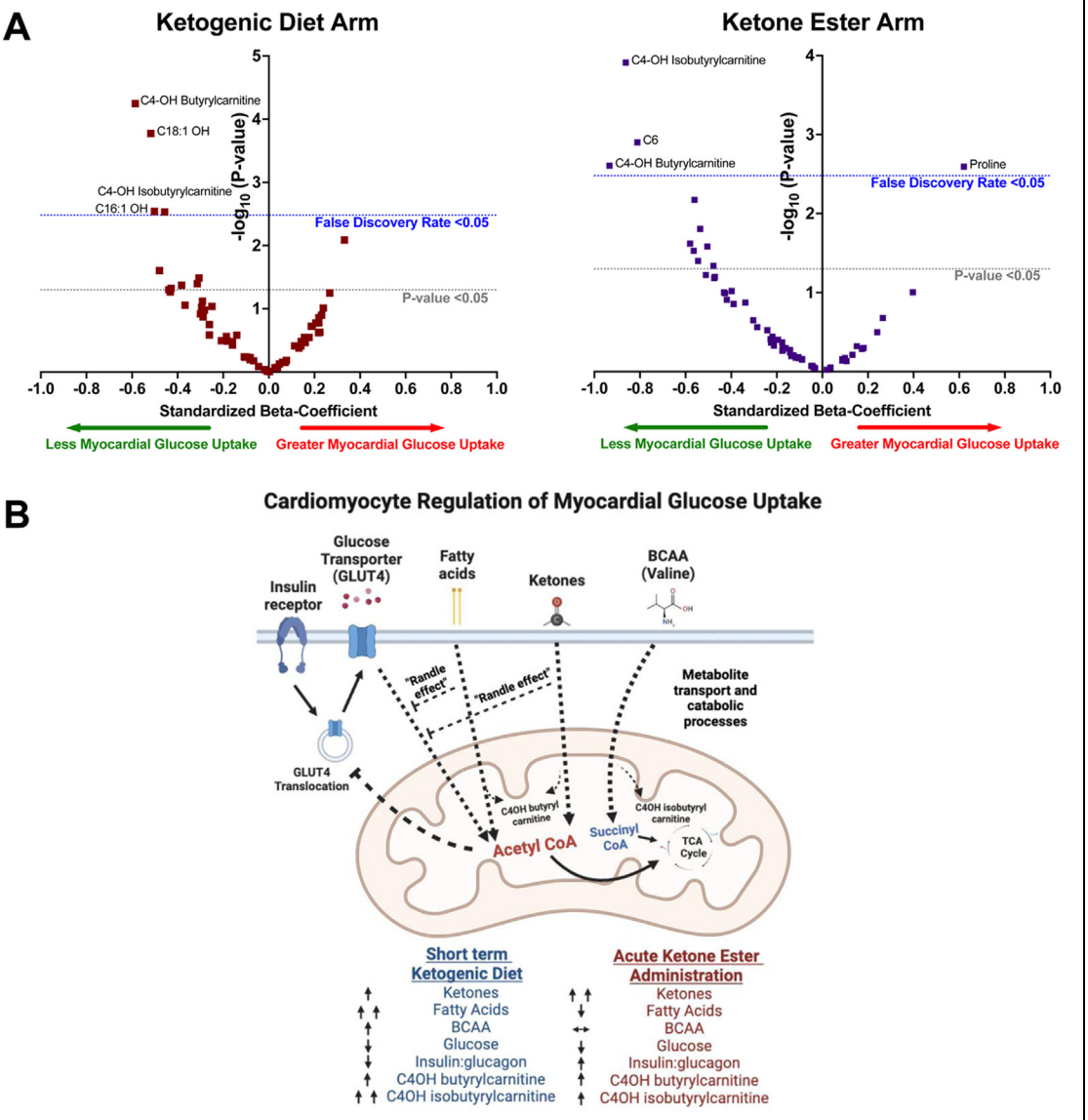
In KEETO-CROSS, participants free of cardiovascular disease (in whom any MGU could be assumed physiological) were randomly assigned to the KE (one visit) or KD (2 visits at 24 and 72 hours) arm, and crossed over after a >1-week washout period.[2] All 20 participants completed the KE arm, whereas 19 and 18 participants completed the 24- and 72-hour KD visits, respectively. The study was approved by an institutional review board, and informed consent was obtained. At these 3 visits, FDG-PET was performed and MGU was analyzed according to a protocol previously described.[2]

Quantitative metabolomic profiling of 30 amino acids and 57 acylcarnitines was performed in fasting, frozen plasma collected at these visits by using liquid chromatography/mass spectrometry (1290/6495 Triple Quadrupole LC/MS, Agilent Technologies). Absolute metabolite quantification was achieved by the addition of stable isotope-labeled internal standards. Twenty-six metabolites with >50% of samples below limits of detection were not analyzed, leaving 61 metabolites for analysis. Metabolite levels below assay limits were analyzed as the lower limit of detection.

Box-Cox transformation and standardization of metabolites into z scores were performed. Linear mixed effects regression models[3] were used to assess the association between each metabolite and MGU, controlling for study visit as a fixed effect, modeling participant as a random effect, and using an independent covariance structure, where applicable. A Benjamini-Hochberg false discovery rate cutoff of 0.05 defined significance. C statistics were calculated to assess the discriminative capability of metabolites for achieving MGS, accounting for clustering at the participant level. Analyses were performed by using STATA version 14 (StataCorp).

The mean age was 30 ± 7 years, 50% were female, 45% were non-White, and the mean fasting time before FDG injection was 16.0 ± 0.9 hours.[2] MGS failure occurred in 11 of 20 scans with the KE and 8 of 37 with the KD. **Figure 1A** depicts volcano plots for the relationship between 61 metabolites and MGU for the 2 KD visits (combined) and the KE visit. During the KD, two C4-OH carnitine species (hydroxybutyrylcarnitine and hydroxy-isobutyrylcarnitine) were negatively associated with MGU. Hydroxybutyrylcarnitines are generated by ketone and fatty acid oxidation, whereas hydroxy-isobutyrylcarnitines are generated during breakdown of the branched-chain amino acid (BCAA) valine. In addition, the long-chain acylcarnitines C16:1-OH and C18:1-OH

FIGURE 1 **Volcano Plot of Targeted Metabolites and Associations With Myocardial Glucose Uptake on Fluorine-18 Fluorodeoxyglucose Positron Emission Tomography**



(A) Volcano plots showing the strength (standardized beta-coefficient) and significance (-$\log_{10}$ [*P* value]) of univariate associations between 61 targeted metabolites with myocardial glucose uptake (ratio of the standardized uptake value of the myocardium to blood pool) during the ketogenic diet arm and the ketone ester arm. **(B)** Simplified schema of metabolic pathways putatively affected by the ketogenic diet and ketone ester and the relationship with myocardial glucose suppression and C4-OH species. BCAA = branched-chain amino acid; TCA = tricarboxylic acid.

(markers of long-chain fatty acid oxidation) were inversely related to MGU. With KE, both C4-OH carnitine species, as well as the short-chain acylcarnitine C6-carnitine, were negatively associated with MGU, whereas proline was positively associated with MGU. Medium-chain acylcarnitines, dicarboxylated acylcarnitine species, and most amino acids (including BCAAs) were not associated with MGU.

Given their common relationship to MGS with both techniques, we assessed C statistics for MGS with C4-OH isobutyrylcarnitine and C4-OH butyrylcarnitine. Both were predictive of MGS (C statistics

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of 0.83 [95% CI: 0.65-1.00] and 0.81 [0.70-0.92], respectively) in all 57 FDG-PET scans, and corresponding odds ratios were 4.93 (95% CI: 0.62-39.43) and 9.59 (95% CI: 3.54-25.97) for MGS per SD increase. Although we previously found that BHB achieved comparable C statistics in the KD and KE arms separately,[2] BHB was not predictive in a combined model (C statistic: 0.57; 95% CI: 0.41-0.74). Furthermore, although BHB levels were significantly higher on KE than the 24- and 72-hour KD visits ($P < 0.001$ and $P = 0.001$), C4-OH isobutyrylcarnitine (although not butyrylcarnitine) levels were higher during KD visits than during KE ($P = 0.018$ and $P = 0.003$).

In KEETO-CROSS, combined markers of enhanced ketone/fatty acid oxidation and BCAA catabolism strongly predicted MGS, had robust discriminatory value, and demonstrated utility in measuring before FDG-PET to reduce the risk of false-positive scans. These findings suggest that strategies to enhance these oxidative pathways, rather than augmenting circulating metabolite levels alone,[1] may be more effective in improving MGS. In addition, our results showing higher C4-OH isobutyrylcarnitine levels with KD explain why, despite achieving substantially higher BHB levels, KE was inferior to KD for MGS (**Figure 1B**). Finally, these biomarkers may act as novel surrogate endpoints during early-phase studies to prioritize high-yield MGS techniques. Limitations include study of a healthy cohort and study of only 2 MGS strategies.

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The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the Author Center.

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