

## Supplementary Online Content

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### **eAppendix.** Supplemental Methods

**eFigure.** Receiver Operating Characteristic (ROC) Curve Analysis for Comparison of the Model Presented Herein and Models Based on Caloric or Carbohydrate Content Alone for Classifying High PPGR Responses

This supplementary material has been provided by the authors to give readers additional information about their work.

## **eAppendix. Supplemental Methods**

The current study uses an approximate methodology to that described in Zeevi (2015)<sup>1</sup>.

### **Human Research Subject Protection**

Participants were recruited from October, 2016 to December, 2017, in Olmsted and Hennepin Counties in Minnesota, and Duval County in Florida, United States, completed this study, which was approved under the Mayo Clinic Institutional Review Board protocol number 16-005208.

### **Inclusion and exclusion criteria**

Inclusion criteria for participation in our study included men and women above 18 years of age, with access to a mobile device and web browser. Individuals were excluded if they were under 18 years of age, were pre-diagnosed with type I or type II diabetes mellitus, used antibiotics in the 3 months prior to the study participation, were pregnant, were substance abusers, had a chronic medical condition, treatment or medication known to affect glucose metabolism, had undergone bariatric weight loss surgery, had taken fertility treatments in the 3 months prior to the study participation, had undergone chemotherapy or radiation treatment for cancer within the last 2

years or had active cancer, had a chronic gastrointestinal disorder, had chronic anemia, or were unable to safely perform finger pricking.

### **Microbiome sample collection, processing and analysis**

In the two days prior to the beginning of the study week, participants were asked to provide a stool sample using an OMNIgene-Gut stool collection kit (DNA Genotek). Samples were shipped to the DayTwo processing facility in Israel. Genomic DNA was purified using PowerMag Soil DNA isolation kit (MoBio) optimized for Tecan automated platform. Illumina compatible libraries were prepared as described<sup>2</sup>, and sequenced on an Illumina Nextera 500 (75bp, single end). Reads were processed with Trimmomatic (to remove reads containing Illumina adapters, filter low quality reads and trim low quality read edges; version 0.32, parameters used: -phred33 ILLUMINACLIP:<adapter file>:2:30:10 LEADING:25 TRAILING:25 MINLEN:50<sup>3</sup>). Reads mapping to host DNA were detected by mapping with GEM<sup>4</sup> (parameters used: -q offset-33 --gem-quality-threshold 26 -e 0.1 --min-matched-bases 0.8 --max-big-indel-length 15 -s 3 -d 200 -D 1 -v -m 0.05]) and removed from downstream analysis. All samples were subsequently down-sampled to a depth of 5M reads. Samples with fewer reads were removed from further analysis, leaving us with a reduced sample of participants that was used for downstream microbiome analyses. Relative

abundances for members of the microbial community were obtained with MetaPhlAn2<sup>5</sup> (default parameters).

### **Connection meeting**

Participants were asked to attend a connection meeting at the beginning of the study week. During this meeting, study staff provided a review of the study purpose and requirements and offered an opportunity for questions. Trained study staff also measured height, weight, waist and hip circumference. They took blood pressure and pulse, drew blood for estimation of glycosylated hemoglobin (HbA1c) levels (for which fasting was not required), attached the continuous glucose monitor and provided standardized meals. Study participants were instructed on the use of the food and activity logging mobile application to be used throughout the week and of the manual blood glucose monitor (Bayer Contour Next Link Glucometer). The latter was used to calibrate the readings obtained with the continuous glucose monitor.

### **Data collection during study week**

During the study week, subjects were asked to wear the continuous glucose monitor, complete manual glucose monitoring at least four times a day for added accuracy, and log food intake (including meal content, duration and time)

on the DayTwo Food and Activity Logger. They were asked to maintain their normal eating habits for the week, except for four breakfasts, which were composed of defined food items and provided by the study team.

### *Food logging*

Meals were logged by the participants using a mobile device application available for both iOS and Android devices. Participants were asked to choose the food items consumed from the MyNetDiary™ database with over 400,000 items for which nutrient content information is available and to log the amount consumed, and the time and duration of the meal. Reported meal times were rounded to the closest 5 minutes interval. Meals logged less than 30 min apart were merged. We also removed meals with very large (>1 kg and >20Kcal) components, meals with incomplete logging and meals with unreasonable nutritional values (defined as meeting one or more of these conditions: > 5000Kcal, >500g Sugar, >1000g carbs, >1000g protein, >500g fat, >5000mg sodium, >600mg caffeine, >300g dietary fibers), as they were likely to be the result of logging errors. Meals logged within 90 min of other meals and meals consumed at the first and last 12 hr of the connection week were not used for algorithm training, to avoid inaccuracies in postprandial sugar response measurements. Lastly, meals with > 40g carbs and postprandial glycemic

response values  $< 5 \text{ mg/dl}\cdot\text{h}$  were excluded from analysis as they likely represent meal misreporting.

### *Standardized meals*

To allow comparison of glycemic responses across all individuals in the study, participants were supplied with two sets of standardized foods representing typical Midwestern breakfast foods that were to be eaten alone, as first meal of the day: plain bagel with cream cheese, cereals (participants chose one of three brands) with or without a cup of milk (milk was not supplied and participants were instructed to use either soy milk or 2% milk).

### *Blood glycemic response measure*

Glucose was measured using the iPro2™ continuous glucose monitor (CGM; Medtronic, MN, USA), which measures interstitial glucose levels every 5 minutes using the subcutaneous Enlite™ sensors. CareLink online software (Medtronic) was used to perform calibration for CGM measurements, as directed by the iPro2 manual. Subjects were connected to the continuous glucose monitor for 6 days.

Subjects with corrupted CGM files that could not be processed were excluded from the dataset used to train the predictive model, as well as from the validation of accuracy step. Only data from subjects that had both a valid CGM and over 5M reads depth in the microbiome samples were used for training the postprandial glycemic response predictive model.

## **Postprandial glyceic responses calculation**

Postprandial glucose responses were computed following Zeevi et al.<sup>1</sup>: logged meal times and continuous glucose measurements were used to calculate the incremental area under the curve following a meal as previously described <sup>6</sup>. To reduce noise, the median of all glucose values from the 30- minute period prior to the meal was taken as the initial glucose level, above which the incremental area was calculated. Missing values in up to 25 consecutive minutes were interpolated. Finally, as in Zeevi et al.<sup>1</sup>, since models were trained with the objective of lowering root mean squared error, PPGR values were truncated to the range of [0, 80] mg/dl\*h to discourage the models from trying to fit rare (< 2%) and extreme values at the expense of the much more common low PPGR values. Following this interpolation, any meals that still had incomplete glucose measurements in the time window of 30 minutes before and 2 hours after the logged meal time were filtered out.

## **Predictive model creation**

We predicted PPGR values based on stochastic gradient boosting regression, using the XGBoost (version 0.6, <https://xgboost.readthedocs.io/en/latest/>,<sup>7</sup>), XGBRegressor class. A total of 72 features were used as the pool of available

features for model, including: (1) Meal features - amount of carbohydrates (g), fat (g), protein (g), sugar (g), dietary fiber (g), energy (kcal), alcohol (g), caffeine (mg), sodium (mg), water (g), and the ratio between carbohydrates and fat in each meal; (2) Meal context features - carbohydrates (g) consumed in 3,6,12 hours prior to meal, energy (kcal) consumed in 2,3,6,12 hours prior to meal, total dietary fibers consumed in 12, 24 hours prior to meal, and the hour at which the meal was consumed; (3) blood tests - HbA1c% values; (4) personal features - age, sex, weight, body-mass index, physical activity frequency and duration, stress, sleep quality, past and current smoking behavior, defecation routines, midday, morning and general hunger levels; (5) CGM derived features - CGM dynamic range, defined as the difference between 95% and 10% overall CGM percentile values, incremental area under the curve of 2,4 hours prior to meal, glucose trends for 1,2,4 hours prior to meal, and the blood glucose level 1 hour prior to meal relative to the overall median glucose level; (6) Microbiome features - metagenome based (metaphlan derived) relative abundances for 23 taxa used by in a previously described predictive model that for an Israeli cohort<sup>1</sup>. These constituted the phyla Actinobacteria, Firmicutes, Proteobacteria, Bacteroidetes and Verrucomicrobia, the family Lachnospiraceae, the genus *Subdoligranulum*, and the species *Eubacterium siraeum*, *Roseburia hominis*, *Akkermansia muciniphila*, *Alistipes finegoldii*, *Alistipes onderdonkii*, *Roseburia inulinivorans*, *Ruminococcus bromii*, *Bacteroides thetaiotaomicron*, *Bacteroides uniformis*,

*Bacteroides vulgatus*, *Bacteroides xylanisolvens*, *Eubacterium eligens*, *Eubacterium rectale*, *Bacteroides dorei*, *Alistipes putredinis* and *Parabacteroides distasonis*. Given the particular microbial characteristics of the Midwestern population, we also used the abundances of *Prevotella* and *Bacteroides* genera as features in the model.

### **Model performance evaluation**

Model performance was assessed by 10-fold cross validation, in which participants are divided to ten parts, the model is trained on nine parts, containing both American and Israeli participants, and the performance is measured by the ability to accurately predict meals reported by the left-out participants (out-of-bag predictions). Prediction results on Midwestern participants from all left folds were aggregated and Pearson's correlation with measured PPGR was reported.

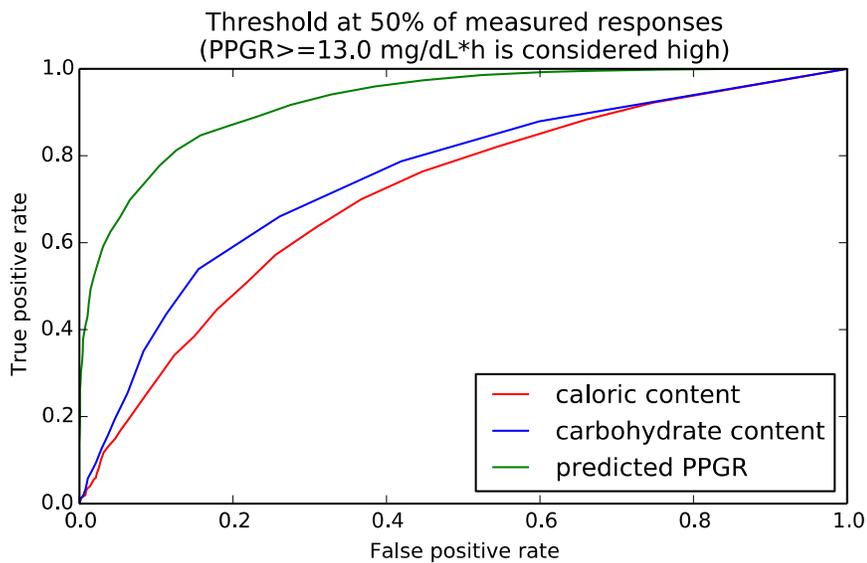
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**eFigure.** Receiver Operating Characteristic (ROC) Curve Analysis for Comparison of the Model Presented Herein and Models Based on Caloric or Carbohydrate Content Alone for Classifying High PPGR Responses, Where "High PPGR" Was Defined as the 50th (A) and 90th (B) Percentile Value of All Measured PPGR Responses in the US Cohort

A



B

