Faecalibacterium predicts postprandial glucose control following potatoes in overweight females

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Abstract

Background: Individual glycemic responses following dietary intake result from complex physiological processes and can be influenced by physical properties of foods, such as increased resistant starch (RS) from retrogradation of starch upon cooling after cooking. Predictive equations are needed to provide personalized recommendations for those individuals most at risk for poor metabolic outcomes.

Methods: Thirty overweight women with no comorbid conditions participated in this randomized crossover trial, in which the women consumed 250g of hot (9.2 g RS) or cold (13.7 g RS) potatoes. Baseline characteristics included demographics, 10-day dietary records, body composition, and the relative abundance (RA) and α-diversity of gut microbiota. Elastic net regression using 5-fold cross-validation predicted postprandial glucose response (PPGR; incremental $AUC_{0\text{-}120min}$) following the potatoes.

Results: Thirty participants (29.6 ± 6.0 yrs; BMI 32.8 ± 3.7 kg/m2) participated in this trial. Most women (70%) showed a favorable PPGR to the cold potato. The model explained 32.2% of the variance in $iAUC_{0\text{-}120min}$ glucose with the equation: 547.65 x (0 [if cold potato], x 1 [if hot potato]) + (BMI[kg/m2] x 40.66) - (insoluble fiber[g] x 49.35) + (*Bacteroides*[RA] x 8.69) - (*Faecalibacterium*[RA] x 73.49) - (*Parabacteroides*[RA] x 42.08) + (α-diversity x 110.87) + 292.52.

Conclusion: This model improves understanding of baseline characteristics that explain interpersonal variation in PPGR following potato intake and offers a tool to optimize dietary recommendations for a commonly consumed food. Larger studies are warranted to expand generalizability and application of the equation.

Trial Registration: The National Clinical Trials number is NCT03310476, and this study was registered with clinicaltrials.gov on Oct 16, 2017.

Background

Resistant starch (RS) is a bioactive fiber found naturally in certain foods. Health benefits associated with RS intake include lower postprandial glucose and insulin and an improvement in enteroendocrine hormones (1–9). Potatoes contain RS that can increase in concentration based on preparation method (10). Cooked then chilled potatoes contain higher amounts of RS compared to cooked, non-chilled potatoes through the process of retrogradation (11). The process of altering preparation methods to optimize RS from potatoes, one of the most commonly consumed high-RS foods (12), had not been investigated to promote a glycemic benefit until recently(13). Previous studies demonstrate an improvement in glycemic biomarkers following the intake of Russet potatoes with varying RS concentrations that resulted from different cooking and preparation methods. Significant reductions in insulin and glucose-dependent insulinotropic peptide (GIP) area under the curves (AUC) after 120 minutes postprandial were found following the cold potato compared to a hot potato; however, not all of the

participants responded favorably to the cold potato in terms of lower postprandial glucose response (PPGR).

To better understand inter-individual differences, the field of precision nutrition identifies factors that contribute to individual responses to dietary interventions. Most studies investigating responses to RS interpret results as collective means and do not consider variability among individuals or factors driving inter-individual differences. Since postprandial physiological responses depend on several complex biological and metabolic processes, this oversimplification of PPGR warrants deeper investigation with consideration for the individuals' specific biology and external influences (i.e., dietary choices). A precision nutrition framework takes inter-individual differences into account using machine learning and have been used to predict PPGR following dietary interventions. For example, Zeevi et al.(14) investigated PPGR in over 800 participants to develop a predictive model for selecting dietary recommendations to lower PPGR. The model's prediction of foods to lower glycemic responses in a prospective cohort was accurate and superior to standard or universal dietary recommendations. Furthermore, the model was reproducible and demonstrated similar results in a different population (15). These results suggest that application of personalized dietary recommendations derived from a predictive model can successfully lower PPGR. Key variables used in these personalized models to predict PPGR include demographics, dietary intake, and the microbiome composition.

The objectives of this study included assessing baseline characteristics to predict PPGR in individuals following an intervention of low- vs high-RS potatoes (hot vs cold Russet potatoes, respectively) and to develop a precision nutrition model to predict PPGR. To achieve these objectives, we performed a *post hoc* analysis and used an elastic net penalty to select variables to be included in the model. Baseline characteristics included demographics, dietary components, body composition, the relative abundance of stool microbiota, and α-diversity of the stool microbiome. We hypothesized that our model would identify predictors of PPGR, and that microbiome features would significantly contribute to the explained variance of the model.

Methods

RS quantification

Quantification of RS was performed using the AOAC Method 2002.02 (Megazyme© RS Assay Kit, Bray, County Wicklow, Ireland) (16). Three potatoes were prepared for each potato type: baked-served hot, boiled-served hot, baked-chilled, and boiled-chilled. Chilled potatoes were stored at 4°C for 5 days. After potatoes were prepared, they underwent lyophilization for 72 hrs to ensure adequate drying. Each potato type was run in duplications for 2 samples taken from each potato (a total of 4 per potato type). The mean concentrations of RS per potato type were compared using the unpaired t-test with significance being $p<0.05$.

Glucose response

Blood samples were drawn at fasting (>8hr, water allowed) and at 15, 30, 60, and 120 minutes postprandial. Blood was collected in one 6mL EDTA vacutainer for glucose. All blood samples were centrifuged at 4000 RPMs for 15 minutes. Serum was immediately aliquoted into cryovials and stored at -80˚C. Serum glucose was determined by colorimetric analysis. Quantification of glucose occurred by a multi-mode reader (Synergy HI, BioTek® Instruments, Inc., Winooski, Vermont, U.S.A.).

The incremental area under the curve (iAUC) was calculated for glucose from fasting until 120 minutes after the final bite of the potato. The area between each time interval (15 – 0 minutes, 30 – 15 minutes, 60 – 30 minutes, and 120 – 60 minutes) was calculated using the trapezoidal method based on 5 different equations depending on if concentrations fell above or below the baseline concentration. Only the area above the baseline value was retained in the iAUC value. Detailed equations and scenarios of iAUC calculations are described elsewhere (17, 18). The iAUC describes the cumulative postprandial response over time; however, important features like the concentration maximums (Cmax) and minimums (Cmin) between each intervention are not independently captured. Therefore, we tabulated the Cmax and Cmin and the time to Cmax and Cmin (as Tmax and Tmin, respectively) of glucose and compared them between potato interventions.

Clinical Trial and Baseline Characteristics

More detailed descriptions of the study participants and the primary analysis can be found elsewhere (13). In brief, participants were all females with a body mass index (BMI) between 25 – 40 kg/m^2. Participants were excluded if they were not between 18-40 years old, were pregnant or lactating, had significant weight change in the past 6 months, or taking medications or supplements that affect metabolism or antibiotics/probiotics within the past 3 months.

Body composition was measured using air displacement plethysmography via the BOD POD® (COSMED USA, Inc., Concord, CA) to determine fat mass and fat free mass. Anthropometrics included height, weight, and BMI. The weight measurement used was derived from the calibrated scale from the BOD POD®.

Dietary records were captured over 10 days throughout the study to better represent a participant's usual diet compared to other methods like 24-hr recalls. One participant had an average energy intake of > 4000 kcals/d and was likely an over-reporter (19); therefore, we applied a crude cutoff value of 4000 kcals/day and adjusted subsequent dietary components to the proportion of energy reduction. Dietary information was entered into the Nutrition Data System of Research (Nutrition Coordinating Center, University of Minnesota, 2016), and the nutrient composition was analyzed. Only dietary components thought to be relative to the study question were included for analysis.

Participants collected stool specimens prior to the first study intervention (OMNIgene® GUT OM-200, DNA Genotek, Ontario, CA). Stool samples were aliquoted and stored in -80˚C freezer and batch-analyzed by MicrobiomeDx (Houston, TX). In brief, microbial DNA was extracted using the Mag-Bind Universal Pathogen DNA Kit (Omega Bio-Tek, Norcross, GA). 16S sequencing libraries were generated by amplifying the v3-v4 hypervariable regions of the 16S gene (20). MicrobiomeDX used BacPro™, a proprietary

algorithm, to generate a comprehensive report that includes α-diversity scores describing community richness, evenness, taxonomic composition with relative abundances.

Statistical Analysis

No participants or periods were excluded following assessments of the carryover, treatment, and period effects, demonstrating an adequate 7-day washout period. We applied simple mean imputation for missing biomarkers of one participant (21). Outliers of biomarkers were evaluated visually by boxplots and calculated by interquartile range (IQR) x 3.

Normality assumptions were evaluated using the Shapiro-Wilks test, and hypothesis testing was performed based on the distribution. Demographics (age, ethnicity, BMI) were described using proportions and mean (standard deviation). The glucose iAUC was calculated using the trapezoid method, and differences in biomarkers between the potato interventions were determined by Wilcoxon signed rank test and described as median (interquartile range). Dietary data were calculated as means and standard deviations or medians and interquartile range, as appropriate, to describe the energy, total and percent of kilocalories of macronutrients (fat, protein, and carbohydrates), available carbohydrate, glycemic index, total fiber, insoluble fiber, soluble fiber, monounsaturated fatty acids, polyunsaturated fatty acids saturated fatty acids and trans fatty acids. Microbiota taxa included in correlative studies and the regression model were selected based on prevalence and use in previous literature. At a minimum, genera had to be present in at least 50% of the participants. Five phyla and 1 family of interest were also included based on prior studies related to PPGR. Correlations between glycemic biomarkers and baseline demographics, body composition, microbiota, and diet, were performed using Spearman's rho. Relationships between the microbiota and biomarkers included a Bonferroni correction for multiple comparisons. All other significance was assigned at $p < 0.05$. Statistical analysis was performed using Stata® v.16.1 (College Station, TX, USA), and figures were generated using Prism v.7.03 (Graphpad, San Diego, CA, USA).

Penalized Regression

We used a data-driven approach to build a penalized regression model with an elastic net penalty and k-fold cross-validation to identify predictors of PPGR following hot and cold potatoes. Baseline demographics, body composition, Shannon and Simpson α-diversity, the relative abundance of key microbiota, and dietary intake were used as input data for the models (**Supplemental Table 1**). Predictors that were sparse or clinically insignificant were removed as input variables.

One model was built to predict glucose iAUC with a variable in the final equation to account for potato type (hot vs. cold). Data was stacked, and the glucose iAUC for hot (1) or cold (0) potatoes were retained as separate input variables; all other variables were duplicated and controlled for by using the vce cluster option in STATA® (variance-covariance matrix of estimators, used commonly in case-control analyses) for matched-paired comparison. A 5-fold cross-validation was used to avoid overfitting the model. The strength of this approach is the out-of-sample prediction (22). This method of k-fold cross-validation randomly partitions data into k-1 samples for training and then tests the model on the 1 held out k-fold.

Elastic net regression was chosen over other penalized regression methods because it applies two penalty terms, a combination of the L1 norm from the least absolute shrinkage and selection operator (LASSO) to provide feature selection and the L2 norm of ridge regression to provide effective regularization. This makes it optimal for analysis using a small sample size and a large number of predictors that are highly correlated. After elastic net variable selection, we performed linear regression with the final input variables to generate β-coefficients and the model equation.

The linearity of the standardized residuals against each of the predictor variables in the regression model was evaluated. Although there is a certain level of non-linearity at the far end values of the BMI, insoluble fiber, and Bacteroides, we believe it is not severe, and it is acceptable that the final model met the linearity assumption. The normality of residuals of the final model has been evaluated using the Shapiro-Wilk test for normality. With a p-value=0.16, the Shapiro-Wilk test indicated that the residual of the final model was normally distributed. Correlations between dietary components and the microbiome were present; however, the implementation of the elastic net penalties controls for multicollinearity by restricting correlated parameters so that only one (the most predictive) is retained in the model (23). To this effect, the variance inflation factor (VIF) revealed no evidence of multicollinearity. Lastly, the vce cluster option to fit the model resulted in no homoscedasticity concerns.

Results

Participants and Study Design

A total of 30 overweight females without comorbid conditions participated in this randomized, crossover study. Participants consumed roughly 9.2 ± 1.1 g of RS during the hot potato intervention and 13.7 ± 3.0 g of RS during the cold potato intervention (p=0.009). The mean age of participants was 29.6 ± 6 years old and the mean BMI was 32.8 ± 3.6 kg/m^2. Participants exhibited a high body fat percentage, averaging 45.5 ± 4.8%. The following results consist of a *post hoc* analysis describing baseline characteristics associated with PPGR and the predictive model developed using key baseline features.

Postprandial Biomarker Response

Postprandial responses following the hot and cold potatoes varied among participants. Previous analysis(13) revealed reductions in glucose concentration following the cold potato in the early postprandial period (15 and 30 min), compared to the hot potato, but no difference in glucose total area under the curve (tAUC) between potatoes occurred. However, when the data were reanalyzed based on incremental AUC (iAUC) to exclude values below the basal fasting concentrations from the AUC calculation (24), PPGR was different between potato intakes, p=0.02.

Despite an overall significant reduction in the median glucose concentration of the group, not all participants demonstrated a lower glycemic response following the cold potato (Table 1). The median reduction in glucose iAUC after consuming the cold potato was 471 mg•hr/ mL, p=0.02. Twenty-one out

of 30 participants (70%) exhibited a lower glucose iAUC after consuming the cold potato compared to the hot potato.

Table 1
Postprandial glucose response of women with overweight (n=30) following hot and cold potato consumption

Postprandial Glucose	Hot	Cold	Delta (Hot – Cold)	*p*-value
iAUC, mg•hr/ mL	1180 (500, 1910)	709 (316, 1038)	471	**0.021**
Concentration maximum, mg/dL	153.2 (129.6, 174.7)	140.93 (124.6, 160.8)	12.3	**0.047**
Concentration minimum, mg/dL	95.93 (85.2, 120.9)	100.70 (89, 109)	-4.8	0.417
Time to peak concentration, minutes	30 (15, 30)	30 (15, 30)	0	0.767
Time to minimum concentration, minutes	120 (22, 120)	90 (30, 120)	30	0.99
All values are presented as median (interquartile range) except for the delta.				
iAUC, incremental area under the curve				

Dietary Patterns

Dietary patterns heavily influence the gut microbial population and epigenetic factors associated with glucose metabolism (25); therefore, key components of dietary intake contributed to the model. Dietary records were captured over 10 days throughout the study to represent the usual diet of participants during the study period (6 weekdays and 4 weekend days). Participants consumed an average of 1828 ± 643 kcals/d (Table 2). Dietary patterns revealed slightly higher fat and sugar intakes than the U.S. Dietary Guidelines for Americans 2020-2025 recommendations (26). The mean percent of kilocalories (%kcals) from fat totaled 36% (recommended between 20-35%), and the mean saturated fat intake was 25.4 ± 12.7 g/day (recommended as <10 g/day). Further, participants consumed a diet high in sugar, with 48 g out of 75 g (64.1%) of sugar ingested as added sugars (sugar not naturally present in the food product consumed). Added sugars composed 10.5% of kcals consumed per day, which slightly exceeds dietary recommendations of 10% of total kcals. Fiber intake did not meet dietary guidelines and averaged nearly half of the recommended intake: 15.24 g/day consumed by the participants vs. recommendations of 25g /d (26).

Table 2
Mean 10-Day nutrient composition.

Dietary variable	Mean (SD)
Energy, kcal	1828 (643)
Total fat, g	78.7 (36.1)
Kcals from fat, %	36.0 (5.1)
MUFA, g	27.9 (12.4)
PUFA, g	18.8 (9.1)
Trans FA, g	2.3 (1.17)
SFA, g	25.4 (12.7)
Protein, g	75.0 (28.0)
Kcals from protein, %	16.6 (3.5)
Total CHO, g	206.4 (69.2)
Kcals from CHO, %	45.5 (8.1)
Total sugar, g	75.4 (36.9)
Added sugar, g	48.1 (27.8)
Available CHO, g	191.1 (66.7)
Total Fiber, g	15.2 (5.7)
Soluble fiber, g	4.5 (1.5)
Insoluble fiber, g	10.7 (4.4)
Glycemic Index	60.3 (4.6)
Dietary record information was inputed into the Nutrition Data System for Research (Nutrition Coordinating Center, University of Minnesota, 2016) to determne nutrient composition.	
Abbreviations: CHO, carbohydrate; kcal, kilocalories; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids	

Microbiome Profile

Microbiome profiling using 16S rRNA occurred from a single stool sample collected prior to the first potato intervention. All 30 participants provided an adequate sample for analysis. The average number of operational taxonomy unit reads was 147,070, with 75% of those reads were mapped to the SILVA database as previously observed microbes (27). Divided by taxonomy, there were 14 different phyla, 62 families, and 221 genera identified. Top phyla detected followed typical Western patterns and included:

Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria. The most prevalent genera (phyla) observed among the samples were *Bacteroides* (Bacteroidetes), *Faecalibacterium* (Firmicutes), *Blauti* (Firmicutes), *Lachnoclsotridium* (Firmicutes), *Ruminococcus* (Firmicutes), *Anaerostipies* (Firmicutes), and *Ruminiclostridium* (Firmicutes; Figure 1). Relative abundance of top taxa varied considerably between participants. Key taxa used for correlative and predictive analyses were determined based on previous studies using microbiota to predict PPGR (**Supplement** Table 1)(15, 28). The genera explored in the penalized regression model accounted for >66% of the total population genera (Figure 2).

Correlative Relationships with Baseline Characteristics and Glucose iAUC

All significant relationships between baseline features and PPGR correlated separately between potato types (Table 3), meaning that the different RS content in the potatoes played a major role in these findings. Following the hot potato, moderate, inverse relationships existed between height and the relative abundance of *Faecalibacterium.* These correlations were not found with the cold potato intervention. Glucose iAUC following the cold potato correlated inversely to insoluble fiber intake and the relative abundance of the Actinobacteria phyla. Positive correlations were seen between %kcals from fat and protein and the cold potato glucose iAUC. Of note, when a Bonferroni correction factor was applied to the microbiome relationships with glucose iAUC, no significant correlations remained. These differences in PPGR following each potato, though consumed by the same participants, further highlights the need for precision nutrition to distinguish personal characteristics that influence PPGR.

Table 3
Significant correlations between baseline characteristics and iAUCs of glucose, Spearman's rho.

	Glucose – Hot Potato		Glucose – Cold Potato	
	Rho	**p-value**	**Rho**	**p-value**
ANTHROPOMETRICS				
Height, cm	-0.38	0.04	-0.23	0.23
Insoluble fiber, g	-0.20	0.28	-0.37	0.04
Kcals from fat, %	-0.13	0.49	0.39	0.03
Kcals from protein, %	-0.20	0.30	0.50	0.005
Actinobacteria (phyla)	-0.16	.67	-0.40	.04
Faecalibacterium	-0.44	0.02	0.03	0.87
Abbreviation: kcal, kilocalories.				
*All microbiome correlations became nonsignificant when the Bonferroni correction factor was applied.				

Predictive Model for PPGR Following Potatoes

A total of 58 input variables (**Supplemental Table 1**) underwent elastic net selection for the final model. Stopping criteria and variable selection were based on 5-fold cross-validation. Sparse taxa were removed, and the top 5 most abundant genera and the taxa noted in previous prediction models for postprandial glucose were evaluated (15, 28–30). Glucose iAUC could be explained by the potato type (hot vs. cold), three genera, Simpson α-diversity, BMI, and insoluble fiber intake. The model accounted for 32.2% of the variance (R^2) in glucose iAUC. Only the type of potato (hot or cold) and the relative abundance of *Faecalibacterium* were significantly associated with glucose iAUC (β = 547.65, 95% CI 131.61, 963.68, p=0.01 and β=-73.49, 95% CI -128.51, -18.47, p=0.01, respectively; Table 4).

Table 4
Unadjusted and adjusted coefficients from linear regression that predict postprandial glucose response

	Univariate		**Multivariate**	
	β coef. (95% CI)	p-value	β coef. (95% CI)	p-value
Hot (vs cold)	547.65 (153.72, 941.58)	0.01	547.65 (131.61, 963.68)	0.01
Faecalibacterium	-69.37 (-124.15, -14.58)	0.02	-73.49 (-128.51, -18.47)	0.01
Bacteroides	11.26 (-11.78, 34.31)	0.33	8.69 (-14.33, 31.72)	0.45
Body mass index (kg/m2)	49.05 (-77.58, 175.68)	0.39	40.66 (-54.21, 135.54)	0.39
Alpha Diversity, Simpson	-5,599.38 (-15,827.10, 4,628.34)	0/27	110.87 (-10,209.57, 10,431.30)	0.98
Insoluble fiber, g	-50.10 (-101.24, 1.05)	0.06	-49.35 (-116.56, 17.86)	0.14
Parabacteroides	-70.90 (-173.86, 32.06)	0.17	-42.08 (-136.35, 52.18)	0.37
Intercept	--	--	292.52 (-9,705.98, 10,291.01)	0.95

The type of potato (hot vs. cold) was the only significant, positive association with glucose iAUC, while the relative abundance of *Faecalibacterium* significantly and negatively associated with glucose iAUC. This model predicts the PPGR following either hot or cold Russet potatoes in this small population of overweight women.

Discussion

This study observed significant reductions of glucose iAUCs following the intake of cold potatoes compared to hot potatoes in 30 overweight or obese women. The study is novel in that a predictive equation to determine baseline characteristics that influenced the glycemic response following a hot or cold potato was developed. The study focused on understanding the glycemic benefits of modifying RS by cooking and refrigeration in a commonly consumed food.

Investigations into the role of RS on glucose homeostasis related to other RS foods mirror the results of the current study. A randomized crossover trial by Nilsson et al.(3) examined a 3-day intervention where high RS bread (barley, ~17 g RS/day) was compared to white bread (2.5 g RS/day) and reported improved glucose in 20 healthy volunteers (85% women). Glucose peaks were reduced following the high-RS bread. Stewart et al.(31) provided acute supplementation of RS type 4 (16.5 g) in a crossover study comparing high fiber vs low fiber scones. After measuring the iAUC for 180 minutes, significant reductions of glucose between 43-45% emerged (31). However, these trials did not examine the influence of food processing on RS levels in whole foods, nor was the influence of the gut microbiome on glycemic response explored. These trials, among many others(1, 2, 6, 7, 31), demonstrate that a higher, acute intake of RS resulted in improved postprandial glucose homeostasis. It is important to note that the present study observed reductions in glucose iAUC following the intake of a cold potato with approximately 13.7 g of RS, which is a lower amount than many of the studies, but was still efficacious. This in part may be due to the combination of RS types, both RS type 2 and RS type 3, present in the cold potato or other fiber components inherent in potatoes. We also studied healthy, overweight females following acute ingestion of a whole food product containing RS, rather than RS as a supplement, which few other studies exclusively investigate.

The usual dietary intake reported by the study participants mostly aligned with the U.S. Dietary Guidelines for Americans 2020-2025 (26). The most concerning dietary pattern from our participants was the lack of fiber intake and excessive added sugar consumption. Studies investigating the postprandial response of RS (either acute or prolonged consumption) usually fail to report the usual dietary intake of the participants in the trial. Our participants' metabolic phenotype was primed by diets high in available carbohydrate, possibly confounding the interaction between epigenetic factors and the hot potato (higher in available carbohydrates). Phenotypic patterns may have affected the availability of digestive enzymes or activated genes related to carbohydrate metabolism or RS degradation. RS causes genetic alterations in carbohydrate metabolism (32), to where participants consuming a higher-fiber diet may have elicited a different response to the intervention than individuals with consistently lower-fiber intake. The importance of this may be evident in the negative association (though not significant, $p=0.14$) between insoluble fiber and glucose iAUC determined in our regression model.

The interplay between diet and microorganisms residing in the intestinal tract provides a potential mechanistic concept of how dietary RS can improve PPGR. Although this study did not measure the fermentation of RS and its byproducts nor the microbiome changes resulting from RS intake, we did measure how baseline microbiota can influence the physiological response to RS. Several genera showed relationships with postprandial glucose iAUC following consumption of hot and cold potatoes. The

Faecalibacterium genus and Actinobacteria phyla showed moderate, negative correlations following the intake of hot and cold potatoes, respectively. Several studies demonstrate similar findings. Zhang et al. sequenced the microbiome of patients with different levels of glucose intolerance, and *Faecalibacteria prausnitzii* was most abundant in the normal glucose tolerant group compared to the participants with prediabetes and type 2 diabetes mellitus (T2DM) (33). The importance of *Faecalibacterium* in determining glucose iAUC became evident in our model as the only significant contributor, other than potato type, associated with PPGR. Other studies have also observed an inverse relationship between *Faecalibacterium* and glucose sensitivity (34), and a recent review (35) found that four out of five studies found a negative association between *Faecalibacterium* and T2DM. To our knowledge, this is the first study to demonstrate the inverse association between *Faecalibacterium* and iAUC glucose following a high-RS whole food.

Various modeling techniques can predict PPGR using baseline characteristics. In a *post hoc* analysis of 106 healthy Danish adults, S□ndertoft et al. used a random forest model focused on clinical features and the microbiome to determine the magnitude of the effect on PPGR (30). The authors noted that a model based solely on microbial components accounted for up to 14% of the variance in PPGR excursions. When clinical features were added to the model, up to 78% of the variance in PPGR excursions was reported. Our model did not have any other clinical laboratory values available, such as serum cholesterol or triglycerides, which may have explained more variance. Because many underlying clinical, physiological, and metabolic features contribute to PPGR, stronger predictors may exist that were not assessed in the present study.

Researchers have replicated the PPGR modeling structure on meal-based interventions. Korem et al.(29) performed a crossover study assessing the PPGR following white bread (low-fiber) versus high-fiber sourdough bread. These authors built a model using stochastic gradient boosting that included solely microbiome features (relative abundance of species, relative abundance of genes, and function) that accurately predicted which type of bread induced a lower glycemic response for individuals (ROC=0.83). Although the analysis of the microbiome in our study did not extend beyond the relative abundance of specific taxa, the microbiome still played a leading role in determining the PPGR following potatoes of different RS concentrations. This further demonstrates the important role the microbiome plays in deciphering the inter-individual PPGR after consuming products with varying fiber content, as we found in our study.

In the present study, a key element in the design was to provide a dietary intervention that could feasibly be achieved in a real-world setting. The amount of potato administered (250 g) was equivalent to a serving size of mashed or baked potato, which can be realistically consumed alone or with a meal. We were unable to determine the actual amount of RS2 and RS3 (RS3 exclusively in the cold potato) for each potato administered to participants, but we did quantify the mean RS in the hot and cold potatoes. Moreover, the volume of potato consumed was equivalent between interventions, yet the proportion of available carbohydrate differed, with a lower amount of available carbohydrate in the cold potato. Another limitation of this study includes that the postprandial time period did not allow for adequate

assessment of bacterial fermentation of RS and further stimulation of incretins located lower in the gut. We were also limited to microbial data at the 16S rRNA level, while whole genome sequencing could provide deeper insight into the functional role of key microbiota and specific species associated with lowering postprandial glucose. Despite these limitations, a robust modeling technique incorporated common baseline features and selected variables with the greatest influence on PPGR. Further, because this study recruited volunteers without chronic disease, the identified predictors of PPGR may be applicable to other healthy populations.

Conclusions

Incorporating simple, modifiable changes, such as increasing the RS in the commonly consumed potato by changing the cooking method, may aid in better controlling glycemic responses to this starchy food. Understanding the interpersonal variation in the glycemic effect of potatoes would allow for appropriate dietary recommendations and optimization of routine food choices. The gut microbiota, especially *Faecalibacterium*, predicted the PPGR following potato intake. Larger studies are needed for the generalizability and evaluation of more diverse populations.

Abbreviations

AUC	Area under the curve
BMI	Body mass index
Cmax	Concentration maximum
Cmin	Concentration minimum
GIP	Glucose-dependent insulinotropic peptide
iAUC	Incremental area under the curve
IQR	Interquartile range
Kcals	Kilocalories
LASSO	Least absolute shrinkage and selection operator
PPGR	Postprandial glucose response
RA	Relative abundance
ROC	Receiver operator curve
RS	Resistant starch
T2DM	Type 2 diabetes mellitus
tAUC	Total area under the curve
Tmax	Time to concentration maximum
Tmin	Time to concentration minimum
U.S.	United States
VIF	Variance inflation factor

Declarations

Ethics approval and consent to participate

The Texas Woman's University Institutional Review Board approved the study (IRB-FY2020-58) and participants consented to the clinical trial.

Consent for publication

Not applicable

Availability of data and materials

De-identified data can be made available upon request to the corresponding author for up to 3 years after publication. Code availability for the modeling algorithm is also available upon request to the

corresponding author.

Competing interests

The authors declare the existence of a financial competing interest. NA worked as the Scientific Director at MicrobiomeDX and had a financial interest in the project.

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Authors' contributions

JNF drafted the manuscript and statistical analysis plan. DM, LWM, CDM, EAG, and DN assisted with statistical analysis and concepts. MAP procured funds and developed the original crossover study. JNF and MAP coordinated the clinical trial and performed biomarker analysis. NA performed microbiome analysis and provided significant input for the manuscript. All authors critically reviewed and approved the manuscript.

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Figures

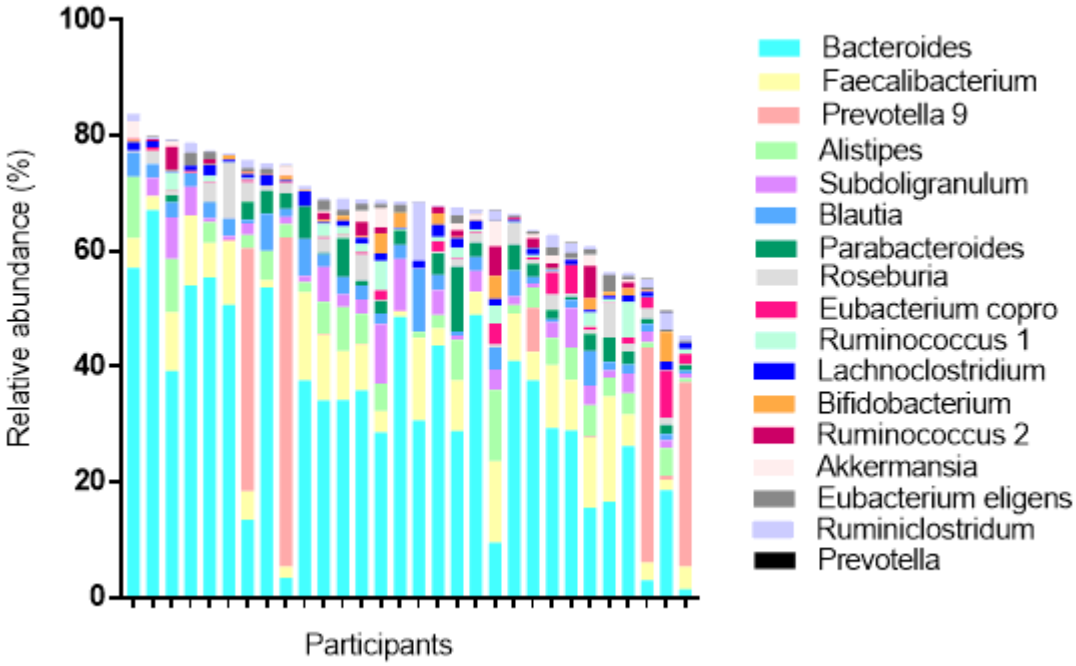

Figure 1

Relative abundance per participant of genera classified as key microbiota.

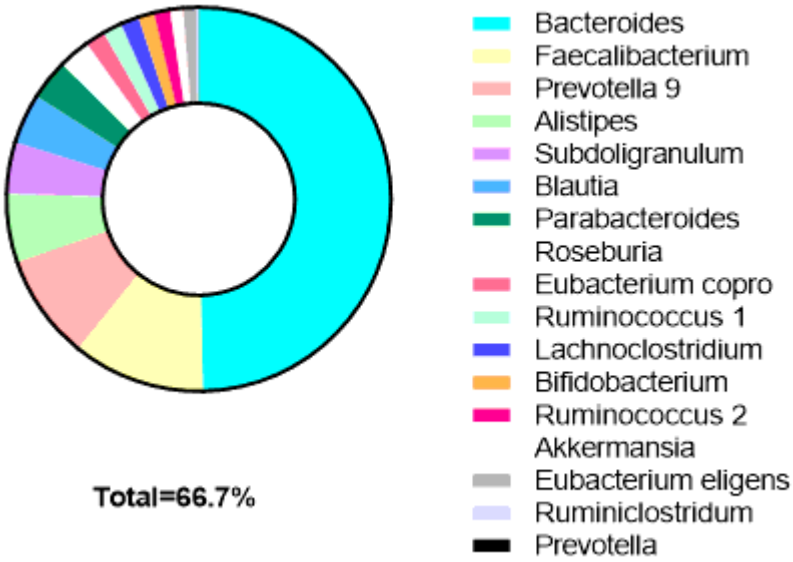

Figure 2

Cumulative relative abundance of genera classified as key microbiota. The selected taxa represent 66.7% of the abundance in participants.

Supplementary Files

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- SupplementTable1.docx