Developing and Evaluating a High Resistant Starch Menu and Glycemic Response in Adults with Prediabetes: A Non-Randomized Pilot Study

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Research

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Abstract

Background

The nondigestible fiber, resistant starch (RS), is found in beans, peas, and legumes, bread, cereals and grains, plantains, and potatoes. Here, we determined the feasibility of developing, preparing, and distributing a 7-day menu with a weekly average of ~30 g RS/d, as well as the likability of the foods.

Methods

First, a menu was formulated using a database, then prepared, stored, and delivered to mimic a future clinical trial. The RS content in each food item was quantified “as consumed” using an assay. Second, adults with prediabetes ($n = 15$) evaluated the likeability of the food items using a 9-point Likert scale. Likeability was acceptable if the overall score was ≥ 7 and $>75\%$ of the food items were consumed. Anthropometrics ($n = 11$), and fasting and postprandial (15, 30, 60, and 120 min) blood were collected to determine changes in weight, BMI, glucose, and insulin before and after the intervention.

Results

The feasibility of preparing, storing, and distributing the menu was verified and a weekly average of ~30 g RS/d at consumption was sustained. The menu provided ~2,000 kcal and 44.1 ± 9.5 g fiber per day. The feasibility of distributing the food items was acceptable where 100% compliance was achieved with the food preparation and participant pick-up schedules. Overall, 78.6% of the food items were consumed with an overall likeability score of 7.1 ± 1.9 , meeting likeability targets. Frozen meals were the highest ($94.1 \pm 5.8\%$; 8.1 ± 1.4) and soups the lowest ($61.4 \pm 6.6\%$; 6 ± 2.4) amount consumed and mean likeability, respectively. Body weight and BMI changed -1.6 kg; 95% CI -2.6 to -0.7 and -0.5 kg/m²; 95% CI -2.8 to 8, respectively. The total area under the curve_(0–120 min) for glucose and insulin changed by -655 and -1178 units, respectively, following high RS menu intake.

Conclusions

It is feasible to formulate, prepare, and distribute a high RS menu that is consumed and liked by adults with prediabetes. Due to small sample size, decreases in body weight, BMI, and glycemic indices should be interpreted with caution and tested using a randomized, cross-over intervention trial.

Trial registration:

Not applicable.

Key Messages Regarding Feasibility

1) What uncertainties existed regarding the feasibility?

To date, a 2,000 kcal/d menu containing a weekly average of ~30 g of resistant starch (RS)/d has not been developed, nor prepared and tested for quality and acceptability in adults with prediabetes. It was unknown if a menu could be formulated to sustain ~30 g RS/d at consumption. The feasibility of preparing and distributing the menu to study participants, as well as whether the menu would be acceptable for consumption in adults with prediabetes, was also unknown.

2) What are the key feasibility findings?

It is feasible to formulate a 7-day menu where a weekly average of ~30 g RS (verified by a commercial assay) and the recommended macronutrient ranges (verified by nutrient analyses) are provided daily. We also determined that it is feasible to prepare, store, and distribute the menu items to participants, which simulated a protocol that would be used in a larger clinical trial of longer duration. The likeability of the menu items was affirmed based on the overall likeability scores and amount consumed by the participants.

3) What are the implications of the feasibility findings for the design of the main study?

Clinical trials using RS as an intervention have historically used a supplement form of RS. Here, through purposeful development, testing, and planning, it is feasible to develop a menu (breakfast, lunch, snack, and dinner) that contains similar amounts of RS (~30 g/d) known to improve glucose homeostasis and insulin sensitivity. The present study found that in just 7 days, the high RS menu improved body weight, glucose, and insulin in adults with prediabetes; however, future clinical trials should be powered to test these outcomes. Using naturally occurring RS in foods will likely have an advantage over RS supplements. The high RS foods contain other nutrients such as fiber and protein that may provide combined positive health effects.

Background

Resistant starch (RS) is not broken down by digestive enzymes and thus enters the large intestine where it undergoes fermentation by resident microbiota. Hence because of this lack of digestibility RS is considered an insoluble fiber. Five types of RS have been identified based on the nature and properties of the RS granule: RS1, granules are resistant because they are trapped in the cell wall or matrix making it inaccessible to digestive enzymes and are found in foods such as seeds and whole grains; RS2, granules are resistant because of its native granule conformation where a tightly packed amylose structure forms a crystallized molecule often seen in raw potatoes and green bananas, as well as legumes and high-amylose corn; RS3 is formed when the starch granules are gelatinized then cooled which causes the starch to crystallize, a process known as retrogradation; RS4 is a chemically modified RS that is not naturally occurring in foods and is formed through processes like esterification, crosslinking or

transglycosylation; RS5 is heat-stable and formed when lipids bind to amylose in the starch granule to prevent expansion of the granule, which is necessary for digestive enzyme hydrolysis [2].

Determination of RS in food has evolved considerably since it was initially identified by Englyst and colleagues, who recognized RS fractions in both plant foods (in vitro) and human ileostomy effluent samples (in vivo) [3]. Presently, RS is quantified using the Association of Official Agricultural Chemists (AOAC) method 2002.02/ American Association of Cereal Chemists (AACC) method 32-40.01, and factors, such as sample preparation, sample pretreatment, enzymes used, and incubation conditions determine the amount of RS quantified. Commercial kits are available to estimate RS in foods [4].

Adequate consumption of RS improves overall metabolic and physiological health due to its role as a prebiotic that supports the growth, viability, and function of certain gut microbes [5]. In addition to improvements in metabolic health, RS can impact quality-of-life measures in diseased populations due to microbiome modulation. Non-motor and depressive symptoms improved and fecal butyrate, an indicator of RS fermentation, increased in adults with Parkinson's disease after consuming 10 g RS3/d for 8 weeks [6].

Strong clinical evidence shows RS improves glycemic control which can attenuate the development of several diseases, including type 2 diabetes [7]. US adults consume ~2.0 g RS/1,000 kcal/d from foods [8] which is much lower than what has been observed in trials that resulted in improved glucose homeostasis and/or insulin sensitivity [9-12]. This highlights the need for higher intake of dietary RS in adults with prediabetes – before type 2 diabetes develops. Because over 88 million adults in the US population were estimated to have prediabetes in 2018 [13] and prediabetes is prevalent in nearly 1 in 5 adolescents aged 12–18 years and 1 in 4 young adults aged 19–34 years [14] it is important address lifestyle and dietary changes when symptoms first arise.

Almost all clinical trials have used the supplement form of RS as an intervention due to ease of administration. Very few trials have examined foods with naturally occurring RS on metabolic outcomes, and none have studied a complete weekly menu high in RS. In a recent randomized cross over study, we reported that consuming baked then chilled potatoes (high RS) resulted in postprandial glucose and insulin reductions at 15 and 30 minutes and lower total area under curve (tAUC_{0–120 min}) insulin than a boiled potato (low RS) in females with elevated fasting glucose [15]. However, only one type of food, a potato, not a full menu was tested in the study. A recent systematic review and meta-analysis of randomized crossover trials (RCT) found that significant decreases in postprandial glucose and insulin were reported to be due to consumption of starchy foods with high amylose content characterized by low gelatinization, increased degree of retrogradation, and large starch particle size [16]. However, the majority of the studies identified in this review included RS as a supplement or as food prepared with RS fortified ingredients thereby noting the need for studies using naturally occurring RS food sources. In addition, a specific dietary plan has not been identified to reduce the progression from prediabetes to type 2 diabetes. The Diabetes Prevention Programs show that following a low-calorie, low-fat diet with

exercise can attenuate the development of type 2 diabetes by 58% [17]. However, a specific dietary menu plan has not been identified.

The focus on consuming nutrients through food, not supplements, prompted the idea of formulating a menu containing foods with similar amounts of RS that have shown to improve glycemic homeostasis in clinical trials. Thus, there are two main phases of the study that occurred in time sequence. The first phase (first objectives) involved the formulation of a 7-day menu where a weekly average of ~30g RS/d would be provided. Because RS can change during storage conditions, we assessed the feasibility of preparing and storing the food items in the menu so the RS content would be sustained upon consumption. The second phase (second objectives) used a non-randomized study design to determine the feasibility of distributing the 7-day menu and the likeability of each food item in the menu in adults with prediabetes. Changes in body weight, BMI, and glycemic response after consuming the 7-day menu were assessed. The data from both phases will serve as pilot and feasibility data for a future 8-week, randomized, cross-over, clinical trial where the physiological outcomes of consuming the RS menu compared to habitual dietary intake will be determined.

Methods

Phase 1 Formulation of a 7-day menu with RS

Developing the menu

Phase 1 includes the methods that address the primary objectives (Figure 1). A 7-day high RS menu was developed using a database that quantified the amount of RS in foods commonly consumed by US adults [1]. In brief, the database was a compilation of 94 peer-reviewed research articles published between 1982 and 2018 that measured the amount of RS in commonly consumed foods in the US. The RS amount in each food in the database was reported with moisture included, not on a dry weight basis, which is how the food would be consumed. The high RS menu was formulated to have a weekly average of ~30g of RS/d, which is based on other clinical trials using RS as a supplement where improvements in glucose homeostasis and/or insulin sensitivity were observed [9, 11, 18].

The energy and nutrient content of the recipes and food items in the high RS menu were analyzed using the Nutrition Data System for Research (NDSR; version 2019, Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN, USA). Recipes and food items were adjusted so the daily energy intake was ~2,000 kcal and the macronutrients fell within the Acceptable Macronutrient Distribution Ranges (45-65% of energy from carbohydrate, 10-35% of energy from protein, and 20-35% of energy from fat) [19].

Preparing the food items

Raw ingredients to prepare the high RS menu were purchased from a single local grocery store using the same brand names (when applicable) throughout the study period. All raw ingredients or products were purchased fresh and stored in the food lab to maintain quality and shelf life. The research team prepared

all food items individually using recipes where each ingredient was weighed to the nearest gram. All food items were packaged in plastic food containers according to serving size weight, were labeled (e.g., food ID and date), and stored in a manner that increased RS while maintaining safe food handling practices based on industry recommendations [20].

The components each meal is shown in **Table 1**. The meals were prepared on Monday, Wednesday, and Friday which would be consumed by participants on Tuesday/Wednesday; Thursday/Friday; and Saturday/Sunday/Monday, consecutively.

Analyzing RS content in the menu

RS in foods can be altered due to several factors such as cooking method, storage temperature and duration, and serving temperature [2]. There is a positive correlation between RS formation and duration of cold storage in some cooked, starchy foods [1]. However, in Phase 2 of this study the participants consumed each food item after a minimum of 1 day of storage. Hence RS was analyzed after the food items were stored for 1 day.

Each food item in the menu was prepared twice on separate occasions, and each were analyzed for RS in duplicate. Dry samples or samples with less than 25% moisture such as corn flake cereal, were finely milled for RS analysis. High moisture foods ($\geq 25\%$) were removed after at least 1 day of storage at 4°C then prepared as would be consumed. For example, rolled oats in milk, plantains, potato salads, sandwiches, and all snack items would be consumed cold; therefore, these items were not heated prior to RS analyses. However, the bean and barley soup, lentil burger, and frozen meals were heated according to instructions and immediately prepared for RS analyses. The rationale for consuming certain items cold but heating other items was to maximize the RS amount in each food item while maintaining food safety and quality. The feasibility of developing, preparing, and storing the 7-day high RS menu was acceptable if the target weekly average of ~30 g RS/d was maintained after one day of storage.

The prepared food items were homogenized, then added to a glass flask filling 1/3 of the container. The flasks were weighed in grams. The food samples were shell-frozen at -80°C for a minimum of 4 hours. Once frozen, the samples were lyophilized for 48-72 hours depending on food sample type. Following lyophilization, the flasks were weighed then moisture content was determined using the following formula: $(\text{wet weight (g)} - \text{dry weight (g)}) / \text{wet weight (g)} * 100$.

The dried food samples were finely milled in preparation for RS analysis. All the food samples were analyzed for RS in the prepared form that was “as eaten” according to the Resistant Starch Assay Kit (Rapid) instructions (Megazyme Ltd., Bray, Ireland). The RS measured using this kit employs the AOAC method 2002.02/AACC method 32-40.01 [21]. The calculations were completed using the Megazyme MegaCalc™ Excel® based calculator provided as supporting documents with the RS analysis kit.

After Initial RS analysis, the recipes and meals were once again slightly adjusted to ensure a weekly RS average of ~30g/d based on RS analysis data and the energy and macronutrient distribution were within

the target ranges (Nutrient Database Software for Research, version 2019, Nutrition Coordinating Center, University of Minnesota, MN). Any ready to eat meals or foods that reported low RS was replaced with a food containing high RS (e.g., Chex cereal was replaced by cornflakes). Once the menus were finalized, the RS analyzed in duplicates and mean values were reported as shown in Table 2

Phase 2: Feasibility assessment of menu distribution and evaluation, and changes in anthropometrics and glycemic response

Feasibility of distributing the menu items

The feasibility of distributing the menu to participants was assessed by preparation start and finish times. Feasibility would be accepted if 100% of the food items were prepared, packaged, and distributed to the participants on the same day (second objectives) as shown in the schedule under “Preparing the food items.”

Participants

All methods associated with phase 2 addressed the second objectives (Figure 1). First, adults with prediabetes evaluated each menu item (breakfast, lunch, snack, dinner) in the menu containing a weekly average of ~30 g RS/d (first objectives) using a non-randomized dietary intervention. Participants were recruited from the Houston, TX metroplex using flyers, social media, and targeted emails. Interested participants contacted the investigators and were screened for inclusion criteria: adults with a self-report prediabetes diagnosis (e.g., fasting blood 100 – 125 mg/dL, oral glucose tolerance test ≥ 140 - <200 mg/dL, or HgA1c 5.7% - 6.4% mg/dL) [22] from a healthcare provider, between 20 and 65 years of age, able to speak and read English, a BMI ≥ 27 kg/m², not following a specific diet or have food allergies or intolerances to any of the study foods, able to safely prepare and consume all study foods. Females were excluded if they were pregnant or lactating. All participants provided written informed consent prior to data collection. This pilot study was approved by the Texas Woman’s University, Houston, TX Institutional Review Board.

Food consumption and evaluation survey

Participants prepared and consumed the food items for each meal and were instructed to avoid consuming additional foods other than non-caloric beverages. Additionally, they received all pre-labeled surveys upon study enrollment. Each breakfast, lunch, snack, and dinner food items were evaluated each day, even if they were consumed more than once during the study. The surveys consisted of six questions that assessed the appearance, texture, smell, consistency or mouthfeel, taste, and overall likeability using a 9-point Likert scale where 1=dislike extremely and 9=like extremely. The survey also contained two open-ended questions regarding what was liked and disliked about the food item and how much they consumed (0%, 25%, 50%, 75%, or 100%). The benchmark established to indicate acceptability for this pilot study was if the participants consumed >75% [23] of the food items in the menu.

The completed surveys were returned to the investigators at the end of the study. To assess the mean response for the question “overall likeability” must be ≥ 7 . Values at or above 7 indicate the food represents sensory characteristics that are acceptable for consumers [23].

Blood analyses

Blood was collected in vacutainers for glucose and insulin analysis. Blood was drawn at fasting (≥ 8 hours; water allowed) and 15, 30, 60, and 120 min after drinking 360 mL reduced-fat chocolate milk (210 kcal; 19.5g carbohydrate, 6.75g fat, and 6.75g protein) within 10 minutes at baseline (day before beginning RS rich menu evaluation) and the day after consuming the menu. The vacutainers were centrifuged at 4,000 rpm for 10 min and plasma was aliquoted in 1.5 mL tubes and frozen at -80°C until analysis. Glucose was determined using the colorimetric method (Stanbio Laboratory, Boerne, Texas, USA) and insulin by enzyme-linked immunoassay (Alpco, Salem, New Hampshire, USA) according to manufacturer instructions. All samples were analyzed in duplicate and repeated if the intra-coefficient of variation (CV) and inter-CV were $\geq 10\%$ and 15% , respectively.

Anthropometrics

Height was measured at baseline using a stadiometer. Body weight (kg) was determined at baseline and the day after consuming the 7-day menu using the scale associated with the air-displacement plethysmograph machine (BOD POD, COSMED USA, Inc., Concord, California, USA). Body mass index (BMI; kg/m^2) was calculated at each time point. The participants wore the same clothing at both measurements.

Sample size

This pilot study first aimed to evaluate the feasibility of preparing and distributing 7-day menu providing a weekly average of $\sim 30\text{g}$ RS/d in order to investigate potential mechanisms of efficacy for this new intervention. The second objectives tested the likeability of a RS rich menu by human participants. Due to the lack of prior data needed to determine sample size, and the general guidelines for pilot food investigations a sample size of 12 is considered appropriate [24]. Considering this rule of thumb in conjunction with a medium standardized effect size of $0.3 \leq \delta < 0.7$ for two time points and one-sided tail with a power of 80%, 15 subjects with prediabetes were considered appropriate to address the second objectives [25].

Statistical analysis

Data were analyzed using Statistical Package for Social Sciences (version 25, IBM SPSS, Armonk, New York, USA). Descriptive data are presented as mean $\pm$ SD. All data were screened for normality using Shapiro-Wilk tests and box plots. The $\text{tAUC}_{(0-120 \text{ min})}$ for glucose and insulin were calculated using the trapezoid formula. Wilcoxon signed rank tests were used to report mean differences and 95% CI for $\text{tAUC}_{(0-120 \text{ min})}$ glucose and insulin.

Results

7-day menu

The high RS 7-day menu can be found in Table 1. The breakfast items consisted of oats, plantains, corn flake cereal, and tacos. Lunch and snack items included soups, sandwiches, and salads with foods containing RS such as potatoes, beans and legumes, pastas, and bread. The dinners consisted of frozen meals and the addition of other side items (e.g., whole wheat bread) if additional kcal were needed.

The energy, nutrient, and RS content of the meals are provided in Table 2. The daily mean caloric value of the meals was $2,082 \pm 82$ kcal where 56.4% of energy came from carbohydrates, 25.2% from fat, and 18.1% from protein. The mean amount of fiber (44.1 ± 9.5 /d) was above the recommended daily amounts of 38 g for men and 25 g for women between 18 and 50 years old and 30 g for men and 21 g for women > 50 years old [19].

The feasibility of maintaining a weekly average of ~30g RS/d

The total RS varied where the lowest amount was provided on Monday (15.6 g) and the highest on Wednesday (39.7 g); however, the weekly average equated to 30.3 ± 7.9 /d (Table 2), which is the daily amount that has resulted in positive health benefits. According to the RS quantification of each menu item after one day of storage, the results indicate that it was feasible to formulate and prepare the 7-day menu items to contain a weekly average of ~30 g RS/d.

Participant demographics

Eighteen adults with prediabetes enrolled in the study but 15 (37.9 ± 14 years, 11 females, 33.4 ± 7 kg/m²) began the 7-day feeding trial. Anthropometrics and blood collections used for biomarker analyses were obtained from 11 (40.3 ± 12.2 years, 7 females, 31.7 ± 2.5 kg/m²) participants (Figure 2).

Baseline participant characteristics are present in Table 3. The majority were female (73.3%), had a mean age of 37.9 ± 14 years and were obese (BMI 33.4 ± 7 kg/m²). The most prevalent racial category was African American (40%) and Hispanic (33.3%). The duration of prediabetes was 34 ± 38.3 months and fasting glucose was the most frequently used method to diagnose prediabetes.

Feasibility of preparing, packaging, and distributing the menu items

The objective in preparing and distributing the high RS menu items was to ensure the food was prepared then stored at 4°C for a minimum of one day to increase RS content. Meal preparation began between 9:00 and 10:00 AM and ended no later than 3:00 PM on Monday, Wednesday, and Friday. We observed 100% compliance with this schedule. With regards to participant pick up and consumption pattern we observed 100% compliance with this schedule. Therefore, it is feasible to prepare the 7-day menu the same day as participant pick-up in the mid-afternoon. This food preparation and pick-up schedule will be implemented in the larger randomized, cross-over feeding trial for 8-weeks.

7-day RS menu evaluation and consumption

Overall, $78.6 \pm 8.3\%$ of the food items were consumed by the participants. On average, they consumed >75% of 15 food items, 50-75% of 6 food items (chickpea pasta salad, southwest barley salad, traditional potato salad, bean and barley soup, Italian bean soup, and lentil patty), and <50% of 2 food items (bean succotash and lima bean soup). Frozen meals had the highest percent consumed (94.1 ± 5.8) followed by sandwiches (86.7 ± 18.4), breakfast (83.9 ± 3.9), salads (67 ± 6.7), and soups (61.4 ± 6.6).

Results from the food evaluation survey can be found in Table 4. The frozen meal category had highest mean likeability for the question “Considering the appearance, aroma, taste, and texture, how much do you like the product OVERALL” followed by sandwiches, breakfast items, salads, then soups. Of all the food items, the lowest likeability scores included lima bean soup, succotash, and chickpea pasta salad, while the most liked food items included the steak portobello frozen meal, shrimp alfredo frozen meal, and hummus.

Common themes from two open-ended questions on the survey provided insight into the overall likeability scores. The lima bean soup was disliked because of the cold temperature ($n = 3$), bland flavor ($n = 3$), texture ($n = 3$), and appearance ($n = 1$). However, some participants thought the soup was refreshing ($n = 2$) and smelled good ($n = 1$). The bean succotash was disliked because of the overall texture ($n = 1$), gassy nature ($n = 1$), and addition of lima beans ($n = 1$) although some thought the dish was very flavorful ($n = 4$). The chickpea pasta salad was disliked because of a “sour or weird flavor,” ($n = 2$) but in contrast was a great snack option ($n = 2$). For the top liked food items, the steak portobello frozen meal had a good sauce ($n = 2$) even though small portion size ($n = 1$) and broccoli and mushroom additions ($n = 2$) were disliked by some. The shrimp alfredo frozen meal had good ingredient combinations and good flavor ($n = 4$); no dislike comments were made for this meal. The participants reported that the hummus was a good snack especially with the carrots ($n = 5$) even though it was grainy ($n = 1$).

Anthropometrics and biomarkers

Data on anthropometrics and biomarkers ($n = 11$) were collected at baseline and after consuming the 7-day high RS menu. Interestingly, in such a short time frame both weight (mean -1.6 kg; 95% CI -2.6 to -0.7) and BMI (mean -0.5 kg/m²; 95% CI -2.8 to 8) changed (data not shown). This suggests consuming the high RS menu may improve body weight in adults with prediabetes.

The biomarker results are found in Table 5. Three participants were excluded from the blood analyses. Two were outliers based on normality tests and one was due to the inability of obtaining postprandial blood. As expected, mean baseline fasting glucose met the criteria for prediabetes diagnosis, further validating that the participants indeed had prediabetes. Following the 7-day menu, glucose decreased at all time points with the largest reductions at 30 and 60 minutes. Insulin also decreased at all time points except at 15 minutes. Similar to glucose, the largest reductions were observed at 30 and 60 minutes.

The $tAUC_{(0-120)}$ for glucose (-655) and insulin (-1178) also decreased after consuming the 7-day menu. These results indicate RS can improve glucose homeostasis in a short period of time.

Discussion

The main findings of this non-randomized pilot study include the following: 1) it is feasible to formulate, prepare, and distribute a 7-day menu with a weekly average of ~30 g RS/d and 2) the food items in the menu had appropriate sensory characteristics that led to high adherence and acceptability in adults with prediabetes measured by the overall percentage of food items consumed (>75%) and a mean overall likeability score ≥ 7 . In addition, following the short 7-day intervention, glucose, insulin, and body weight were reduced although this pilot study did not test for significance. Resistant starch may not be solely responsible for these changes as a reduction in energy intake and increased dietary fiber likely contributed. These pilot data established preliminary results for future larger randomized, cross-over feeding trials sampling a larger number of participants for a longer duration. Appropriately powered interventions including menus providing ~30 g RS/d through whole foods could lead to changes in dietary recommendations that attenuate, or even prevent, the progression of prediabetes to type 2 diabetes.

High resistant starch (RS) menu

Although several caveats in developing high-RS menus exist, we were able to use a published database [1] to develop recipes that contained ~2,000 kcal/d, recommended macronutrient ranges, and a weekly average of ~30g RS/d. The caveats are associated with the many factors that may influence RS content: plant breed, growing and storage conditions, preparation, storage, and consumption temperature [2]. These challenges have steered researchers away from whole-food RS dietary interventions; therefore, using RS supplements instead. However, we have now shown it *is* feasible to develop a high RS menu and maintain health-benefiting RS amounts in foods. To develop the menu, foods from a database [1] such as potatoes, lentils, white and lima beans, chickpeas, plantains, pasta, and barley were utilized as they provided the highest amount of RS per gram serving size. This pilot study is unique as it ensured the RS contribution of each menu item “as consumed” from the RS analyses in our lab. To the best of our knowledge, we are not aware of any studies estimating the naturally occurring RS content in test meals after production.

We have previously reported that the usual intake of RS in US adults is 1.9 g/1,000 kcal [8], and 2.1 g/1,000 kcal in adults with prediabetes [26], which is <15% of the RS contained in our menu. The most frequent contributors of RS in the US diet were French fries and other fried white potatoes, rice, and beans, legumes, and peas [8]. Our high-RS menu replaced the fried potatoes and white rice with more nutrient-dense options that more closely align with the Dietary Guidelines for Americans 2020-2025 [27].

This is the first pilot study evaluating the adherence and likeability of a full menu high in RS in adults with prediabetes. Over $\frac{3}{4}$ of the high-RS menu items were consumed where frozen meals had the highest

adherence and salads and soups had the lowest. Adherence to the frozen meals could be due to familiarity as they are commonly consumed foods in the US. The steak portabella and shrimp alfredo received the highest likeability score where a “flavorful sauce” was mentioned. Of the salads, the chickpea pasta and succotash were consumed the least and had the lowest likeability scores. Some participants stated the chickpea pasta had a “weird flavor” and “sour taste” indicating the seasoning will need to be adjusted for future studies. The “overall texture” and “lima beans” were comments indicating dislike for the succotash. The lima bean soup had the lowest adherence and likeability due to “cold consumption, bland, texture, appearance” mentions. Future randomized crossover trials will modify this recipe by pureeing the soup thoroughly, adding chives and paprika for color, and adding crackers or croutons for added texture.

Adherence to the 7-day high RS menu was higher than other dietary interventions of similar populations. One study in adults with type 2 diabetes found 67% met adherence criteria to a vegan diet compared to only 44% in the American Diabetes Association group at 22 weeks with no difference diet likeability, or satisfactory rating, between groups [28]. The vegan diet included many high RS foods, such as legumes, beans, and peas, potatoes, bread, and whole grains. The higher adherence in this pilot study could be due to differences in study duration where adherence rates typically decline over time. Obtaining food preferences (e.g., dislike salmon but like tuna) will be necessary to maintain adherence in longer feeding trials. The seasonings could be adjusted in some recipes (e.g., replace lemon and herbs with curry in potato salad) to reduce monotony without altering the RS amount.

Body weight

Following the consumption of the 7-day menu, a -1.6 kg and -0.5 kg/m² change in body weight and BMI, respectively, occurred. Several mechanisms may be associated with weight loss. First, the net energy content of RS is less than digestible starch (2.59 kcal/g vs 4 kcal/g) thus reducing the caloric contribution from the diet [29]. Next, increased fat and decreased carbohydrate oxidation have been observed following RS intake [30]. Lastly, RS fermentation by specific gut microbiome taxa produce metabolites that lead to decreased adipogenesis, fat accumulation, and release of satiety-promoting hormones such as glucagon-like peptide and peptide YY [31] [32]. We did not measure satiety in the present pilot study; however, participants (n=2) stated “could not eat, too full” in the open-ended questions. Fiber also contributes to slower digestion rates and feelings of fullness and could work synergistically with RS to reduce body weight [33]. The 7-day menu provided 44.1 ± 9.5 of dietary fiber per day, which is almost 2.5 times higher than normal fiber intake among adults with prediabetes [34]. In addition, a reduction in total daily energy intake cannot be ruled out since the high RS menu provided ~2,000 kcal/d, which may be lower than what the participants habitually consume. It is unclear if weight loss would be sustained since our intervention only lasted 7 days. One study found that high RS foods, specifically dried beans and high-fiber low-fat grains contributing to a high-carbohydrate and fiber diet has been shown to predict the most weight loss at 1y in adults at high risk of type 2 diabetes [35] suggesting the high RS menu could have lasting health benefits. This pilot study assessed the feasibility

of preparing and delivering the 7-day menu; therefore, the impact of RS on body weight and glycemia will be examined in future research.

Glycemia

Some of the most profound outcomes associated with consuming the high RS 7-day menu were improved glucose and insulin concentrations. Strikingly, in 8 participants, the $\text{tAUC}_{(0-120)}$ glucose and insulin changed by -4.2% and -18.6%, respectively. Fasting glucose was reduced by -3.8% with greater reductions shown at the 30 (-6.7%) and 60 (-4.9%) min postprandial periods. Insulin showed a more drastic decrease after consuming the 7-day high RS menu than glucose. While fasting insulin had the greatest change (-37.3%), postprandial changes also occurred at 30 (-16.7%), 60 (-24.1%), and 120 (-28.4%) min. Although this pilot study did not test for level of significance in glucose or insulin changes, a meta-analysis [23] found a significant fasting glucose effect size of -0.16 (95% CI -0.24, -0.08) mmol/l in trials where ≥ 28 g RS/d were consumed [36].

Changes in glucose and insulin may be associated with several mechanisms. Metabolites resulting from RS fermentation have been shown to improve insulin resistance and *de novo* glucose production through several signaling pathways [31]. In addition, meta-analyses have found that foods with higher amylose, retrograded starch, and starches with less ability to form gels improve postprandial glycemia [16]. Many of these trials included only one food item, such as lentils, rice, pasta, breads, or grains, each having different starch properties than the control [16]. And to date studies involving RS rich test meals have focused on RS content defined by the rate of gelatinization, particle size, and level of retrogradation and its effect of glycemic index [8]. Lower blood glucose and insulin concentrations resulted after consumption of coarse porridge with a structurally intact starch than after the smooth porridge prepared from wheat endosperm in healthy ileostomy participants [37]. Other randomized trials have also observed the effect of high amylose white rice, [38] high amylose noodles, [39] and high amylose bread [40] where improved glycemic response was reported. In contrast, only one trial found no change in glucose homeostasis after adults with prediabetes consumed 45 g RS2/d as a supplement in yogurt for 12-weeks [41]. It is unknown if consuming high amounts of RS in whole foods would have elicited the same results.

While our pilot study included a menu that targeted a specific amount of RS, one dietary intervention women with polycystic ovarian syndrome (PCOS) included foods rich in RS. A lentil, bean, split pea, and chickpea-rich diet reduced tAUC insulin and improved cardiometabolic outcomes compared to a therapeutic lifestyle changes diet after 16 weeks [42]. Women with PCOS have symptomology encompassing impaired glucose metabolism and insulin resistance – like prediabetes. The RCT did not analyze RS in the foods, but 33.3 g/d of fiber and lower glycemic index foods over time may have contributed to the results. One cautionary note is that 24-hr recalls assessed nutrient composition which is subject to self-report bias and misreporting [43]. Weight did not change in the RCT and would not have influenced the biomarker outcomes.

These studies demonstrate the effect of RS structurally and functionally on glycemic response; however, are limited in addressing the gap in translating the scientific rigor to real world practice. The majority of studies are based on the manipulation of RS particles by either storage, supplementation, or genetically altering amylose content. In comparison our study has utilized naturally occurring RS rich foods commonly available to US consumers. In addition, by providing all meals for 7 days, the likelihood of consuming external foods that could alter glycemia was small, even though these data were not collected. Results from this 7-day pilot test intervention are comparable with studies that observed RS effects on glycemic response as we observed similar $\text{tAUC}_{(0-120 \text{ min})}$ glucose and insulin reductions. It is also important to note that our study was conducted on adults with prediabetes with obesity and hence providing a unique insight to potential changes in biochemical responses after consuming a RS rich diet. This is vital because a prospective study assessing dietary intake every 4 years found that adults consuming a high total starch and starch: low-fiber diet, after adjusting for several dietary and lifestyle factors, had higher risk for developing type 2 diabetes [44]. These results can be misleading since the starchy foods are likely from highly refined, low fiber sources - many of which are also low in RS. In this pilot study, using a similar population, starchy foods rich in both RS and fiber elicited positive health outcomes.

Limitations And Future Research

Some limitations of this non-randomized pilot and feasibility study should be noted. The sample size was small thus the level of significance for post RS meal consumption data was not assessed. The primary purpose of the pilot study was to develop a high RS menu and determine the likeability and acceptability in adults at risk for developing type 2 diabetes. Assessing changes in glycemic response and anthropometrics were not the primary goal of this study. However, the decreases observed in this study will help us determine the sample size for randomized trials that would improve glycemic control. We were not able to identify the mechanistic action of the test meals with high RS and its association with the metabolic outcomes, as well. For example, it is unknown if the RS, dietary fiber, or reduced energy contributed more to the weight and glycemia changes or if they worked synergistically together. To reduce participant burden, we did not collect information on external foods consumed during the 7-day trial. Based on these results, adjusting some of the menu items to improve taste, texture, and appearance will be necessary to improve adherence. A longer feeding trial with additional biomarker analysis such as satiety hormones will be beneficial to identify longer-term metabolic impacts. Fecal samples to test changes in microbiome structure and function would provide additional insight to the mechanisms associated with consuming a high RS diet provided through foods.

Conclusions

Developing, preparing, and distributing a menu with the same amount of RS known to improve glycemic health ($\sim 30 \text{ g/d}$) is feasible and liked overall among adults with prediabetes. Body weight and BMI decreased, as well as fasting, postprandial, and $\text{tAUC}_{(0-120 \text{ min})}$ glucose and insulin following the 7-day

feeding trial, although significance was not tested. However, due to the pilot nature of the study, the contribution of RS (compared to reduced energy and increased fiber) cannot be determined, nor the mechanisms associated with the outcomes. Future studies should include a control group or employ a randomized, cross-over design to assess group comparisons. Trials of longer duration are also necessary, and microbiome and satiety-hormones data should be obtained.

Abbreviations

AACC - American Association of Cereal Chemists

AOAC - Association of Official Agricultural Chemists

BMI – Body Mass Index

GIP - glucose-dependent insulintropic peptide

RS – Resistant starch

RCT – Randomized crossover trial

tAUC – Total area under curve

US – United States

Declarations

Ethics approval and consent to participate

This study was approved by the Texas Woman's University – Houston Institutional Review Board (IRB_FY2021-135). All participants provided written informed consent prior to involvement in the research study.

Consent for publication

Not applicable.

Availability of data and materials

Not applicable.

Competing interests

The authors declare they have no competing interests.

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Author's contributions

MAP secured the funding, designed the study, developed the recipes and menus, and conducted the nutrient analyses for the primary objective. For the secondary objectives MAP and MM secured the funding. For both objectives, MAP and MM led the intervention, facilitated data collection, and analyzed the data. ADC, KAF, and CL prepared the food items for the intervention. SR assisted with data collection. MAP and MM prepared the first draft. All authors reviewed and approved the final version of the manuscript.

References

1. Patterson MA, Maiya M, Stewart ML. Resistant starch content in foods commonly consumed in the United States: A narrative review. *J Acad Nutr Diet*. 2020;120(2):230-244.
2. Birt DF, Boylston T, Hendrich S, Jane JL, Hollis J, Li L, McClelland J, Moore S, Phillips GJ, Rowling M et al. Resistant starch: promise for improving human health. *Adv Nutr*. 2013;4(6):587-601.
3. Englyst HN, Kingman SM, Cummings JH. Classification and measurement of nutritionally important starch fractions. *Eur J Clin Nutr*. 1992;46 Suppl 2:S33-50.
4. Resistant starch assay kit. [www.megazyme.com]
5. Zaman SA, Sarbini SR. The potential of resistant starch as a prebiotic. *Crit Rev Biotechnol*. 2016;36(3):578-584.
6. Becker A, Pierre Schmartz G, Gröger L, Grammes N, Galata V, Philippeit H, Weiland J, Ludwig N, Meese E, Tierling S et al. Effects of resistant starch on symptoms, fecal markers and gut microbiota in Parkinson's Disease – the RESISTA-PD trial. *Genomics, Proteomics & Bioinformatics*. 2021;S1672-0229(21)00245-X. doi:0.1016/j.gpb.2021.08.009
7. Lockyer S, Nugent A. Health effects of resistant starch. *Nutrition Bulletin*. 2017;42(1):10-41.
8. Miketinas DC, Shankar K, Maiya M, Patterson MA. Usual dietary intake of resistant starch in US adults from NHANES 2015-2016. *J Nutr*. 2020;150(10):2738-2747.
9. Gower BA, Bergman R, Stefanovski D, Darnell B, Ovalle F, Fisher G, Sweatt SK, Resuehr HS, Pelkman C. Baseline insulin sensitivity affects response to high-amylose maize resistant starch in women: A randomized, controlled trial. *Nutr Metab (Lond)*. 2016;13:2.
10. Martinez I, Kim J, Duffy PR, Schlegel VL, Walter J. Resistant starches types 2 and 4 have differential effects on the composition of the fecal microbiota in human subjects. *PLoS One*. 2010;5(11):e15046.

11. Robertson MD, Bickerton AS, Dennis AL, Vidal H, Frayn KN. Insulin-sensitizing effects of dietary resistant starch and effects on skeletal muscle and adipose tissue metabolism. *Am J Clin Nutr.* 2005;82(3):559-567.
12. Maziarz MP, Preisendanz S, Juma S, Imrhan V, Prasad C, Vijayagopal P. Resistant starch lowers postprandial glucose and leptin in overweight adults consuming a moderate-to-high-fat diet: a randomized-controlled trial. *Nutr J.* 2017;16(1):14.
13. Centers of Disease Control and Prevention. National Diabetes Statistics Report, 2020. Atlanta, GA.
14. Centers for Disease Control and Prevention. Diabetes Report Card, 2019. Atlanta, GA.
15. Patterson MA, Fong JN, Maiya M, Kung S, Sarkissian A, Nashef N, Wang W. Chilled potatoes decrease postprandial glucose, insulin, and glucose-dependent insulintropic peptide compared to boiled potatoes in females with elevated fasting glucose and insulin. *Nutrients.* 2019;11(9).
16. Cai M, Dou B, Pugh JE, Lett AM, Frost GS: The impact of starchy food structure on postprandial glycemic response and appetite: a systematic review with meta-analysis of randomized crossover trials. *Am J Clin Nutr.* 2021;114(2):472-487.
17. Knowler WC, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, Nathan DM: Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med.* 2002;346(6):393-403.
18. Maki KC, Pelkman CL, Finocchiaro ET, Kelley KM, Lawless AL, Schild AL, Rains TM: Resistant starch from high-amylose maize increases insulin sensitivity in overweight and obese men. *J Nutr.* 2012;142(4):717-723.
19. Institute of Medicine: Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids,. In. Washington D.C., 2005.
20. Food Safety and Inspection Service. Cooking for groups: A volunteer's guide to food safety. United States Department of Agriculture, 2011.
21. McCleary BV, McLoughlin C, Charmier LMJ, McGeough P: Measurement of available carbohydrates, digestible, and resistant starch in food ingredients and products. *Cereal Chemistry* 2019, 97(1):114-137.
22. Association AD: 2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes –2020. *Diabetes Care* 2019, 43(Supplement_1):S14-S31.
23. Everitt M. Consumer-targeted sensory quality. In: *Global issues in food science and technology.* Elsevier. 2009;117-128.
24. Julious SA: Sample size of 12 per group rule of thumb for a pilot study. *Pharmaceutical Statistics. Journal of Applied Statistics in the Pharmaceutical Industry.* 2005;4(4):287-291.
25. Whitehead AL, Julious SA, Cooper CL, Campbell MJ. Estimating the sample size for a pilot randomised trial to minimise the overall trial sample size for the external pilot and main trial for a continuous outcome variable. *Statistical methods in medical research.* 2016;25(3):1057-1073.

26. Patterson M, Tucker W, Douglas C, Miketinas D. Usual intake of resistant starch by diabetes group in US adults: NHANES 2013–2018. *Curr Develop Nutr.* 2021;5(Supplement_2):358-358.
27. US Department of Agriculture and US Department of Health and Human Services. Dietary Guidelines for Americans, 2020-2025. 9th Ed. [DietaryGuidelines.gov](https://www.dietaryguidelines.gov).
28. Barnard ND, Gloede L, Cohen J, Jenkins DJ, Turner-McGrievy G, Green AA, Ferdowsian H: A low-fat vegan diet elicits greater macronutrient changes, but is comparable in adherence and acceptability, compared with a more conventional diabetes diet among individuals with type 2 diabetes. *J Am Diet Assoc.* 2009;109(2):263-272.
29. Giles ED, Brown IL, MacLean PS, Pan Z, Melanson EL, Heard KJ, Cornier MA, Marden T, Higgins JA. The in vivo net energy content of resistant starch and its effect on macronutrient oxidation in healthy adults. *Nutrients.* 2019;11(10).
30. Higgins JA, Higbee DR, Donahoo WT, Brown IL, Bell ML, Bessesen DH: Resistant starch consumption promotes lipid oxidation. *Nutr Metab (Lond).* 2004;1(1):8.
31. Liu H, Zhang M, Ma Q, Tian B, Nie C, Chen Z, Li J: Health beneficial effects of resistant starch on diabetes and obesity via regulation of gut microbiota: a review. *Food Funct.* 2020;11(7):5749-5767.
32. Vital M, Howe A, Bergeron N, Krauss RM, Jansson JK, Tiedje JM. Metagenomic insights into the degradation of resistant starch by human gut microbiota. *Appl Environ Microbiol.* 2018;84(23).
33. Lattimer JM, Haub MD. Effects of dietary fiber and its components on metabolic health. *Nutrients.* 2010;2(12):1266-1289.
34. Miketinas D, Tucker W, Douglas C, Patterson MA. Usual dietary fiber intake in US adults with diabetes: NHANES 2013–2018. *Curr Devel Nutr.* 2021;5(Supplement_2):1061-1061.
35. Sylvetsky AC, Edelstein SL, Walford G, Boyko EJ, Horton ES, Ibebuogu UN, Knowler WC, Montez MG, Temprosa M, Hoskin M et al. A high-carbohydrate, high-fiber, low-fat diet results in weight loss among adults at high risk of type 2 diabetes. *J Nutr.* 2017;147(11):2060-2066.
36. Xiong K, Wang J, Kang T, Xu F, Ma A. Effects of resistant starch on glycaemic control: a systematic review and meta-analysis. *Br J Nutr.* 2021;125(11):1260-1269.
37. Edwards CH, Grundy MM, Grassby T, Vasilopoulou D, Frost GS, Butterworth PJ, Berry SE, Sanderson J, Ellis PR: Manipulation of starch bioaccessibility in wheat endosperm to regulate starch digestion, postprandial glycemia, insulinemia, and gut hormone responses: a randomized controlled trial in healthy ileostomy participants. *Am J Clin Nutr.* 2015;102(4):791-800.
38. Zenel AM, Stewart ML. High amylose white rice reduces post-prandial glycemic response but not appetite in humans. *Nutrients.* 2015;7(7):5362-5374.
39. Ang K, Bourgy C, Fenton H, Regina A, Newberry M, Diepeveen D, Lafiandra D, Grafenauer S, Hunt W, Solah V. Noodles made from high amylose wheat flour attenuate postprandial glycaemia in healthy adults. *Nutrients.* 2020;12(8).
40. Behall KM, Hallfrisch J: Plasma glucose and insulin reduction after consumption of breads varying in amylose content. *Eur J Clin Nutr.* 2002;56(9):913-920.

41. Peterson CM, Beyl RA, Marlatt KL, Martin CK, Aryana KJ, Marco ML, Martin RJ, Keenan MJ, Ravussin E. Effect of 12 wk of resistant starch supplementation on cardiometabolic risk factors in adults with prediabetes: a randomized controlled trial. *Am J Clin Nutr.* 2018;108(3):492-501.
42. Kazemi M, McBreaity LE, Chizen DR, Pierson RA, Chilibeck PD, Zello GA. A comparison of a pulse-based diet and the therapeutic lifestyle changes diet in combination with exercise and health counselling on the cardio-metabolic risk profile in women with polycystic ovary syndrome: A randomized controlled trial. *Nutrients.* 2018;10(10).
43. Naska A, Lagiou A, Lagiou P: Dietary assessment methods in epidemiological research: current state of the art and future prospects. *F1000Res.* 2017;6:926-926.
44. AlEssa HB, Bhupathiraju SN, Malik VS, Wedick NM, Campos H, Rosner B, Willett WC, Hu FB. Carbohydrate quality and quantity and risk of type 2 diabetes in US women. *Am J Clin Nutr.* 2015;102(6):1543-1553.

Tables

Table 1-5 is available in the Supplemental Files section.

Figures

Figure 1

Outline of phase 1 and 2.

Figure 2

CONSORT flow diagram

Figure 3

Mean total area under the curve ($tAUC_{(0-120 \text{ min})}$) values before and after consuming the 7-day high resistant starch menu. n = 8.

Supplementary Files

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