



RESEARCH ARTICLE

A resistant-starch enriched yogurt: fermentability, sensory characteristics, and a pilot study in children [version 1; referees: 2 approved]

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Abstract

The rising prevalence of obesity and the vulnerability of the pediatric age group have highlighted the critical need for a careful consideration of effective, safe, remedial and preventive dietary interventions. Amylose starch (RS2) from high-amylose maize (HAM) ferments in the gut and affects body weight. One hundred and ten children, of 7-8 (n=91) or 13-14 (n=19) years of age scored the sensory qualities of a yogurt supplemented with either HAM-RS2 or an amylopectin starch. The amylopectin starch yogurt was preferred to the HAM-RS2-enriched yogurt by 7-8 year old panelists ($P<0.0001$). Appearance, taste, and sandiness scores given by 13- to 14-year-old panelists were more favorable for the amylopectin starch yogurt than for HAM-RS2-enriched yogurt ($P<0.05$). HAM-RS2 supplementation resulted in acceptable (≥ 6 on a 1-9 scale) sensory and hedonic ratings of the yogurt in 74% of subjects. Four children consumed a HAM-RS2-enriched yogurt for four weeks to test its fermentability in a clinical trial. Three adolescents, but not the single pre-pubertal child, had reduced stool pH ($P=0.1$) and increased stool short-chain fatty acids (SCFAs) ($P<0.05$) including increased fecal acetate ($P=0.02$), and butyrate ($P=0.089$) from resistant starch (RS) fermentation and isobutyrate ($P=0.01$) from protein fermentation post-treatment suggesting a favorable change to the gut microbiota. HAM-RS2 was not modified by pasteurization of the yogurt, and may be a palatable way to increase fiber intake and stimulate colonic fermentation in adolescents. Future studies are planned to determine the concentration of HAM-RS2 that offers the optimal safe and effective strategy to prevent excessive fat gain in children.

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Clinical relevancy statement

Resistant starch (RS) is a type of dietary fiber that people cannot digest, diluting caloric density, but is fermented by bacteria in the intestines into short chain fatty acids that have been shown in other studies to stimulate the production of appetite reducing hormones (see the text). We incorporated resistant starch into a yogurt that was generally accepted by children, increased their dietary fiber consumption and increased colonic fermentation in adolescents. This pilot data suggest the need for a study testing the ability of this yogurt to treat childhood obesity, a vulnerable group where non-food solutions are limited.

Introduction

The rapidly-growing prevalence of obesity in adults and children requires urgent remedial measures to avert individual and societal health care crises¹. Few adult treatment strategies exist², treating children is more challenging, yet childhood obesity is a growing health concern³. Pediatric vulnerability severely limits the use of pharmacological or surgical interventions. Even dietary treatment with energy and nutrient restriction for weight reduction may be detrimental to growth. Dietary resistant starch (RS) supplementation in food may offer a therapeutic opportunity to attenuate excessive fat gain in infants and children by reducing the caloric density while improving dietary quality^{1,2}.

RS are dietary carbohydrates that resist cooking processes and enzymatic digestion in the small intestine, are fermented by colonic microbiota and modify the gut flora^{4,5}. The amount of RS in the human diet has progressively decreased with modern milling and food preparation methods. RS intake in medieval Europe was 50–100g/day⁶, it is estimated at 30–40g/day⁷ in developing countries, and has dropped to 3–8g/day in developed countries^{7–9}. It is unlikely that modern human society will return to a diet of coarsely ground grains and legumes high in RS. However, RS is now available as an ingredient that can be incorporated into breads, cereal products and baked goods that are acceptable to the US population.

Microbiota-derived enzymes are needed to digest complex plant polysaccharides¹⁰ and RS-enriched diets increase butyrate-producing Clostridia in rodent feces¹¹. A natural, granular, type 2 RS from high-amyllose maize (HAM-RS2) decreases plasma cholesterol and triglycerides, increases satiety, increases insulin sensitivity^{12–22}, and is anti-adipogenic in adult populations^{23–30}. HAM-RS2 fermentation in the colon of rodents produces short chain fatty acids (SCFAs) such as acetate, propionate and butyrate that are absorbed through colonocytes, and change colonic microbiota composition^{25,31,32}. Butyrate treatment increases gene expression of peptide tyrosine tyrosine (PYY) and proglucagon in ileal, primary colon and cecal epithelial cells of rats; elevates plasma Glucagon-like peptide-1 and -2 (GLP-1, GLP-2), and raises gene expression and protein production of Glucose transporter 2 (GLUT2)^{27,31,33–35}. Clinical studies show that SCFAs increase in response to consumption of HAM-RS2 or RS from potatoes^{36–38}, that the microbiota of rodents were modified³⁹, and that butyrate was increased in rodents after dietary introduction of human feces⁴⁰. Since yogurt can deliver dietary fibers to treat constipation in children⁴¹, the aim of the current work is to develop a palatable yogurt delivery vehicle for HAM-RS2 that will withstand pasteurization and demonstrate an increase in fecal pH and SCFAs in children and adolescents.

Methods

The RS yogurt manufacture

Yogurt mixes were made by incorporating the starches individually into skim milk. The yogurt mixes were pasteurized at 65.5°C for 30 min, cooled to 40°C, inoculated with freshly thawed *Streptococcus thermophilus* (ST-M5) (3.1E+10 cfu/g, 1ml) and *Lactobacillus bulgaricus* (LB-12) (3E+10 cfu/g, 1ml) (Chr. Hansen Inc., Milwaukee, WI) per 3.785L (1 gallon), then incubated at 40°C until they reached a pH 4.5, and held at 4°C overnight. Blueberry puree (20% w/w) was incorporated into the yogurt the following day and amylopectin starch (15g, control, AMIOCA® corn starch, Ingredion Incorporated, Bridgewater, NJ) or HAM-RS2 (15g, HI-MAIZE® 260 resistant starch, Ingredion Incorporated, Bridgewater, NJ) per 237ml serving was added to the yogurt (Creamery, College of Agriculture, LSU). A high performance liquid chromatography (HPLC) peak was detected in our HAM-RS2 sample and RS accounted for 38.2% of the sample.

In vitro testing

HAM-RS2 30g/237ml yogurt was used for *in vitro* testing. Six samples were prepared, coded, and tested blindly with half subjected to pasteurization. A modified Englyst method was used to quantify glucose release⁴. Intact granular structure of the starch was evaluated using birefringence light microscopy.

Sensory study

The Institutional Review Board (IRB) granted an exemption #HE13-1 (January 16, 2013) from continued oversight for the sensory study conducted in two groups of children evaluating the two yogurts. Ratings of satisfaction with the appearance, color, aroma, taste, thickness, sandiness, and palatability of each type of yogurt were scored by 110 children without communication. Ninety-one children were 7–8 years old (younger) and 19 were 13–14 years old (elder). The younger children were more willing to volunteer for the sensory study than the elder.

Subjects with no dairy or starch-related allergies were recruited from The Louisiana State University Laboratory School and parental consent to participate was obtained along with the children's assent. Participants were given yogurt samples in 85g cups with a snap-on lid. Cups were coded with a random three-digit number. Disposable plastic spoons and napkins were provided to prevent contamination between samples. Prior to the sensory evaluation, the children were provided with a "warm-up" yogurt sample to avoid the "first sample effect" due to possible previous consumption of other food items, and a cup of drinking water was provided to rinse their palate between samplings. Two evaluation forms were used, one with a face scale for the younger panelists and the other with a preference rating form for the older panelists, and clearly explained to each age group. The younger panelists indicated their yogurt preference by circling "smiling face (☺) as yes", scored as 3, "neutral face (⊖) as neither like nor dislike", scored as 2, or "sad face (☹) as no", scored as 1. The elder panelists evaluated the yogurt on a 1–9 scale (1-dislike extremely, 2-dislike very much, 3-dislike moderately, 4-dislike slightly, 5- neither like nor dislike, 6-like slightly, 7-like moderately, 8-like very much, 9-like extremely) for appearance, color, aroma, taste, thickness, and sandiness. The elder panelists evaluated the yogurt thickness by checking 1-too thin, 2-just about right, or 3-too thick; and the sandiness as 1-not grainy, 2-just about

right, or 3-too grainy. Elder panelists answered the question “Is this product acceptable?” with 2-“yes” or 1-“no” answer.

Clinical study

The four-week pilot clinical trial was approved by the PBRC Institutional Review Board (IRB28012) and registered (<http://clinicaltrials.gov/>, NCT01338571) to determine the effects of consuming HAM-RS2-enriched yogurt on fecal pH and fecal SCFAs, pre- and post-consumption, in a healthy child and three healthy adolescents. The subjects (a 6-year-old female, two 10-year-old African-American females, and a 14-year-old Caucasian male) were recruited through the PBRC recruiting department. Parents signed a consent form and subjects signed an assent form. Subjects with gastrointestinal disease, on medications with the potential to alter the intestinal bacterial microbiota such as antibiotics and subjects with allergies to corn were specifically excluded.

Subjects were weighed in the morning on an electronic scale in light street clothing without shoes or outer clothing and with pockets emptied. The electronic scale (Model 450, GSE Inc., Livonia, MI, USA) was calibrated daily using standardized weights and quarterly by an external service. Parents were given stool-collecting kits and instructed to collect a stool specimen from their child for 3 consecutive days at baseline and after 4 weeks of yogurt consumption. They were provided with ice packs, coolers and were instructed to return stool samples in the coolers to the research site on the day they were collected so they could be stored at -70°C until analysis.

Children were given HAM-RS2 10g plus 1g per year of age daily⁴² which was 16, 20, or 24g for the four subjects. A fresh supply of yogurt was given to the parents weekly and the daily yogurt was divided into servings at breakfast and dinner.

Measurements of fecal SCFAs and pH were previously published elsewhere¹⁸. Briefly, the frozen fecal specimens were thawed, homogenized and further diluted to wet sample in distilled water (0.5g/5ml). The pH was measured using a combination electrode. Samples were then acidified with metaphosphoric acid (250g/L, 1ml) containing ethyl-butyrlic acid (2g/L) as an internal standard. The mixture was vortexed, and centrifuged at 4°C for 10 minutes at 8,000 rpm to remove solids in the homogenized samples and syringe-filtered (33mm, Millipore, Billerica, MA). The filtrate was put into a gas chromatograph (GC) auto-sampler vial and capped. SCFAs in the effluent were analyzed using gas-liquid chromatography. The GC conditions (115°C for 0.1 min) were increased to 150°C for 0.1 min in increments of 10°C, then to 170°C for 2 min at increments of 11°C. The injector temperature was 250°C. Helium was the carrier gas with a flow rate of 60 ml/min and splitless injection was 60 ml/min. Single SCFAs were determined by retention time based on standards and the relative concentrations calculated based on the ratio of the peak areas of the sample to the internal standard.

Statistical analysis

The RS content differences of yogurt samples prepared with or without pasteurization were determined using the Student *t*-test (SAS 9.3, SAS Institute Inc., Cary, NC). The sensory data obtained from children were analyzed with a Randomized Block Design using panelists as blocks (GLM, SAS 9.1), and the paired *t*-test for HAM-RS2 score minus amylopectin score was performed.

Differences between the types of yogurt were determined by differences of least squares mean \pm SEM. The clinical data analysis of feeding yogurt to the four subjects was performed with the Student *t*-test (weight) and paired *t*-test (change in weight) (SAS 9.1). Alpha was set at 0.05.

Ethics

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000.

Results

Resistant starch in yogurt

The glucose release was detected by a modified Englyst method. A light microscope (200x, Leitz Wetzlar, Ortholux II, Ernst Leitz GmbH, Wetzlar, Germany) revealed morphologies of the starch granules in the yogurts as having an equal presence of birefringence indicating an intact granular structure (Figure 1). The RS content of the six yogurt samples varied minimally (from 45% to 51% on a dry weight basis with values of 51%, 45%, or 48% for the unpasteurized samples, and 45%, 45%, or 46% for the pasteurized samples ($P>0.05$).

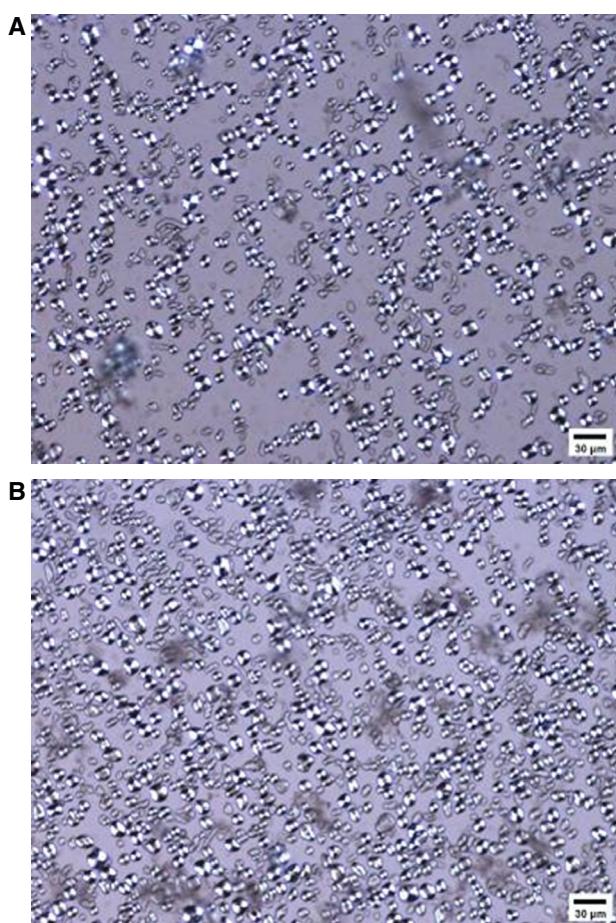


Figure 1. HAM-RS2-enriched yogurt observed using polarized light microscopy (200x). Bar=30μm.

Sensory results for HAM-RS2 versus amylopectin starch

Group 1: Ninety-one 7- and 8-year-old panelists. The average scores were 1.538 for the HAM-RS2-yogurt and 2.143 for the amylopectin starch yogurt. The difference was -0.604 ± 0.1 ($t=6.05$, $P<0.0001$) which indicated that the amylopectin starch yogurt was preferred over the HAM-RS2-yogurt.

Group 2: Nineteen 13- and 14-year-old panelists. No score differences were detected in color (6.95 ± 0.36 vs. 7.05 ± 0.28) and aroma (7.84 ± 0.24 vs. 7.47 ± 0.32) for the amylopectin starch compared to the HAM-RS2-yogurt, respectively (Table 1, $P>0.05$). However, appearance (6.84 ± 0.34 vs. 4.58 ± 0.38), taste (6.95 ± 0.32 vs. 4.84 ± 0.49), thickness (6.74 ± 0.48 vs. 4.47 ± 0.37), and sandiness (6.26 ± 0.37 vs. 3.05 ± 0.36) scores for the amylopectin starch yogurt were higher than for the HAM-RS2-yogurt ($P<0.005$).

On a 1–3 scale (1-too thin, 2-just about right, or 3-too thick), the amylopectin starch yogurt (2.26 ± 0.13) was judged slightly thicker than just about right while the HAM-RS2-yogurt (1.16 ± 0.09) was judged as too thin ($P<0.0001$, Table 2).

Using a 1–3 scale (1-not grainy, 2-just about right, and 3-too grainy), the HAM-RS2-yogurt (2.84 ± 0.12) was judged as too grainy but was acceptable to 74% of the children of 13–14 years of age, while the amylopectin starch yogurt (1.95 ± 0.12) was judged as just about right (Table 2, $P<0.0001$).

The amylopectin starch yogurt was always judged as acceptable (Table 2) and its acceptability on a 1–2 scale (1-not acceptable or 2-acceptable) was significantly higher than for the HAM-RS2-yogurt

($P<0.05$). The sensory study indicated that children preferred the amylopectin starch yogurt more than the HAM-RS2 added yogurt.

Dataset 1. Sensory raw data from study participants

<http://dx.doi.org/10.5256/f1000research.6451.d47918>

Participants were asked to rate amylopectin starch- and HAM-RS2-containing yogurts for preference (7–8 years old; 1–3 scale, 1 being dislike, 3 being like) or appearance, color, aroma, taste, thickness, and sandiness (13–14 year olds; 1–9 scale, 1 being dislike extremely, 9 being like extremely)⁴⁵.

Clinical study

All adolescent participants finished the HAM-RS2-yogurt and returned the empty containers during the weekly clinic visits with no complaints regarding taste or compliance issues related to consumption of the yogurt.

One 10-year-old had a BMI of 19.8 kg/m^2 and the other had a BMI of 27.1 kg/m^2 . The 14-year-old had a BMI of 31.5 kg/m^2 . All were otherwise healthy. The pre-pubertal child gained 1.9kg (39.5 to 41.4 kg). One of the adolescent females gained 3.2kg (49.6 to 52.8kg), the other one gained 0.4kg (69.5 to 69.9kg) and the adolescent male gained 1.7kg (89.9 to 91.6 kg) ($P>0.05$).

SCFAs ($\mu\text{g/g}$ wet stool weight) from carbohydrate fermentation were increased in the adolescent participants; in ascending order, butyrate (23%, $2,410 \pm 691$ to $3,144 \pm 1,509 \mu\text{g}$, $P=0.09$), acetate (26%, $5,078 \pm 492$ to $6,870 \pm 515 \mu\text{g}$, $P=0.02$), but not propionate

Table 1. Comparison of the appearance, color, aroma, taste, thickness, and sandiness score (on a 1 to 9 scale in which 1 was the least desirable ranking and 9 was the most desirable ranking) of the HAM-RS2 (treatment) versus the amylopectin starch (control) yogurt when evaluated by 13- and 14-year-old children.

Type of starch present in yogurt	Appearance	Color	Aroma	Taste	Thickness	Sandiness
Amylopectin starch	$6.84^{\text{a}} \pm 0.34$	$6.95^{\text{a}} \pm 0.36$	$7.84^{\text{a}} \pm 0.24$	$6.95^{\text{a}} \pm 0.32$	$6.74^{\text{a}} \pm 0.48$	$6.26^{\text{a}} \pm 0.37$
HAM-RS2	$4.58^{\text{b}} \pm 0.38$	$7.05^{\text{a}} \pm 0.28$	$7.47^{\text{a}} \pm 0.32$	$4.84^{\text{b}} \pm 0.49$	$4.47^{\text{b}} \pm 0.37$	$3.05^{\text{b}} \pm 0.36$

^{ab}Means without a common superscript are significantly ($P<0.05$) different from each other.

Table 2. Comparison of the thickness score (1 = too thin, 2 = just about right, or 3 = too thick), the sandiness score (1 = not grainy, 2 = just about right, or 3 = too grainy), and the acceptability score (1 = no (not acceptable) or 2 = yes (acceptable)) of the HAM-RS2 (treatment) versus the amylopectin starch (control) yogurt when evaluated by 13- to 14-year-old children.

Type of starch present in yogurt	Thickness Score 1 = too thin 2 = just about right 3 = too thick	Sandiness Score 1 = not grainy 2 = just about right 3 = too grainy	Acceptability Score 1 = no (not acceptable) 2 = yes (acceptable)
Amylopectin starch	$2.26^{\text{a}} \pm 0.13$	$1.95^{\text{b}} \pm 0.12$	2.00^{a}
HAM-RS2	$1.16^{\text{b}} \pm 0.09$	$2.84^{\text{a}} \pm 0.12$	1.74^{b}

^{ab}Means without a common superscript are significantly ($P<0.05$) different from each other.

($2,387 \pm 645$ to $1,889 \pm 120$ µg, $P > 0.05$). The isobutyrate from protein fermentation increased (39%, 285 ± 31 to 471 ± 58 µg, $P = 0.01$) (Figure 2). The stool pH of the adolescents was mildly reduced at the end of the fourth week with a trend toward a lower pH (2.8%, from 7.2 ± 0.4 to 7.0 ± 0.35 , $P = 0.1$, Figure 3).

The pre-pubertal participant responded to HAM-RS2-enriched-yogurt differently than the three adolescent children with an increase in stool pH (from 6.89 to 7.62). The stool SCFAs were decreased; in ascending order, isobutyrate (35%, from 526 to 186 µg), butyrate (39%, from 4,028 to 1,571 µg), acetate (52%, from 8,328 to 4,336 µg), and propionate (65%, from 2,870 to 1,877 µg) over the 4-week study.

Dataset 2. Raw data for clinical study

<http://dx.doi.org/10.5256/f1000research.6451.d48004>

Four participants (a 6-year-old female, two 10-year-old African-American females, and a 14-year-old Caucasian male) were weighed and their weights recorded. Parents were given stool-collecting kits and instructed to collect a stool specimen from their child for 3 consecutive days at baseline and after 4 weeks of yogurt consumption. They were provided with ice packs, coolers and were instructed to return stool samples in the coolers to the research site on the day they were collected so they could be stored at -70°C until analysis. Children were given HAM-RS2 10g plus 1g per year of age daily which was 16, 20, or 24g for the four subjects. SCFA content of samples was analysed by GL-chromatography and determined by retention time based on standards and the relative concentrations calculated based on the ratio of the peak areas of the sample to the internal standard⁴⁶.

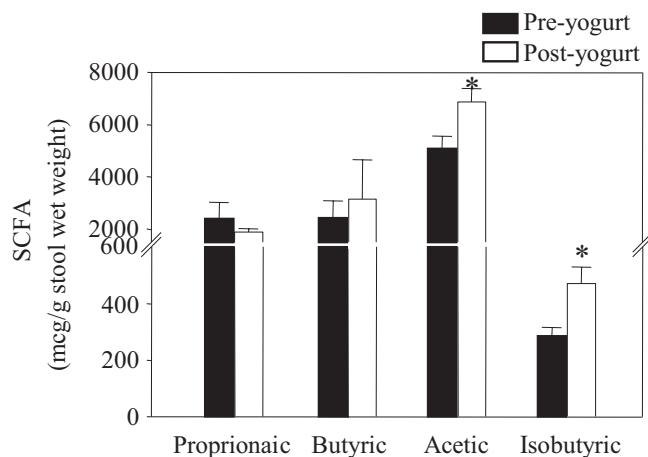


Figure 2. Stool SCFAs increased ($P < 0.05$) in adolescents post-yogurt treatment. The pre-pubertal child was not included.

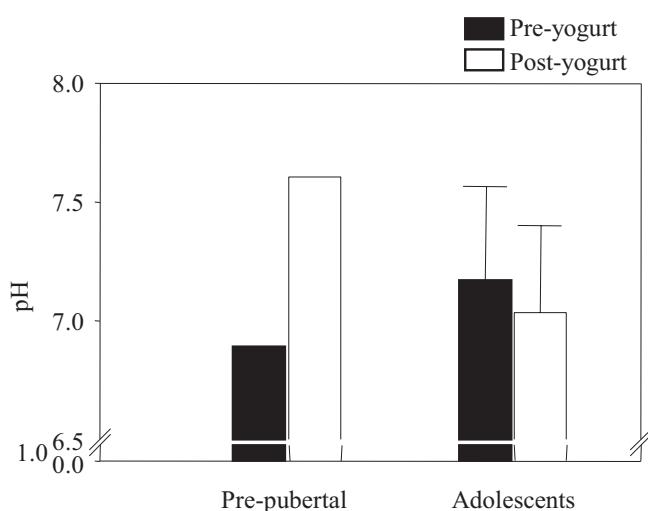


Figure 3. Stool pH was reduced ($P = 0.1$) in adolescents post-yogurt treatment. The pH was increased in the pre-pubertal child.

Discussion

The prevalence of childhood obesity has increased 2- to 3-fold in just the last 25 years globally (see review 2). Childhood obesity is associated with co-morbidities similar to adults including hypertension, dysglycemia, dyslipidemia, inflammation and endothelial dysfunction². Supplementing other treatment approaches with behavioral interventions may increase long term participation and is felt to be more important in the pediatric population than for adults², but meta-analyses show prevention or treatment strategies to be ineffective. Currently, no truly effective pharmacological options are available for weight management, and surgery is restricted to a highly selected subgroup of very obese adolescent individuals. Medication and surgery have safety concerns in growing children and their efficacy is uncertain in the pediatric age group⁴³. Novel treatments for childhood obesity offering safety, efficacy and acceptability are urgently needed⁴⁴. Desirable attributes of an intervention for pediatric obesity include a preventive measure that attenuates excess fat accumulation while allowing for normal growth. RS is a natural food ingredient with a low risk profile that attenuates body fat accretion in experimental animal models, and is an excellent candidate to effectively combat childhood obesity. This feasibility study suggests that HAM-RS2-enriched foods likely alter microbiota composition, and this is supported by the increase in fecal SCFA content and lower pH. Yogurt was a generally acceptable vehicle for providing HAM-RS2. The yogurt cultures fermented lactose (milk sugar) and the RS granules in the final yogurt product were not damaged.

Enriching the diet with RS which has been refined out of the US diet will improve dietary quality and may help to ease the severity of pediatric obesity. Although the amylopectin starch yogurt was preferred, our studies confirmed the general acceptability of incorporating HAM-RS2 into yogurt through taste and sensory testing in 91 7- to 8-year-olds and 19 13- to 14-year-old volunteers. The four subjects in our pilot study that consumed the HAM-RS2-enriched yogurt twice a day for weeks established the feasibility of feeding the HAM-RS2-enriched yogurt to children. We demonstrated a trend toward a reduction of pH and documented a significant increase in the SCFA content of the stools of the adolescent

children. This agrees with previous studies that have found that adding HAM-RS2 to rodent diets reduced abdominal fat in association with increased fermentation^{20,21,37,44}. Supplementing the diet with RS will need to be acceptable and palatable or children are likely to reject it in favor of low-fiber alternatives. Overall, the HAM-RS2-yogurt in the taste testing was acceptable to 74% of the children in the 13- to 14-year-old group, but 24% less acceptable in younger children. The knowledge that RS is healthy may increase the adoption of RS fortified foods, such as yogurt.

People eat for volume and consume fewer calories when food has a lower caloric density³⁸. RS and other dietary fibers reduce the caloric density of food^{12,13,44}. RS is present in many different sources, which offers the opportunity to choose the RS with the greatest success in reducing or controlling body weight¹⁷. We have previously shown that the HAM-RS2 supplementation produces a 30% reduction in intestinal fat deposition in wild type *C. elegans*²⁸ and in rodents the same also reduced body fat^{25,44}. Longer-term controlled studies are needed to determine if the reduced adiposity seen in animal models will occur in human populations. In a human pilot study, a HAM-RS2 (15g/day) supplemented diet enhanced insulin sensitivity by 56.5% in men over two to three months, which suggests that lower amounts of HAM-RS2 may also be efficacious. Beneficial changes in adiposity may occur over longer treatment periods^{12,13,21,22}, and lower amounts of RS may further improve palatability – an important factor for long-term consumption.

Children maintain weight loss better than adults¹¹. Although it is not clear why the pre-pubertal child in this study did not respond in the same way as the adolescents, it could represent differences in her intestinal microbiota or her pre-treatment diet which was not controlled nor queried. Further research will be necessary to explore the differential role of diet and the intestinal microbiota on the fermentation of RS before puberty. Weight gain in all of the children during the 4-week study may reflect the fact that they were growing.

Conclusion

The current study showed the acceptability and feasibility of using yogurt to deliver RS to adolescents which caused a change in SCFA and probably changed the gut microbiota. These preliminary data suggest the need to evaluate differences that may exist in the microbiota before and after puberty to determine whether the non-response of the pre-pubertal child represented an outlier or a real effect in pre-pubertal children. These preliminary results will need confirmation in a controlled trial so that the effects of growth can be taken into account in evaluating weight changes in longer-term studies using yogurt as a vehicle to deliver the functional food component HAM-RS2 in a range of doses into everyday foods that consumers enjoy. Our data encourage controlled studies in children and adolescents testing insulin sensitivity, effects on body weight, and potential differences between pre-pubertal and adolescent children in their microbiota response to RS. Hopefully, increased consumption of reduced-calorie foods in combination with increased physical activity¹² will reduce weight gain, help to maintain a healthier weight, and lead to future improvements in public health for adults and adolescents alike.

Data availability

F1000Research: Dataset 1. Sensory raw data from study participants, [10.5256/f1000research.6451.d47918](https://doi.org/10.5256/f1000research.6451.d47918)⁴⁵

F1000Research: Dataset 2. Raw data for clinical study, [10.5256/f1000research.6451.d48004](https://doi.org/10.5256/f1000research.6451.d48004)⁴⁶

Consent

Informed consent was obtained from all patients being included in the study.

Author contributions

Each individual has contributed to this manuscript as a qualified author and meets ALL of the requirements following the American Medical Association (AMA) manual guidelines. KJ Aryana, C Pelkman, and D Olson contributed to the conception and design of sensory study, acquisition of data, analysis and interpretation of the data, and drafting or editing the manuscript. R Tulley performed stool test and the SCFA analysis. FL Greenway and NV Dhurandhar designed the clinical study, contributed to its conception, acquisition of data, analysis and interpretation of the data, and drafting the manuscript. JW Finley, MJ Keenan, RJ Martin, and J Zheng contributed to designing of the sensory study, data analysis and interpretation, and drafting or editing the manuscript. All authors have approved the final version of the manuscript.

Competing interests

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The authors are addressing an important issue -- the development of foods for children that will not impede their growth, but also not contribute to adiposity, that children will like to eat, and that will withstand pasteurization. In their case they have chosen to add a specific resistant starch (HAM-RS2)- to yogurt. There was a good rationale for them to select this resistant starch as previous studies done in rats, and some done in humans, have shown beneficial results. They performed a series of sensory tests comparing the yogurt with resistant starch to their control yogurt (containing amylopectin). Except for the color of the product all other sensory attributes were rated better for the control than the yogurt containing resistant starch. The statistical analyses appear to be acceptable except there was no stated allowance for multiple T tests. Despite this, it is clear that the children liked the control better than the one containing resistant starch. Instead of calling this product a "bust" the authors have chosen to call this a success as a certain percentage still liked the experimental product sufficiently to eat it. One wonders how the abstract would have read if the resistant starch containing product scores were reversed with the controls. One would imagine that they would have been delighted if not ecstatic.

The "clinical trial" in children was important to do, as it provided information as to the acceptability of the resistant starch over time. However, there were only 4 children in this trial and one, who was pre-pubescent (age 6), was different from the others (2) 10 y and (1) 14, and the results from that individual were also different from the other 3. In one way this is really a preliminary test of the resistant starch, not a trial, as there was no control. All 4 children ate the resistant-starch yogurt, in amounts based, in part, on their ages. Again, the results of this trial were disappointing if weight gain was a primary outcome measure. All gained weight, and for some this was substantial. The resistant starch appeared to be fermented to SCFA, but the values for pH and SCFA were reported in a previous paper. If weight gain, or gain of muscle mass and not fat stores was an important endpoint and these children were still growing, the study would have benefitted by a control group of the amylopectin containing yogurt.

The title of the paper seems incomplete as it doesn't mention the aim of the pilot study. I've tried to come up with the purpose of the clinical trial, but the fact that I'm unsure what the goal was is impeding me. Is it testing the fermentability of the resistant starch product? If so, it should provide pH and SCFA data. Is it testing whether or not children will eat this product for a month, then it should say that. If it's about weight gain/loss then it needed a control.

In summary, I think this idea of accepting a paper and then asking experts in the field to comment is excellent, and I'm glad to be part of the process. These authors are experts in what they are doing, and they have found some important information about a natural, granular, type 2RS from high-amylose maize

(HAM-RS2). Others working in this field can benefit from the fact that they presented their data in what could be characterized as a preliminary study. However, there are two points that bother me about the interpretation of their data. First, they put a very positive spin on the HAM-RS2 intervention, when by almost all accounts this was not a positive outcome. This really should be toned down. Second, the authors make statements about the benefits of fermentability that come across as "facts" when rather this is an open and unresolved issue. For example, when discussing HAM-RS2 they say "decreases plasma cholesterol and triglycerides, increases satiety, increases insulin sensitivity and is anti-adipogenic in adult populations." Although they show several citations that have shown this, they have not reviewed the entire literature, and they make the statement as if it is fact. This needs to be qualified. In a different place they say "People eat for volume and consume fewer calories when food has a lower caloric density." This is Barbara Rolls's hypothesis, but not everyone would agree with this statement. In fact, Rolls herself says it mostly applies to men, not women. So, please go back through your comments that are stated as FACTS and perhaps modify them.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Competing Interests: No competing interests were disclosed.

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There is much current attention to the role of the gut, especially gut microbiota, in the regulation of body weight. At the same time the US and other countries face escalating rates of childhood and adolescent obesity, suggesting the importance of obesity prevention efforts, of which there have been few shown to have significant impacts. This study examines one innovative potential approach to preventing obesity targeting food components, specifically the incorporation of dietary resistant starch into a common food item, yogurt. The study focused on the acceptability of the sensory characteristics of yogurt enhanced with one such resistant starch, compared to yogurt enhanced with a control amylopectin starch, among pre-adolescent and adolescent subjects. A small pilot 4-week trial also looked at the effects of the resistant starch when consumed by 4 subjects.

The sensory evaluation study was well-designed with tight controls and a sizable N. Assessment of various sensory attributes was rather comprehensive among the adolescent sample, which unfortunately was substantially smaller than the sample of 7- to 8-year olds who could not be expected to make the finer sensory distinctions on which the adolescents were queried. The actual resistant starch content of the various yogurt samples was verified by analyses.

The sensory evaluation study showed that the subjects consistently preferred the control yogurt to that containing the resistant starch. The adolescents found the resistant starch yogurts to be inferior to the control yogurts on appearance, taste, thickness, sandiness and acceptability. About 25% of the adolescent subjects rated the resistant starch as unacceptable. Among the younger children, whose only ratings were a smiling face, neutral face or sad/frowning face, the resistant starch yogurt was more

strongly disliked, with nearly two-thirds of subjects awarding it a frowning face. The discussion does not adequately address these results, which show consistently that the sensory characteristics of the resistant starch yogurt preparation are not well received by children and adolescents. The conclusion that "...our studies confirmed the general acceptability of incorporating HAM-RS2 into yogurt..." seems to fly in the face of these results.

The very small pilot clinical trial provided some support for the hypothesis that the resistant starch would produce changes in gut microbiota, findings worth following up with a larger study. It also showed that despite the limited acceptability ratings, the products could be consumed over a 4-week period. However, the weight gain seen among all four subjects is disturbing. Although the discussion dismisses this finding by saying that it may reflect growth, the amounts of weight gain seen over 4 weeks were 0.4, 1.7, 1.9 and 3.2 kg. Certainly the latter three gains, if continued over a year, would be quite excessive. Given that the point of this dietary intervention is to prevent excessive weight gain, these preliminary findings are cause for considerable concern.

The authors have conducted a well-designed study of the feasibility of this innovative dietary intervention which has the purpose of obesity prevention. The findings show that it is possible to get children and adolescents to consume yogurt containing resistant starches, but more development is necessary to produce a food product with adequate acceptability. Aside from that, however, the assumption that this product, if consumed regularly, might avert excess weight gain needs further study, as the limited results here suggest its effect may be just the opposite of that which was expected and desired.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Competing Interests: No competing interests were disclosed.
