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Resistant starch wheat increases PYY and decreases GIP but has no effect on self-reported perceptions of satiety

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ABSTRACT

Dietary fiber has numerous health benefits, such as increasing satiety, and is regularly included in healthy dietary recommendations. However, different types and sources of fiber vary in their chemical properties and biological effects. This double-blind, randomized, placebo-controlled, crossover study investigated the effects of resistant starch type 2 (RS2) from wheat on self-reported perceptions of satiety and associated gut hormones in 30 healthy adults ages 40–65 years of age. Participants consumed rolls made using either RS2-enriched wheat flour or a wild-type flour for one week before a test day during which they ate a mixed meal containing the same roll type. Both self-reported perceptions of satiety and plasma concentrations of gut hormones were measured following the meal to assess whether the RS2-enriched wheat enhanced satiety and suppressed hunger for a longer period than the control wheat. Exploratory analysis indicated that fasting and peak concentration of peptide $YY_{3\text{-}36}$ ($PYY_{3\text{-}36}$; $q_{fast} = 0.02$, $q_{peak} = 0.02$) increased, while peak concentration and iAUC of glucose-dependent insulinotropic peptide (GIP; $q_{peak} < 0.001$, $q_{iAUC} < 0.001$) decreased after ingesting RS2-enriched wheat. However, self-reported perceptions of hunger or fullness using visual analog scales (VAS) did not differ following the test meal.

1. Introduction

Obesity is a leading public health problem in the United States and increasingly worldwide. An important aspect of weight management is satiety, which refers to the suppression of appetite and food intake between meals (Blundell et al., 2010). Satiety is thought to be regulated by numerous hormonal signals such as leptin, ghrelin, glucagon-like peptide-1 (GLP-1), peptide YY (PYY), and glucose-dependent insulinotropic peptide (GIP), many of which originate in the gut. However, the causal role of these hormones in hunger and satiety are unclear (Steinert et al., 2017). Ghrelin, known as the "hunger hormone," has been shown to increase food intake (Manning & Batterham, 2014). Though the role of GIP in regulation of satiety is less well-established, it has been negatively correlated with fullness and positively correlated with prospective food consumption, suggesting it may also increase hunger (Raben et al., 1994). Conversely, leptin, GLP-1, and PYY have demonstrated an opposite effect (Chaudhri, Field, & Bloom, 2006; Manning & Batterham, 2014; Seino, Fukushima, & Yabe, 2010; Troke, Tan, & Bloom, 2014). The inclusion of foods or ingredients that promote satiety has the potential to aid in healthy weight management as hunger has been cited as a barrier to the success of diets intended to promote weight loss (López-Nicolás et al., 2016).

Fiber appears to increase satiety by increasing gastric distention, delaying gastric emptying, blunting glycemic responses, and/or stimulating GLP-1, GIP, and PYY secretion (Howarth, Saltzman, & Roberts, 2001; Keenan et al., 2006; Shimada, Mochizuki, & Goda, 2008; June Zhou et al., 2008). However, studies indicate that fiber types or sources may differentially affect satiety based on physicochemical properties,

Abbreviations: AUC, area under the curve; BMI, body mass index; CA, cholic acid; CDCA, chenodeoxycholic acid; C, Control; DCA, deoxycholic acid; FXR, farnesoid X receptor; FGF-19, fibroblast growth factor-19; GCA, glycocholic acid; GCDCA, glycochenodeoxycholic acid; GDCA, glycodeoxycholic acid; GIP, glucose-dependent insulinotropic peptide; GLP-1, glucagon-like peptide-1; iAUC, incremental area under the curve; IRB, Institutional Review Board; LCA, lithocholic acid; PYY, peptide YY; RIA, radioimmunoassay; RS, resistant starch; RS2, resistant starch type 2; TCDCA, taurochenodeoxycholic acid; TDCA, taurodeoxycholic acid; TILLING®, Targeting Induced Local Lesions IN Genomes; UDCA, ursodeoxycholic acid; VAS, visual analog scales.

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including solubility and viscosity (Gidley & Yakubov, 2019; Poutanen et al., 2017; Slavin & Green, 2007; Willis, Eldridge, Beiseigel, Thomas, & Slavin, 2009). Resistant starch (RS) is a type of dietary fiber defined as the portion of starch resistant to digestion by amylases (Sajilata, Singhal, & Kulkarni, 2006). When RS reaches the large intestine, it can be fermented by the resident intestinal microbiota and may yield positive effects on postprandial glycemia, weight management, and digestive tract health (Birt et al., 2013; Keenan et al., 2015; Sajilata et al., 2006). However, results from investigations into the effects of RS on satiety outcomes have been mixed; some have shown increased satiety scores, reduced hunger, and/or reduced energy intake while others have reported no effect (Belobrajdic et al., 2019; J. A. Higgins, 2014; Lockyer & Nugent, 2017; White, Peterson, Beyl, Martin, & Ravussin, 2020). These conflicting results may be due to differences in dosage, population-specific effects, or other aspects of study design. Variation may also be due to differences between types and sources of RS. RS types (e.g. I, II, III, and IV) differ in their physicochemical properties and can be derived from numerous sources (e.g. corn, wheat, and potato) (Sajilata et al., 2006). Most studies investigating satiety-related hormones have measured only a few select hormones rather than a comprehensive panel, precluding a clear understanding of the cascade of satiety signals in response to RS intake (Klosterbuer, Thomas, & Slavin, 2012).

The gut microbiota plays a major role in health and metabolism [2,6]. The intestinal microbiome is comprised of over 10^{12} bacterial cells from up to 1000 or more species with diverse impacts on host physiology such as from energy harvest, immunomodulation, regulation of mood and behavior, and satiety The gut microbiota influences the bile acid pool by deconjugation and dehydroxylation of primary bile acids to form secondary bile acids (Sonne, Hansen, & Knop, 2014). In addition to their function as detergents for lipid digestion, bile acids are hormones that trigger a variety of signaling pathways via the farnesoid X receptor (FXR) and the G protein-coupled receptor TGR5. The activation of TGR5 on enteroendocrine L cells, which are abundant in the cecum and large intestine, stimulates secretion of GLP-1 and PYY (Penney, Kinross, Newton, & Purkayastha, 2015; Sonne et al., 2014). Because the secondary bile acids lithocholic acid (LCA) and deoxycholic acid (DCA) are the most potent agonist of TGR5 (Sonne et al., 2014), changes in the microbiota and microbial metabolism by RS may mediate changes in the bile acid pool and potentially satiety. Additionally, circulating bile acids may be involved in central regulation of appetite (Keitel et al., 2010; Ogundare et al., 2010; Quinn et al., 2014; Ryan et al., 2013) and have been shown to indirectly upregulate leptin gene expression in adipose tissue (Levy, Heuman, Pandak, & Stevens, 1998).

In this crossover, randomized controlled trial, 30 healthy adults aged 40–65 years consumed RS2-enriched wheat rolls and wild-type wheat rolls to determine the effect of the RS2-enriched wheat on both self-reported perceptions of satiety and associated gut hormones to evaluate the correlations between these outcomes and the changes in fecal bile acids.

2. Methods

2.1. Study design

The trial was conducted in adherence with the Good Clinical Practice guidelines (Vijayananthan & Nawawi, 2008) and ethical standards of the Helsinki Declaration. The trial protocol was approved and ethical clearance to conduct the study was granted by the University of California Davis Institutional Review Board (IRB) (Protocol #: 984621). The trial was registered on ClinicalTrials.gov (NCT03082131). Informed consent was obtained from each participant before being enrolled into the study.

Participants were healthy males and females, aged 40–65 years. Participants were excluded if they had body mass index (BMI) < 18.5 or >39.9 kg/m^2 due to the effects of underweight and obesity on satiety and appetitive hormones. Participants were also excluded if they had untreated or uncontrolled metabolic diseases, any gastrointestinal disorders (e.g., Crohn's disease, irritable bowel syndrome, colitis), cancer or other serious chronic disease, or dietary restrictions that interfered with consuming the intervention foods. Participants were also excluded if they were pregnant, lactating, used tobacco, or any other prescribed or over-the-counter medications that impacted weight loss or metabolism. A total of 30 subjects completed the study as previously described (Hughes et al., 2021). In this crossover design, subjects were randomly assigned treatment order to first receive either RS2-enriched wheat first or wild-type wheat (Hughes et al., 2021). Full details regarding the development of the RS2-enriched wheat, and the formulation and nutritional content of the roll product have been provided previously (Hughes et al., 2021).

Products (rolls) made from RS2-enriched wheat and wild-type wheat were provided to supplement participants' usual diet for seven days. Women were asked to eat three rolls per day (a half roll at breakfast and lunch, two at dinner) while men were asked to eat four rolls per day (one at breakfast and lunch, two at dinner). The RS2-enriched wheat products provided 14–19 g of RS per day, whereas the regular refined wheat products provided only 2–3 g of RS per day. The amount of RS provided was chosen so that, when added to the typical fiber intake of the American diet (~15 g) (Quagliani & Felt-Gunderson, 2017), the RS2-enriched wheat products would increase dietary fiber intake to recommended levels. Subjects kept a log of the products eaten and returned unused products at the week's end. Participants performed a total of seven dietary recalls over the course of the study: one at baseline, two during each week-long intervention arm, and one at each test day. The meal challenge test day was scheduled on the 8th day. A two-week washout period separated the treatments. A fecal specimen was collected prior to and at the end of each treatment.

2.2. Meal challenge and test protocol

Metabolic responses to a mixed breakfast meal containing either RS wheat or regular wheat were evaluated. GLP-1, PYY, leptin, ghrelin, and GIP were measured as biological indicators of satiety. The test protocol was approximately 4 h in duration. The meal challenge consisted of a standard breakfast meal (an egg, cheese, and turkey sausage sandwich served on either RS wheat roll or wild-type wheat roll) prepared in the WHNRC metabolic kitchen. The nutritional composition of each test meal is shown in Table 1.

While controlling for calories, the RS meal provided 19.68 g of total dietary fiber and 9.57 g of RS, and the control meal provided only 4.69 g and 1.84 g, respectively. Over the course of the test day, four blood samples were obtained by venipuncture: one while subject was fasting, and three following the test meal at 1, 2, and 3 h post-meal. For gut

Table 1
Test meal nutrition.

Nutrient	Resistant Starch (RS) Meal	Control Meal
Calories (kcal)	780.1	789.3
Carbohydrate (g)*	79.2	88.9
Total dietary fiber (g)	19.7	4.7
Resistant starch (g)	9.6	1.8
Insoluble fiber (g)	11.8	2.3
Soluble fiber (g)	7.8	2.4
Protein (g)	38.0	35.2
Fat (g)	33.7	32.3
Monounsaturated (g)	11.6	11.2
Polyunsaturated (g)	5.9	5.3
Saturated (g)	13.4	13.2

Footnote: Nutrition information for meal components (excluding the rolls) was calculated using Nutrition Data System for Research (NDSR) software, which was then added to the custom nutrition information for the RS2-enriched roll and wild-type wheat roll in the RS meal and Control Meal, respectively. *Total carbohydrate was determined by difference and is therefore an estimate; includes dietary fiber, digestible sugars, and other unmeasured carbohydrates.

hormone analyses, blood was drawn into vacutainers containing EDTA and protease inhibitors (DPPIV inhibitor and aprotinin). Samples were immediately put on ice before being centrifuged and plasma aliquoted into cryotubes. For ghrelin analysis, hydrochloric acid was also added to plasma. Aliquots were stored at −80 °C for further analysis.

2.3. Measurement of gut hormones and self-reported indices of appetite, satiety, and food intake

Biologically active GLP-1 and leptin were measured using Meso Scale Discovery (MSD) Multi-Spot Assay System containing human active GLP-1 (7–36) amide, insulin, glucagon, and leptin. GIP and acylated (active) ghrelin were also analyzed using the MSD Multi-Spot Assay System. Multi-spot assays were conducted according to manufacturer instructions and plates were read on the MSD QuickPlex SQ 120 imager.

Active PYY was measured using Millipore's PYY (3-36) Radioimmunoassay (RIA) Kit prepared according to manufacturer's instructions and sample pellets were counted using a gamma counter (2480 Wizard^2, PerkinElmer).

The intra- and inter-assay coefficients of variation for standard samples for our laboratory were as follows: GLP-1: 11.0%, 20.9%; leptin: 5.2%, 8.4%; GIP: 6.3%, 20.6%; ghrelin: 5.8%, 13.3%; PYY: 5.9%, 14.3%. Samples were measured in duplicate. Due to the potential for inter-assay variation, all samples from the same subject were analyzed on one plate.

Self-reported perceptions of appetite were collected throughout the test day, once right before the meal challenge as well as every 30 min afterwards for a total of seven measurements. Participants were required to rate their appetitive sensations using a visual analogue scale (VAS) presented on a handheld device. The VAS had end anchors ranging from "not at all" to "extremely" and used standard appetite questions, as described by Hill and Blundell (Hill & Blundell, 1982). Values were reported on a linear scale with 0 = not at all to 100 = extremely.

An automated 24-h dietary recall system (ASA-24, NIH–NCI) was used to capture dietary intake during each arm of the intervention. One recall was obtained during screening to assess baseline dietary intake. Two recalls were obtained at home during each treatment period as well as an additional recall during each test day. Average daily calorie (kcal) intake was assessed for differences between treatment arms to determine whether RS reduced food intake.

Subjects recorded all stools passed during treatment, evaluated consistency with Bristol stool chart, and assessed degree of nausea, bloating, GI rumbling, gas/flatulence, abdominal pain, diarrhea or constipation. This allowed us to assess whether the RS wheat increased gastrointestinal distress in participants.

2.4. Stool sample collection

Fecal samples were collected prior to (Pre-RS, Pre-Control) and at the end (RS, Control) of each treatment. Fecal samples were delivered to the WHNRC within 24 h of collection. Participants were provided with collection kits, which included a cooler, ice packs, commode specimen collection system, empty tubes, zipper plastic bags, pen, and instructions. If not immediately delivering samples to the WHNRC after collection, participants stored a portion of the stool samples in tubes in the freezer, while the remaining stool sample was kept refrigerated within multiple zipped bags to ensure no cross-contamination occurred. Samples were transported using the cooler and ice packs to the WHNRC where the tubes were immediately placed in freezer storage at −80 °C, and the remaining sample was stored at 4 °C. The remaining sample was homogenized using a stomacher (Seward Model 80 Stomacher; Tekmar Company, Cincinnati, OH, USA) before being divided into aliquots and stored at −80 °C. Aliquots were used for stool analyte profiling, including bile acids, and pH, while tube samples were used for microbiota analyses (Hughes et al., 2021).

2.5. Fecal bile acid analysis

Bile acids were extracted from approximately 50 mg of fecal sample homogenate using 1:1 methanol/acetonitrile (v/v) (MeOH/ACN), enriched with isotopically labeled standards and quantified via UPLC-MS/MS. In short, 50 mg of sample was homogenized with 200 μL of MeOH/ACN using GenoGrinder 2010 (SPEX Sample Prep; Metuchen, NJ) vertical ball mill for 8 min at 1200 rpm. Samples were then centrifuged for 10 min at 10,000 g at 4 °C and 150 μL supernatant was filtered at 0.1 μm in a 96-well PVDF filter plate by centrifugation. A 20 μL aliquot of filtrate was mixed with 5 μL of 20 μM BA surrogate mixture containing GCA-d4, TCDCA-d4, GCDCA-d4, CDCA-d4, DCA-d4, and LCA-d4, and diluted with 175 μL of MeOH/ACN. A 2 μL sub-aliquot of the 200 μL extract was further diluted with 98 μL of MeOH/ACN containing 100 nM 1-cyclohexyl ureido, 3-dodecanoic acid (Sigma Aldrich; St. Louis, MO) and 1-phenyl ureido 3-hexanoic acid (kind gift from Dr. B. D. Hammock, University of California Davis, Davis CA, USA) and stored at −20 °C until analysis.

Bile acids were separated on a 2.1 mm × 100 mm, 0.17 μm Acquity BEH C18 column (Waters, Milford, MA), detected by electrospray ionization on an API 4000 QTRAP (Sciex; Redwood City, CA) and quantified against a 7-9-point calibration curve in multireaction monitoring as previously described (La Frano et al., 2017). A study-wide pooled sample was prepared and assayed in duplicate in each of the five analytical batches to assess analytical precision. For metabolites >1 nM, the aggregate inter-assay CV and intra-assay CVs were 25.6 ± 8.5% and 23.76 ± 6.5%, respectively. All data were analyzed using Multiquant 3.0.3 software (Sciex; Redwood City, CA).

Bile acids included in the current analysis were: cholic acid (CA), chenodeoxycholic acid (CDCA), deoxycholic acid (DCA), lithocholic acid (LCA), glycocholic acid (GCA), glycodeoxycholic acid (GDCA), glycochenodeoxycholic acid (GCDCA), taurochenodeoxycholic acid (TCDCA), taurodeoxycholic acid (TDCA), and ursodeoxycholic acid (UDCA). To facilitate analysis, bile acids were also grouped intro primary (CA, CDCA), secondary (DCA, LCA, UDCA), and conjugated (GCA, GCDCA, TCDCA, GDCA, TDCA) bile acids.

2.6. Statistical analysis

All statistical analyses were conducted in R version 3.6.1. For outcomes assessed over the course of the test days, fasting, area under the curve (AUC) or incremental AUC (iAUC) (Wolever & Jenkins, 1986), and peak values were computed and added as separate variables.

The effect of the RS intervention was assessed using a linear model, controlling for treatment sequence and, if available, baseline value prior to the intervention. The outcome in the regression model was the difference between the outcome value after RS treatment and the value after Control treatment (e.g., RS – C). Sequence was coded as −0.5 and + 0.5 so the regression intercept would give the mean treatment effect (possibly adjusted for baseline). The baseline value used in the linear models was calculated as the individual's baseline value subtracted from the mean baseline value across all subjects. Correction for multiple hypothesis testing was performed using Benjamini-Hochberg correction with a false discovery rate (FDR) of 0.15 and results are reported as q-values. Outcomes for which initial p-values were not significant are reported as p-values. The bile acid analysis was exploratory and therefore not corrected for multiple hypothesis testing.

For data containing outliers (i.e., outside 1.5 times the interquartile range above the upper quartile and below the lower quartile), sensitivity analyses were conducted to determine the effect of removing outliers. For data demonstrating a change in statistical significance as a result of outlier removal, results of both analyses are reported. All variables and outcomes were assessed for normality using quantile-quantile (Q-Q) plots. For normally distributed data, the linear model described above was used. For data demonstrating a pronounced and consistent departure from a normal distribution (Schützenmeister, Jensen, & Piepho,

2012), data were log transformed and normality was re-evaluated before analyzing using the above linear model. Outcomes examined with log transformed values included leptin (fasting, peak). Pearson and Spearman correlations of gut hormone concentrations and self-reported indices of satiety were analyzed during both RS and control treatments. Outliers were removed for correlation analyses.

3. Results

3.1. Food intake

ASA24 food intake records were analyzed to determine whether reported calorie (kcal) intake differed from baseline or between treatment periods. There were no significant differences in calorie intake between treatment periods or from baseline although calorie intake was slightly elevated during both treatments compared to baseline ($p_{control} = 0.07$, $p_{RS} = 0.06$).

Fiber, but not carbohydrate, intake was elevated during the RS treatment compared to the Control and compared to baseline ($q < 0.001$, Fig. 1) due to the higher fiber content of the RS rolls. There were no significant correlations with baseline fiber intake and the effect of RS on either gut hormone concentrations or self-reported perceptions of hunger and satiety ($p > 0.05$).

3.2. Appetite and satiety

Self-reported measures of appetite, measured using the VAS, showed no significant effects of the RS intervention. AUC of hunger, fullness, desire to eat, and prospective consumption on test days all showed insignificant p-values for the difference between treatments (Supplementary Table 1).

Appetitive hormones were measured as potential biological indicators of satiety. GIP was lower while PYY_{3-36} was higher after RS compared to Control. GIP iAUC (pg·min/mL) and peak GIP concentration (pg/mL) were significantly lower after the test meal when participants were fed the RS wheat rolls compared to the control rolls ($q_{iAUC} < 0.001$, $q_{peak} < 0.001$, Fig. 2). Fasting PYY_{3-36} was significantly higher during RS treatment compared to control ($q = 0.02$, Fig. 3). Peak PYY_{3-36} showed no significant difference between treatments in the full dataset ($q = 0.31$) but, after sensitivity analysis and removal of a single outlier, peak PYY_{3-36} was significantly higher during the test day when participants were fed the RS wheat rolls compared to the control rolls ($q = 0.02$, Fig. 3). PYY_{3-36} iAUC showed no significant difference between treatments. There were no significant differences between RS and control for ghrelin, leptin, or GLP-1, with or without sensitivity analysis ($p > 0.05$, Supplementary Fig. 1). There were significant effects of treatment sequence on peak GIP ($p = 0.01$) and fasting PYY_{3-36} ($p = 0.03$). The effect of treatment sequence on fasting GIP was insignificant in the full dataset ($p = 0.29$) but became significant after removing three outliers in sensitivity analysis ($p = 0.02$). However, these sequence effects were not significant ($q > 0.15$) following correction for multiple hypothesis testing. There were no significant differences between treatments in the time at which peak hormone levels occurred for any of the appetitive hormones.

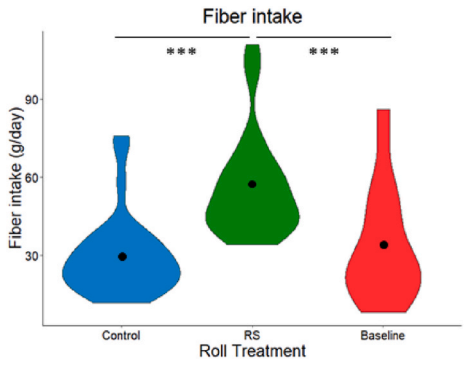

Fig. 1. Differences in fiber intake. Participants' fiber intake was significantly increased during RS supplementation ($q < 0.001$). RS (resistant starch).

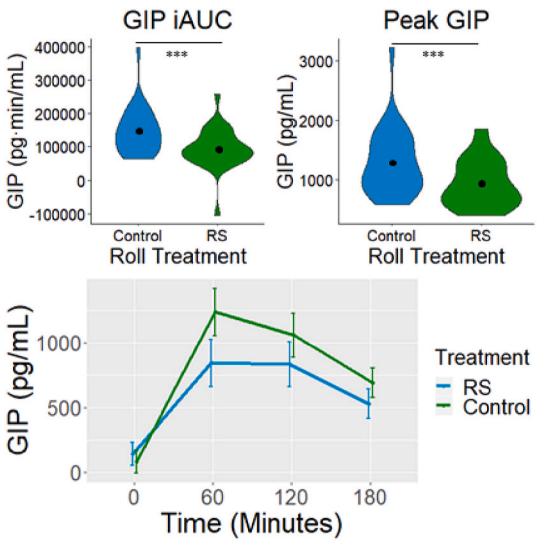

Fig. 2. Differences in GIP concentrations between RS and Control. Violin plots depicting the difference in GIP iAUC and peak GIP between RS and control display the median (black dot) and a kernel density distribution (outline) to show the distribution of the data. GIP iAUC and peak were significantly lower during RS compared to Control ($q_{iAUC} < 0.001$, $q_{peak} < 0.001$). GIP (glucose-dependent insulinotropic polypeptide), iAUC (incremental area under the curve), RS (resistant starch).

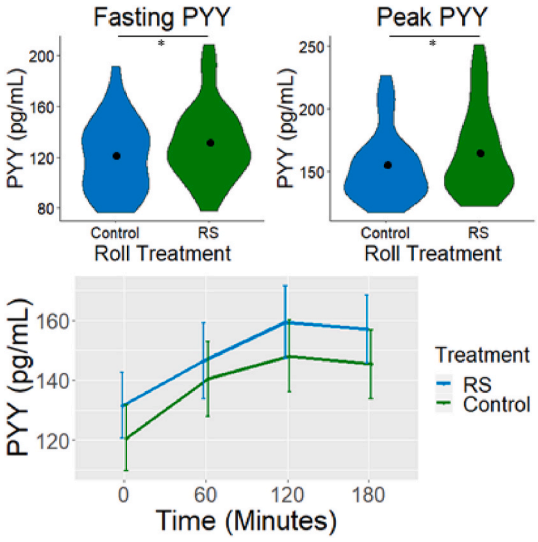

Fig. 3. Differences in PYY concentrations between RS and Control. Fasting PYY was higher during RS compared to control ($q = 0.02$) while peak PYY was higher during RS after sensitivity analysis ($q = 0.02$). PYY (peptide YY), RS (resistant starch).

Pearson correlation analysis showed a negative correlation between ghrelin iAUC and fullness during the RS treatment only ($r = -0.39$, $p = 0.03$), consistent with its orexigenic effects (S. C. Higgins, Gueorguiev, & Korbonits, 2007), but this effect was not observed during the control treatment ($r = -0.03$, $p = 0.87$) and ghrelin was not lowered by the RS treatment ($p = 0.49$). No other significant correlations between appetitive hormone concentrations and self-reported perceptions of satiety

were shown in the current dataset.

3.3. Bile acids

Concentrations of fecal bile acids at each time point are shown in Supplementary Table 2. Linear mixed models were used to analyze pairwise effects of treatments (Pre-Control, Control, Pre-RS, RS) on bile acids. Prior to sensitivity analysis, initial findings suggested a significant difference between treatments for total bile acids, total primary bile acids, and total secondary bile acids as well as CA, CDCA, DCA, LCA, and UDCA ($p < 0.05$). There was a significant main effect of time on fecal bile acids following the Control intervention, but not following the RS intervention. Mean concentrations of bile acids all followed the same general pattern with Control samples having the highest concentrations followed by RS while Pre-Control and Pre-RS samples were lowest and roughly equivalent (Supplementary Table 2). The only bile acids to break this pattern were the glycine conjugates GCA, GDCA, and GCDCA, concentrations of which were highest in Pre-Control samples followed by Control samples, RS samples, and Pre-RS samples. Compared to RS, only total bile acid concentration was significantly higher after Control ($p = 0.04$).

Due to presence of outliers, sensitivity analysis was done to determine the effect of outlier removal. From the 120 samples, eleven outliers were removed from total bile acids, eleven from total primary bile acids, eight from total secondary bile acids, twelve from total conjugated bile acids, nineteen from CA, fifty-one from CDCA, seven from DCA, and nine from LCA. Outliers tended to cluster within individuals, suggesting that the presence of outliers were due to individual biological variability rather than lab analytic techniques. After removal of outliers, only DCA showed significant effects of treatment. DCA was significantly higher after RS versus Pre-Control (0.13 ± 0.11 pmol/g versus 0.10 ± 0.07 pmol/g, $p = 0.04$) (Supplementary Fig. 2).

Due to the potential for bile acids to stimulate secretion of appetitive hormones, correlations in the current dataset were assessed. Spearman correlation of fecal bile acid concentrations and both fasting GLP-1 and GLP-1 AUC revealed a negative association between total primary bile acids and fasting GLP-1 ($\rho = -0.35$, $p = 0.015$) (Supplementary Fig. 3). Spearman correlation analyses also demonstrated a positive association between concentrations of total bile acids ($\rho = 0.35$, $p = 0.012$), total secondary bile acids ($\rho = 0.31$, $p = 0.024$), DCA ($\rho = 0.33$, $p = 0.013$), and LCA ($\rho = 0.27$, $p = 0.053$) and leptin iAUC (Supplementary Fig. 4). There were no significant correlations between bile acids and self-reported perceptions of hunger or fullness ($p > 0.05$).

3.4. Gastrointestinal symptoms

Subject evaluation of gastrointestinal symptoms and bowel movements showed some effects of the RS treatment. Average ratings of fecal hardness, straining during bowel movement, discomfort during bowel movement, sensation of incomplete evacuation, and Bristol stool scale showed insignificant p-values for all measures ($p > 0.05$). However, the RS treatment significantly increased the number of bowel movements per week ($q = 0.12$) (Fig. 4). While there were no significant differences in participant ratings of gastrointestinal pain, rumblings, nausea, or diarrhea; participant ratings of gastrointestinal gas and bloating were significantly higher during the RS treatment ($q_{gas} = 0.002$, $q_{bloat} = 0.08$) (Fig. 4).

4. Discussion

This analysis demonstrates that, while RS2-enriched wheat showed no significant effects on self-reported indices of satiety and fullness or food intake, we did observe a decrease in GIP and increase in $PYY_{3\text{-}36}$ with RS2-enriched wheat supplementation. However, GLP-1, leptin, and ghrelin were unaffected by RS.

The increase in $PYY_{3\text{-}36}$ observed in the current analysis corroborates previous findings on the effects of RS type 2 in both animals (Ingerslev et al., 2017; Keenan et al., 2006; Jun Zhou et al., 2006; June Zhou et al., 2012; June Zhou et al., 2008) and humans (Maziarz et al., 2017; Sandberg, Björck, & Nilsson, 2017). PYY has been shown to induce feelings of satiety and may be attenuated in individuals with obesity (Batterham et al., 2003; le Roux et al., 2006; Manning & Batterham, 2014). This suggests that an impaired PYY response may lead to increased food intake and, potentially, weight gain (Karra, Chandarana, & Batterham, 2009). However, in the human studies listed above (Maziarz et al., 2017; Sandberg et al., 2017) as well as in our dataset, participants' daily food intake did not differ between RS and Control and there were no differences between treatments in self-reported perceptions of appetite and satiety.

GIP response decreased following the test meal containing RS after participants had consumed RS2-enriched wheat for one week. The observed decrease in GIP is in agreement with previous findings showing decreased GIP as well as GIP mRNA expression after RS supplementation (Raben et al., 1994; Shimada et al., 2008; Shimada, Mochizuki, & Goda, 2009a, 2009b). GIP may increase gastric emptying, though the effects of this on self-reported perceptions of hunger and satiety are unclear (Edholm et al., 2010). GIP has been linked to increased fat deposition in adipose tissue and impaired fat metabolism (Robertson, 2012; Seino et al., 2010), which may influence weight management.

The current findings of the effect of RS2-enriched wheat on fecal bile acids suggest that RS2-enriched wheat mitigates the increase in fecal bile acids that was observed after consuming wild-type wheat. This is in agreement with previous findings, which suggest that fiber intake is negatively correlated with fecal bile acid concentrations (Trefflich et al., 2020). However, the secondary bile acid DCA was elevated ~30% after RS2-enriched wheat intake, which contradicts previous findings of the effects of high-amylose maize and other dietary patterns on fecal bile acid excretion (Reddy, 1981; van Munster, Tangerman, & Nagengast, 1994). Fecal excretion of total primary bile acids was negatively associated with fasting GLP-1, in contrast to previous findings (Sonne et al., 2014). Fecal concentrations of total bile acids, total secondary bile acids, DCA and LCA were positively associated with leptin iAUC. In plasma,

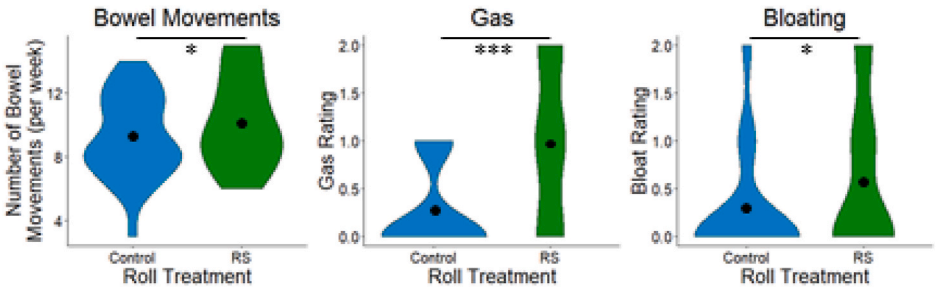

Fig. 4. Differences in gastrointestinal symptoms. The number of bowel movements were increased during RS ($q = 0.12$) as well as participants' subject ratings of gas and bloating ($q_{gas} = 0.002$, $q_{bloat} = 0.08$). RS (resistant starch).

studies comparing circulating bile acids and leptin have had mixed results, with some findings indicating a positive association (Levy et al., 1998) but others finding a negative association (Pierre et al., 2016). The current study analyzed only fecal bile acid excretion, rather than the composition in bile or the liver, which are more representative of the bile acid pool (Chiang, 2017). Bile acids in enterohepatic circulation are re-absorbed in the terminal ileum with high efficiency (~95%) (Ferrebee & Dawson, 2015). Therefore, fecal bile acid excretion may not adequately reflect the composition of the circulating biliary pool (Chiang, 2017) and provide an incomplete picture of the effects of circulating bile acids.

The increased number of bowel movements per week is in agreement with previous findings on the effect of RS on laxation in healthy adults (Maki et al., 2009), suggesting that RS2-enriched wheat contributes to bulking of stool and speeding of passage through the bowel (Dreher, 2018). The observed increase in bloating may be due to the production of intestinal gases such as carbon dioxide, hydrogen, and methane from fermentation of non-digestible carbohydrates, which results in gas excretion and bloating (Klosterbuer et al., 2013; Suarez & Levitt, 2000).

Several limitations of the current study should be noted. First, the average fiber intake of participants at baseline was higher than expected (33.67 g), and it is unclear whether RS2-enriched wheat would have had a different effect on satiety in a more fiber-deficient population. However, we did not observe any correlations between either baseline fiber intake or RS2-enriched wheat on hunger and satiety. Second, measurement of participants' sensations of hunger and satiety were measured using VAS, and it is well known that different people interpret the descriptive anchors differently (Hayes, Allen, & Bennett, 2013). Thus, alternative scales, such as the labeled magnitude scales (Cardello, Schutz, Lesher, & Merrill, 2005), present another option that could have been used in this study. Both approaches to capturing perceptions have the limitation that we do not know what individual responses represent (Schifferstein, 2012). Another limitation of the current study is that participants were required to eat the full test meal, rather than allowing participants to eat *ad libitum* until fully satiated. This would have allowed for more acute measurement of effects on food intake than with a standardized meal. However the standardized meal posed a better digestive challenge that could be more easily compared between treatments, providing 31.4% of the recommended daily intake of 2500 calories for men and 41.3% of the recommended daily intake of 1900 calories for women ages 40–65 years of age according to the 2015 Dietary Guidelines for Americans (Dietary Guidelines Advisory Committee, 2015). A potential solution for future research may be to have participants finish the meal but indicate at what point during the meal they feel satiated. It would also be useful for future studies to assess how the size of participants' habitual meals compare to the test meal and whether this influences subsequent measures of hunger and fullness. Finally, the period of data collection on the test days was also limited to 3 h postprandial, which might not have been enough time for the RS2-enriched wheat to make it to the lower bowel, limiting our ability to assess acute meal effects on the colon. This analysis demonstrates a moderate effect of one week of RS2-enriched wheat on satiety hormones. However, self-reported perceptions of hunger and satiety were not affected, suggesting no significant effect of short-term intake of RS2-enriched wheat on satiety. The long-term effects of RS2-enriched wheat bread on food intake, satiety, and weight management remain unknown and require future research.

Declarations of competing interest

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.appet.2021.105802.

Author contributions

RLH recruited participants, conducted the trial, analyzed the data, and composed the manuscript. WFH provided assistance with data collection on test days. BR and AW developed the fecal bile acid analysis protocol for this study and trained analytical staff in the application of these methods. LRW and JWN supervised quantification of blood and fecal analytes, respectively. NLK advised and consulted during the trial and edited the final manuscript. All authors have read and approved the final manuscript. USDA is an equal opportunity provider and employer.

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Ethical statement

The trial was conducted in adherence with the Good Clinical Practice guidelines and ethical standards of the Helsinki Declaration. The trial protocol was approved and ethical clearance to conduct the study was granted by the University of California Davis Institutional Review Board (IRB) (Protocol #: 984621). The trial was registered on ClinicalTrials.gov (NCT03082131). Informed consent was obtained from each participant before being enrolled into the study.

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Abbreviations

area under the curve: (AUC)
body mass index: (BMI)

cholic acid: (CA)
chenodeoxycholic acid: (CDCA)
Control: (C)
deoxycholic acid: (DCA)
farnesoid X receptor: (FXR)
fibroblast growth factor-19: (FGF-19)
glycocholic acid: (GCA)
glycochenodeoxycholic acid: (GCDCA)
glycodeoxycholic acid: (GDCA)
glucose-dependent insulinotropic peptide: (GIP)
glucagon-like peptide-1: (GLP-1)
incremental AUC: (iAUC)
Institutional Review Board: (IRB)
lithocholic acid: (LCA)
peptide YY: (PYY)
radioimmunoassay: (RIA)
resistant starch: (RS)
resistant starch type 2: (RS2)
taurochenodeoxycholic acid: (TCDCA)
taurodeoxycholic acid: (TDCA)
TILLING®: (Targeting Induced Local Lesions IN Genomes)
ursodeoxycholic acid: (UDCA)
visual analog scales: (VAS)