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ORIGINAL PAPER

Comprehensive nutrient consumption estimation and metabolic profiling during ketogenic diet and relationship with myocardial glucose uptake on FDG-PET

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Aims The ketogenic diet (KD) is standard-of-care to achieve myocardial glucose suppression (MGS) for assessing inflammation using fluorine-18 fluorodeoxyglucose–positron emission tomography (FDG-PET). As KD protocols remain highly variable between centres (including estimation of nutrient intake by dietary logs for adequacy of dietary preparation), we aimed to assess the predictive utility of nutrient intake in achieving MGS.

Methods and results Nineteen healthy participants underwent short-term KD, with FDG-PET performed after 1 and 3 days of KD (goal carbohydrate intake <20 g/day). Nutrient consumption was estimated from dietary logs using nutrition research software. The area under receiver operating characteristics (AUROC) of macronutrients (carbohydrate, fat, and protein intake) for predicting MGS was analysed. The association between 133 nutrients and 4 biomarkers [beta-hydroxybutyrate (BHB), non-esterified fatty acids, insulin, and glucagon] with myocardial glucose uptake was assessed using mixed effects regression with false discovery rate (FDR) correction. Median (25th–75th percentile) age was 29 (25–34) years, 47% were women, and 42% were non-white. Median (25th–75th percentile) carbohydrate intake (g) was 18.7 (13.1-30.7), 16.9 (10.4-28.7), and 21.1 (16.6-29.0) on Days 1–3. No macronutrient intake (carbohydrate, fat, or protein) predicted MGS (*c*-statistic 0.45, 0.53, 0.47, respectively). Of 133 nutrients and 4 biomarkers, only BHB was associated with myocardial glucose uptake after FDR correction (corrected *P*-value 0.003).

Conclusions During highly supervised, short-term KD, approximately half of patients meet strict carbohydrate goals. Yet, in healthy volunteers, dietary review does not provide reassurance for adequacy of myocardial preparation since no clear thresholds for carbohydrate or fat intake reliably predict MGS.

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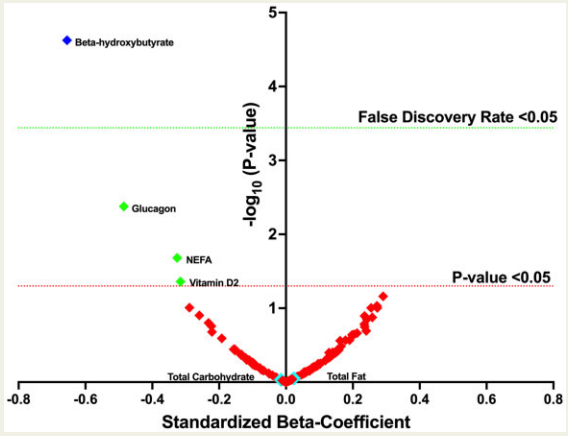
Graphical Abstract



Volcano plot of relationship between nutrient intake, metabolites, and hormones with myocardial glucose uptake on FDG-PET.

Keywords ketogenic diet • FDG • PET • myocardial glucose uptake • carbohydrate

Introduction

Radiolabeled glucose markers [fluorine-18 fluorodeoxyglucose (FDG)] are employed to detect inflammation using positron emission tomography (PET).[1,2] However, such a diagnostic strategy is inherently plagued by the simultaneous, physiologic uptake of glucose that impedes distinction from inflammatory pathologies such as sarcoidosis or myocarditis.[3] Patient preparation through high fat and very low carbohydrate consumption [also known as the ketogenic diet (KD)] facilitates down-regulation of physiologic glucose uptake through the insulin-dependent GLUT4 transporter, while highlighting pathologic uptake through inflammatory cells that constitutively express insulin-independent glucose transporters (GLUT1 and GLUT3).[4] A growing number of cardiovascular pathologies, such as coronary plaque characterization, cardiac tumours, and prosthetic valve endocarditis, likewise rely on suppressing physiologic glucose uptake of the myocardium to optimize diagnostic assessment.[5,6] Appropriate reduction of physiologic glucose uptake is critical to reducing non-diagnostic or false positive scans that may lead to misdiagnosis, inappropriate immunotherapy, repeat scans with excess radiation exposure, and unnecessary costs to the healthcare system.

The KD is standard-of-care for reducing physiologic glucose uptake prior to evaluating myocardial inflammation using FDG-PET.[1] Expert consensus documents recommend a high fat diet that minimizes carbohydrate intake for 24 h prior to FDG-PET to accomplish this metabolic switch.[7] However, ~20% of scans have non-specific myocardial glucose uptake despite dietary modification, though rates range widely.[1,7–10] Recommendations for dietary composition are scant since dietary guidance has not been clearly defined or standardized in the literature.[7] Significant variation exists in how dietary adherence is assessed clinically (if at all). For example, some centres broadly screen dietary logs maintained by participants for significant deviations from the KD, such as high carbohydrate intake above certain thresholds, prior to FDG injection. Understanding the utility of assessing nutrient intake to predict appropriate myocardial glucose suppression (MGS) upstream of FDG-PET remains critical to address unmet needs. Further, we have recently shown that ketone levels strongly predict MGS,[11] and the relationships between comprehensively estimated nutrient intake during the KD and ketosis are less well established.

To bridge these gaps in knowledge, we prospectively evaluated the relationship between 133 nutrients with MGS in healthy participants (in whom any myocardial glucose uptake could be presumed physiologic) after 1 and 3 days of the KD using both hypothesis-driven as well as unbiased approaches. Our goals were to (i) comprehensively detail dietary composition among participants undergoing the KD, (ii) assess discrimination of specific nutrient intake for MGS, and (iii) model the relationship between nutrient intake and achieved ketosis. While our analyses are targeted towards the diagnostic applications of the KD for FDG-PET, these data may also be broadly relevant to therapeutic applications of ketosis (such as in weight loss and epilepsy).[12,13]

Methods

Study design

We enrolled 19 participants aged 18–60 at the University of Pennsylvania between January 2020 and January 2021. We excluded individuals with any reported history of cardiovascular disease (including hypertension, hyperlipidaemia, and diabetes mellitus) or pregnant/breast feeding women. The study was approved by an institutional review board and informed consent was obtained. The deidentified data underlying this article will be shared on reasonable request to the corresponding author with the appropriate ethical approval.

Participants presented for two visits: first after 1 day of KD and second after 3 days of KD. The day before the 1-day visit, participants began a

low-carbohydrate, high-fat diet intended to achieve <20 g of carbohydrate intake per day (Supplementary data online, *Methods*), and fasted from at least midnight until FDG-PET scan the next day. Thereafter, they continued the KD for two additional days, fasting again from at least midnight before the 3-day FDG-PET visit. A total of 18 of the 19 participants completed both study visits, as one participant dropped out due to adverse symptoms from the KD.

A detailed dietary log was maintained by study participants during the dietary arm and reviewed by a nuclear cardiologist prior to FDG injection to broadly screen for significant dietary indiscretions. All participants were considered to have adequate dietary adherence by dietary log review.

Dietary record analysis

Dietary intake data were collected and analysed using Nutrition Data System for Research (NDSR 2020) by a registered dietitian with significant experience with the software who was blinded to other clinical data.[14] In brief, the NDSR is a comprehensive calculation software developed for research purposes which provides a complete nutrient profile for over 18,000 foods in the database. More than 160 nutrients, nutrient ratios, and food components are reported by the software system. General nutrient categories include proximate nutrients, fat components, carbohydrate fractions, protein components, vitamins, minerals, nutrient ratios, and other food components. If an analytic value is not available for a nutrient in a food, NCC calculates the value based on the nutrient content of other nutrients in the same food or on a product ingredient list or estimates the value based on the nutrient content of similar foods. The NDSR software utilizes the multiple-pass system of the interview methodology. Interview prompts guide and standardize data entry.

Metabolite and hormone level analysis

Venous blood was collected via peripheral phlebotomy at the time of FDG injection at each study visit; serum BHB levels were then determined on freshly processed samples using a Beckman–Coulter analyzer (AU5800 or AU680). The reportable range for BHB is 0.02–13.5 mmol/L, and the coefficient of variation through multilevel, internal quality control ranges from 0.8% to 2.6%. Insulin, glucagon, and non-esterified fatty acid (NEFA) were analysed in bulk at the Penn Radioimmunoassay and Biomarkers Core. Blood samples were collected on ice into tubes on ice containing EDTA and protease inhibitor cocktail (Sigma–Aldrich, St. Louis, MO, USA). Samples were centrifuged at 4°C, separated, and frozen at -80°C for subsequent analysis. NEFA levels (range 0.125–2.9 mM) were measured in duplicate using enzymatic colorimetrics (Wako Chemicals, Richmond, VA, USA). Plasma insulin (range 2–100 mIU/mL) and glucagon (range 20–400 pg/mL) were measured in duplicate by double-antibody radioimmunoassay (Millipore, Billerica, MA, USA).

Baseline laboratory analysis

In addition to metabolite and hormone data, a basic metabolic panel, complete blood count, and lipid panel were also obtained after fasting overnight for baseline data on a visit outside of the KD protocol. Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) was calculated as the product of glucose (mg/dL) and insulin (mIU/L) levels divided by 405.

FDG-PET protocol and study outcomes

Participants underwent FDG-PET using a PET/CT scanner (Philips Ingenuity TF, Cleveland, OH). At study visits, FDG [~185 MBq (~5.0 mCi)] was injected and PET images of the chest were acquired 1 h later in 3D list mode. Low-dose CT images were also acquired for attenuation correction purposes and to aid in distinguishing FDG activity in the myocardium and blood pool on fused PET/CT images. Images were analysed using MIM software (Cleveland, OH) by a board-certified nuclear cardiologist blinded to participant characteristics. We primarily evaluated complete MGS, defined by FDG activity in all segments of the myocardium lower than the blood pool.[1] We secondarily evaluated the ratio of the average myocardial to blood pool standardized uptake value (SUV) in the septal and lateral walls.

Statistical analysis

Baseline characteristics were described using medians and 25th–75th percentiles or counts and percentages as appropriate. Biomarker levels were compared using the Wilcoxon signed-rank test.

We assessed receiver operating characteristics of macronutrients using *c*-statistics to predict achievement of MGS, accounting for clustering at the participant level as participants contributed longitudinal biomarker values paired with FDG-PET data at the 1- and 3-day visits. *c*-Statistics for carbohydrate consumption were displayed modelling the risk for MGS failure since higher carbohydrate intake is theoretically inversely related to MGS,while other nutrients were modeled for the risk of MGS success. *P*-values are displayed with the null hypothesis of an area under the receiver operating characteristics curve (AUROC) of 0.5. We also calculated correlation coefficients using repeated measures techniques according to a previously established methodology to account for participant-level effects.[15]

We next applied Box-Cox transformation and standardization of variables into *Z*-scores[16] to assess the association between 133 nutrients and 4 biomarkers with glucose uptake on FDG-PET using a longitudinal, mixed effects, linear regression model. We controlled for study visit and modelled study participant as a random effect. Dietary intake was defined using estimated consumption in the day preceding each visit at 1 and 3 days, and only unique nutrients in whom intake was present in at least 50% were analysed. A Benjamini–Hochberg false discovery rate (FDR) cut-off of 0.05 defined significance to control for multiple comparisons.

Finally, we performed linear regression to assess the relationship between change in macronutrient intake levels with change in log transformed ketone levels (from the 1-day to 3-day visits), adjusting for Day 1 visit values.

Analyses were performed using STATA version 14 (STATA Corp, College Station, TX, USA), RStudio (Boston, MA), and GraphPad Prism v.9 (San Diego, CA, USA). Analyses used a two-sided *P*-value <0.05 for statistical significance.

Results

Baseline characteristics

Baseline characteristics of the 19 participants are shown in *Table 1*. The median (25th–75th percentile) age was 29 (25–34) years, 47% were women, and 42% were non-white. Body-mass index [23.6 (22.2–25.3) kg/m^2] and systolic blood pressure [120 (107–126) mmHg] fell within normal ranges. Laboratory testing was consistent with a healthy cohort. Time of fasting prior to FDG injection was 16.4 (15.6–17.9) h at the 24-h visit and 15.5 (13.9–17.1) h at the 72-h visit. Changes in average myocardial glucose uptake by participant during the two visits are shown in Supplementary data online, *Figure S1*.

Table 1 Baseline characteristics of the study population

	All study participants N = 19
Age (years)	29 (25–34)
Women, *n* (%)	9 (47)
Race, *n* (%)	
White	11 (58)
Black	3 (16)
Asian	4 (21)
Other	1 (5)
Number of alcohol drinks (per week)	7 (4–10)
Current smoker	1 (5%)
Physical characteristics	
Systolic blood pressure (mmHg)	120 (107–126)
Diastolic blood pressure (mmHg)	74 (70–82)
Heart rate (beats/min)	71 (60–82)
Body mass index (kg/m^2)	23.6 (22.2–25.3)
Laboratory testing	
Creatinine (mg/dL)	0.8 (0.7–0.9)
Glucose (mg/dL)	82 (76–89)
Haemoglobin (g/dL)	13.6 (12.1–14.3)
Low-density lipoprotein cholesterol (mg/dL)	104 (88–112)
High-density lipoprotein cholesterol (mg/dL)	54 (50–63)
Triglycerides (mg/dL)	80 (62–95)
HOMA-IR	2.0 (1.7–2.4)
Beta-hydroxybutyrate (mmol/L)	0.11 (0.10–0.26)
Glucagon (pg/mL)	65 (48–82)
Insulin (mIU/L)	10.8 (8.1–12.4)
Non-esterified fatty acids (mmol/L)	0.39 (0.30–0.54)

Continuous variables presented as median (25th–75th percentile).
HOMA-IR, homeostatic model assessment of insulin resistance.

Nutrient intake and biomarker levels during the KD

Selected nutrient consumption by day is shown in *Table 2*. Of note, Day 2 intake levels were generally lower than other days because participants were fasting prior to FDG-PET performed on that day. Median kilocalorie levels were <1500 during each day of dietary modification. Median percent calories from carbohydrates (5–7%), protein (35–37%), and fat (56–57%) were consistent with the KD. All carbohydrate intake was derived from plants and vegetables, and none were reported from grains and legumes. Consumption of simple sugars was infrequent. The goal of <20 g of carbohydrate consumption per day was achieved in 53% on Day 1 and 33% on Day 3. Fat consumption tended to be from monounsaturated and saturated fat sources. Comprehensive profiling of a total of 133 nutrients during the 3 days of the KD is detailed in Supplementary data online, *Table S1*.

Utility of nutrient intake and biomarker levels for predicting MGS

We next calculated *c*-statistics for predicting MGS using macronutrient levels combining both visits on Days 1 and 3. Of the 37 scans occurring at these two visits, there were 8 failures (22%) of MGS, with 5/19 (26%) occurring after 1 day of KD and 3/18 (17%) occurring after 3 days of KD. Surprisingly, carbohydrate intake during the KD was not predictive of MGS [*c*-statistic 0.45 (95%CI 0.13, 0.78)], and neither were fat [0.53 (95% CI 0.27, 0.79)] nor protein 0.47 (95% CI 0.23, 0.71) intake ($P > 0.70$ for all comparisons). *Figure 1* shows AUROC curves for predicting MGS among macronutrients. *Figure 2* demonstrates poor discrimination for MGS by carbohydrate and fat intake, depicting the relationship between individual data points and MGS success or failure.

Since we have previously shown BHB as a strong discriminator for MGS, we assessed the relationships between biomarkers and macronutrient consumption using correlation heatmap analysis (*Figure 3*). BHB was directly correlated with glucagon ($r = 0.79$, $P < 0.05$), though relationships with other biomarkers and estimated macronutrient consumption among participants undergoing the KD were not statistically significant ($P > 0.05$ for all comparisons).

In an unbiased approach, we next assessed the relationship between 133 nutrients (Supplementary data online, *Table S1*) and 4 biomarkers (BHB, insulin, glucagon, and NEFA) with myocardial glucose uptake, which is depicted in *Figure 4* as a volcano plot. Of these 137 variables, only BHB was significantly associated with myocardial glucose uptake after FDR correction (beta coefficient = -0.66, corrected *P*-value 0.003). Two biomarkers (glucagon and NEFA) and 1 nutrient (vitamin D2) were negatively associated with glucose uptake at a nominal (uncorrected $P < 0.05$) significance level (beta-coefficients -0.49, -0.33, and -0.32, respectively). Notable nutrients that were not associated with glucose uptake at even nominal significance included total carbohydrate, total fat, glycaemic index, glycaemic load, and starch intake.

Individual-level relationship between ketosis and macronutrient intake

Since there was no significant relationship between carbohydrate intake and ketosis achieved at each study visit, which may be due to significant inter-participant variability in biological factors affecting ketogenesis, we then assessed intra-participant level changes in carbohydrate intake and ketosis. Between the 1- and 3-day visits, there was no association between change in carbohydrate intake and change in log BHB levels ($P = 0.90$). Likewise, there was no association between change in fat intake ($P = 0.29$) or change in the ratio of fat/carbohydrate intake ($P = 0.79$) with change in log BHB levels.

Discussion

The KD is a standard-of-care intervention for achieving MGS for FDG-PET, yet data from a rigorous evaluation of the relationship between nutrient intake and MGS has not been performed. In our study of healthy participants who underwent detailed nutrient phenotyping during short-term dietary modification, our analyses highlighted several important findings for clinical care. First, most patients in a highly supervised setting with dietary guidance do not achieve strict carbohydrate reduction that is often recommended. Regardless, carbohydrate and fat intake poorly discriminated MGS, compared with BHB which we have shown to be a strong predictor of MGS.[11] Next, BHB poorly correlated with macronutrient intake, and intra-individual,

Table 2 Select nutrient consumption and biomarker levels during short term ketogenic diet

	Day 1 (*N* = 19)	Day 2 (*N* = 18)[a]	Day 3 (*N* = 18)
Nutrient intake			
Energy (kcal)	1324.1 (891.2–1616.2)	983.4 (661.9–1196.7)	1426.6 (1094.2–1640.1)
Total grams	1557 (1088–2447)	1050 (712–1852)	1471 (1030–2375)
Calories from carbohydrate (%)	5.6 (3.3–8.4)	6.7 (4.0–12.7)	5.7 (3.7–9.2)
Total carbohydrates (g)	18.7 (13.1–30.7)	16.9 (10.4–28.7)	21.1 (16.6–29.0)
Carbohydrate intake <20 g	10 (52.6%)	11 (57.9%)	6 (33.3%)
Fructose (g)	2.4 (1.2–3.6)	2.1 (1.1–2.6)	2.5 (1.6–3.2)
Glucose (g)	3.2 (2.4–7.0)	2.7 (1.6–5.3)	4.3 (3.6–5.8)
Lactose (g)	0.2 (0.0–3.1)	0.0 (0.0–0.0)	0.8 (0.0–3.1)
Sucrose (g)	0.5 (0.3–1.1)	0.7 (0.2–1.0)	0.7 (0.3–1.4)
Starch (g)	0.4 (0.0–2.1)	1.0 (0.1–2.6)	1.2 (0.0–1.8)
Total dietary fibre	7.3 (3.8–11.7)	5.7 (3.2–9.9)	7.0 (5.7–11.2)
Calories from protein (%)	36.6 (31.7–45.0)	35.7 (27.9–46.1)	35.5 (29.5–41.8)
Total protein (g)	110.4 (71.6–178.3)	85.6 (46.0–138.7)	111.2 (89.9–150.2)
Animal protein (g)	105.0 (66.9–165.7)	78.0 (40.7–128.7)	103.5 (82.9–147.3)
Vegetable protein (g)	5.2 (2.7–8.1)	3.8 (3.1–10.1)	5.3 (3.5–8.2)
Calories from fat (%)	56.2 (49.1–62.6)	56.8 (50.4–62.2)	56.4 (51.6–65.0)
Total fat (g)	77.6 (57.8–97.9)	54.3 (41.6–86.2)	89.0 (59.4–116.1)
Total saturated fat (g)	19.6 (14.8–24.2)	17.5 (12.1–21.2)	24.7 (18.0–37.1)
Total monounsaturated fat (g)	37.0 (27.0–51.0)	23.3 (17.6–34.9)	37.7 (25.5–46.7)
Total polyunsaturated fat (g)	13.3 (8.1–17.7)	9.3 (4.9–14.6)	13.1 (9.2–18.6)
Metabolites and hormones			
Beta-hydroxybutyrate (mmol/L)	0.76 (0.57–1.02)	NA	1.30 (0.80–2.24)
Glucagon (pg/mL)	67 (56–78)	NA	93 (75–118)
Insulin (mIU/L)	7.6 (5.2–9.7)	NA	6.6 (5.1–10.2)
Non-esterified fatty acids (mmol/L)	0.83 (0.63–0.97)	NA	0.96 (0.78–1.09)

Continuous variables presented as median (25th–75th percentile).
NA, not applicable.
[a]Day 2 nutrient intake values are lower than other days due to patients fasting prior to positron emission tomography scan.

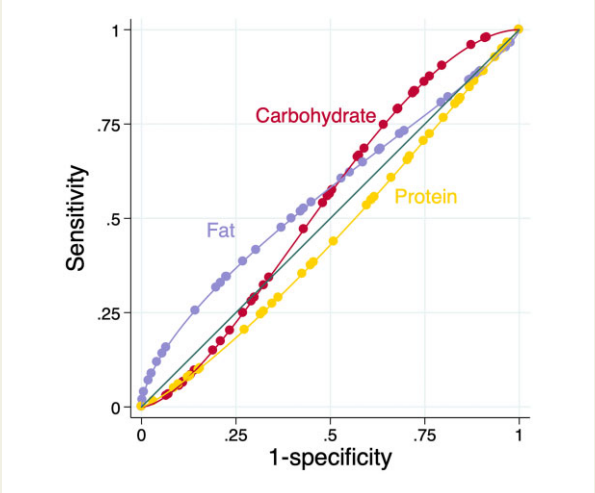


Figure 1 AUROC for macronutrient intake to predict myocardial glucose suppression during ketogenic diet. Receiver operating characteristics curve shown for total carbohydrates, fat, and protein during the ketogenic diet to predict the primary outcome. AUROC, area under the receiver operating characteristics curve.

longitudinal changes in carbohydrate and fat intake were not associated with changes in BHB levels. Finally, comprehensive analysis of 133 nutrients and 4 biomarkers revealed that only BHB was associated with myocardial glucose uptake after correction for multiple hypothesis testing. Despite common practice to review dietary logs for carbohydrate and fat intake, our findings indicate that (i) dietary review should not provide reassurance for adequacy of myocardial patient preparation, (ii) no clear thresholds for carbohydrate or fat intake reliably result in MGS, and (iii) ketone levels are substantially stronger markers for MGS than estimated nutrient intake and centres should consider assaying these levels prior to FDG-PET instead of dietary review.

Expert consensus documents provide some guidance on dietary goals of the KD for patient preparation before FDG-PET based upon scant data.[7] While a goal of at least two meals of <3 g of carbohydrates and >35 g fat in the day prior to FDG-PET combined with 4–12 h of fasting has been proposed,[7] this is based upon a synthesis of several non-standardized studies with varying protocols for dietary modification.[1] Our results indicate that, among participants undergoing the KD, carbohydrate and fat intake poorly predicts adequacy of patient preparation. We further assessed 133 nutrients to parse out whether certain elements of the KD (such as carbohydrate or fat

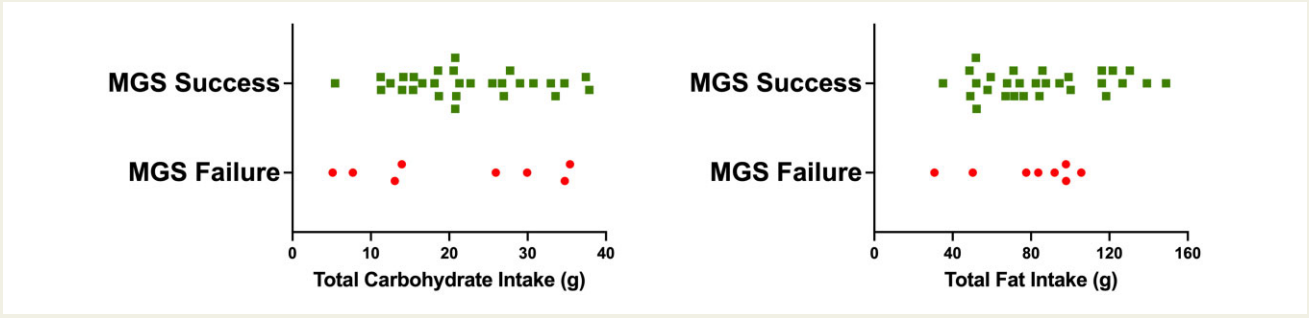


Figure 2 Visit level data of fat and carbohydrate intake for predicting myocardial glucose suppression. Levels of carbohydrate (left) and fat (right) intake in the day prior to positron emission tomography and relationship to myocardial glucose suppression. MGS, myocardial glucose suppression.

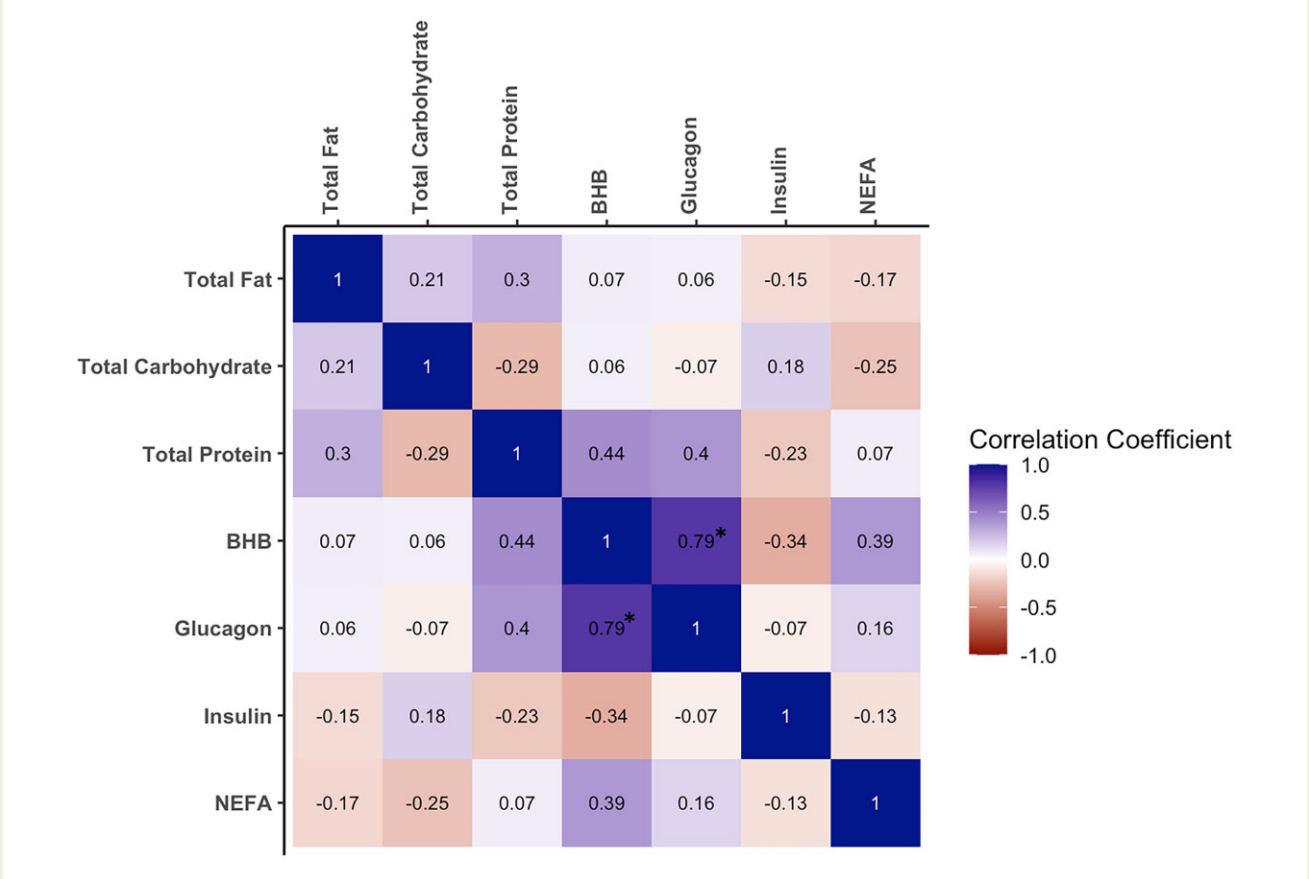


Figure 3 Correlation matrix heatmap for macronutrient intake, metabolites, and hormones. Correlation heatmap using repeated measures correlation among selected macronutrients as well as serum biomarkers. Asterisk denotes statistical significance ($P < 0.05$). BHB, beta-hydroxybutyrate; NEFA, non-esterified fatty acid.

source, glycaemic load, or ketogenic amino acids) were more useful for reducing myocardial glucose uptake. Yet, none met significance after multiple hypothesis testing. We have previously shown ketone levels to strongly discriminate MGS,[11] and now demonstrate here the magnitude of their superiority to nutrient predictors. The use of point-of-care devices may further improve clinical throughput.[17,18]

It is important to note that all participants underwent KD in our analysis. While we did not find a relationship between carbohydrate and fat intake with ketosis, this does not imply that the KD is superfluous (or that dietary carbohydrate restriction does not result in ketosis). Indeed, the relationship between carbohydrate reduction (from high levels >100 g to <30 g) with ketogenesis has been known for

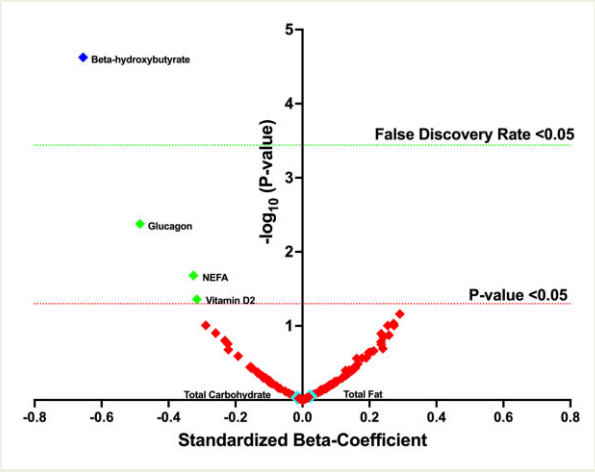


Figure 4 Volcano plot of macronutrient intake, metabolites, and hormones associated with myocardial glucose uptake on FDG-PET. Volcano plots showing the strength (standardized beta-coefficient) and significance (-$\log_{10}$ (*P*-value)) of univariate associations between 133 nutrients and 4 biomarkers with myocardial glucose uptake on FDG-PET (assessed using the ratio of the standardized uptake value of the myocardium to blood pool). Parameters reaching at least nominal significance are labelled, as well as total carbohydrate and fat as parameters of interest (the latter are highlighted in light blue). FDG, fluorine-18 fluorodeoxyglucose; NEFA, non-esterified fatty acid; PET, positron emission tomography.

decades.[19] However, in a restricted range of carbohydrate intake, no further relationship existed in our study.

We offer two hypotheses to account for this finding. First, despite the instructions and guidance for dietary logging and use of dedicated research software for nutrient intake estimation, sources of error exist through incomplete capturing of dietary intake (at the participant level) and estimation of nutrient consumption (at the software level). Dietary intake estimation through analysis of dietary logs is a crude and arduous process, particularly in comparison with the precision and ease of biomarker analysis. This is critical to consider since dietary logs are commonly performed in the clinical setting to evaluate adequacy of patient preparation. Second, significant individual level factors regulate ketogenesis, such as epigenetics, post-translational modification, insulin sensitivity, mitochondrial function, and beta-oxidation.[20] To attempt to control for adynamic, inter-individual level factors by examining intra-individual changes in carbohydrate intake and ketone levels, we still found no significant relationship. Static factors (such as age or genetics) are therefore unlikely to account for the lack of association between carbohydrate intake and ketosis in a restricted range. These data may also be relevant to individuals who employ the KD for therapeutic reasons, for example, for weight loss,[12] or in certain situations, epilepsy management.[13] As monitoring for ketosis has been increasingly common through point-of-care tests,[21] a strategy of very strict reduction in carbohydrates, as opposed to strict reduction, may not engender greater ketosis in these situations.

There are some potential limitations. First, our study sample is modest in size and therefore we cannot rule out associations between specific nutrients and MGS. However, our findings suggest they are unlikely to predict MGS as strongly as BHB. Second, our findings require separate validation in patients with systemic inflammatory diseases, infections, and cardiomyopathy.[22] Third, scans were not protocoled to assess more rigorous measures of dynamic FDG uptake, such as the Patlak slope, and thus, we are unable to compare dynamic patterns of glucose uptake in non-suppressed myocardium with nutrient intake data and BHB levels.[23] However, this limitation is unlikely to change the overall study findings. Strengths include a strict definition of complete MGS, longitudinal design with 1- and 3-day assessments with FDG-PET, and (to our knowledge) the first study employing comprehensive phenotyping including 137 relevant nutrients and biomarkers.

In summary, among healthy participants undergoing short-term KD prior to FDG-PET, most participants do not achieve strict carbohydrate intake reduction recommended. Yet, nutrient consumption still poorly predicted MGS. While serum ketone levels are known to be strongly associated with reducing myocardial glucose uptake, they were not significantly associated with macronutrient intake of carbohydrates or fat in a restricted range. These data do not support current practice to base clinical decisions for adequacy of patient preparation on dietary history, though need to be confirmed in clinical populations referred for testing.

Supplementary data

Supplementary data are available at *European Heart Journal - Cardiovascular Imaging* online.

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