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ORIGINAL CONTRIBUTION

Activation of gastrointestinal ileal brake response with dietary slowly digestible carbohydrates, with no observed effect on subjective appetite, in an acute randomized, double-blind, crossover trial

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Abstract

Purpose To test the hypothesis that oral ingestion of slowly digestible carbohydrates (SDCs) that reach the ileum triggers the ileal brake as indicated by delayed gastric emptying, reduced glycemic response, and decreased subjective appetite.

Methods The study was a five-arm, randomized, double-blind, crossover trial with a 1-week washout period between treatments ($n = 20$; 9 females, 11 males). Five treatments consisted of three SDC ingredients [raw corn starch, isomaltooligosaccharide (IMO), sucromalt], and an IMO/sucromalt combination, shown in vitro to have slow and extended digestion profiles, and a rapidly digestible carbohydrate control (maltodextrin). Carbohydrates (26 g) were incorporated into yogurt [300 g total; carbohydrate (~ 77 g), fat (~ 0.2 g), and protein (~ 9 g)] with closely matched energy content (346 kcal) and viscosity (~ 30,000 cP). Outcomes were measured in a 4 h postprandial period.

Results Mean gastric half-emptying times were moderately though significantly increased for the raw corn starch and IMO treatments ($P < 0.05$), but they could be sub-divided into larger effect responder ($n = 11$) and non-responder groups ($n = 9$). Longer time for glycemic response to return to baseline was associated with increased gastric half-emptying time in an exploratory subset of data removing gastric half-emptying times > 3.5 h ($P = 0.02$). No significant differences in appetite ratings were observed.

Conclusion SDCs caused slower gastric emptying rate through activation of the ileal brake, as closely matched semi-solid yogurts were used and only rate of carbohydrate digestion differed. Extending glycemic response through consumption of SDCs was associated with triggering the ileal brake.

Trial registration ClinicalTrials.gov NCT03630445, August 2018, retrospectively registered.

Keywords Slowly digestible carbohydrates · Gastric emptying · Glycemic response · Appetitive response · Ileal brake · Human participants

Abbreviations

AUC	Area under the curve
BMI	Body mass index
CGM	Continuous glucose monitoring
CPDR	Cumulative percent dose recovery
DOB	Delta over baseline
GLP-1	Glucagon-like peptide-1
IMO	Isomaltooligosaccharide
PDR	Percent dose recovery
PYY	Peptide tyrosine tyrosine
RDC	Rapidly digestible carbohydrate
SDC	Slowly digestible carbohydrate
VAS	Visual analog scale

Tahnia D. Gonzalez is not currently affiliated with General Mills; Tanhia D. Gonzalez contributed to this study while she was affiliated with General Mills.

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Introduction

Designing foods that prolong satiety and provide extended energy through a slow and sustained release of glucose to the body has the potential to aid in weight management and help regulate food intake, and could be a key target for food researchers. We recently showed in rats that fabricated slowly digestible carbohydrates (SDCs) made to be sufficiently slowly digestible such that they reach the ileal portion of the small intestine trigger the gut–brain axis and ileal brake to decrease food intake [1, 2]. This provided impetus to understand how dietary carbohydrates or carbohydrate-based foods can be selected or made to have the same response in humans. In the current study, we examined the potential role of SDCs (though different than we used in rats) on the ileal brake. The ileal brake is a feedback mechanism that leads to the inhibition of gastrointestinal motility and potentially increased feeling of satiety, and it can ultimately help regulate digestion and food intake [3–5]. Hormones released from L-cells, such as glucagon-like peptide-1 (GLP-1) and peptide tyrosine tyrosine (PYY), can also trigger the gut–brain axis, the two-way communication system between the gastrointestinal tract and the brain, to regulate gastrointestinal function and modulate food intake [6–8]. In humans, infusion of glucose into the ileum was shown to increase secretion of GLP-1 and PYY, indicative of triggering the ileal brake and gut–brain axis, and decreased ad libitum energy intake at a subsequent meal [9]. Little is known about the ability of, and extent to which, different types of orally ingested dietary SDCs trigger the ileal brake in humans.

The aim of this study was to test the hypothesis in humans that SDCs with potential to digest in the ileum can trigger the ileal brake. A semi-solid yogurt food matrix was used with closely matched energy content and viscosity, so that the single test variable was carbohydrate digestion rate. Gastric half-emptying time was used as a proxy indicator for activation of the ileal brake, as previous research has shown that activation of the ileal brake results in dose-dependent delays in gastric emptying of solid and liquid foods [10–12]. The study evaluated four SDC treatments (each α-glucan, being an oligomer or polymer of glucose units with different linkages): raw corn starch, isomaltooligosaccharide (IMO), sucromalt, and a mixture of IMO and sucromalt. These were compared to maltodextrin, a rapidly digestible carbohydrate (RDC), as the control. The SDCs possessed slow, but distinct in vitro digestion rates and were hypothesized to reach the ileum in different proportions (Fig. 1) due to either their microstructure (raw corn starch) or arrangement of glucosidic linkages (IMO, sucromalt). Raw corn starch is considered an ideal type of slowly digestible starch due to its microstructure of both crystalline and amorphous lamellae, which permits but impedes enzyme digestion [13, 14]. IMO, an oligosaccharide with α-1,6 linkages, is known to have a carbohydrate portion resistant to the mucosal α-glucosidases, but also has large digestible components [15, 16], as is shown by similar in vitro digestion profiles between IMO and raw corn starch (Fig. 2). Sucromalt is an SDC composed of fructose and an alternan-oligosaccharide with alternating α-1,3 and -1,6 linkages, which is slowly but completely digested in the small intestine [17]. We reasoned that carbohydrates with slow in vitro digestibility (Fig. 2) could potentially activate the ileal brake, and that the

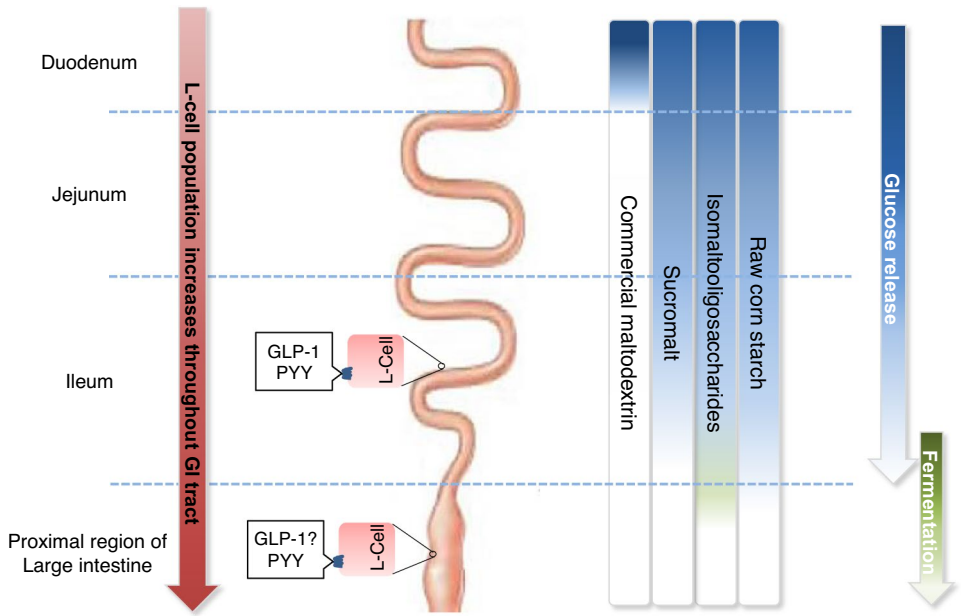

Fig. 1 Hypothetical schematic of locational delivery of glucose with slowly digestible carbohydrates. *GI* gastrointestinal, *GLP-1* glucagon-like peptide-1, *PYY* peptide tyrosine tyrosine

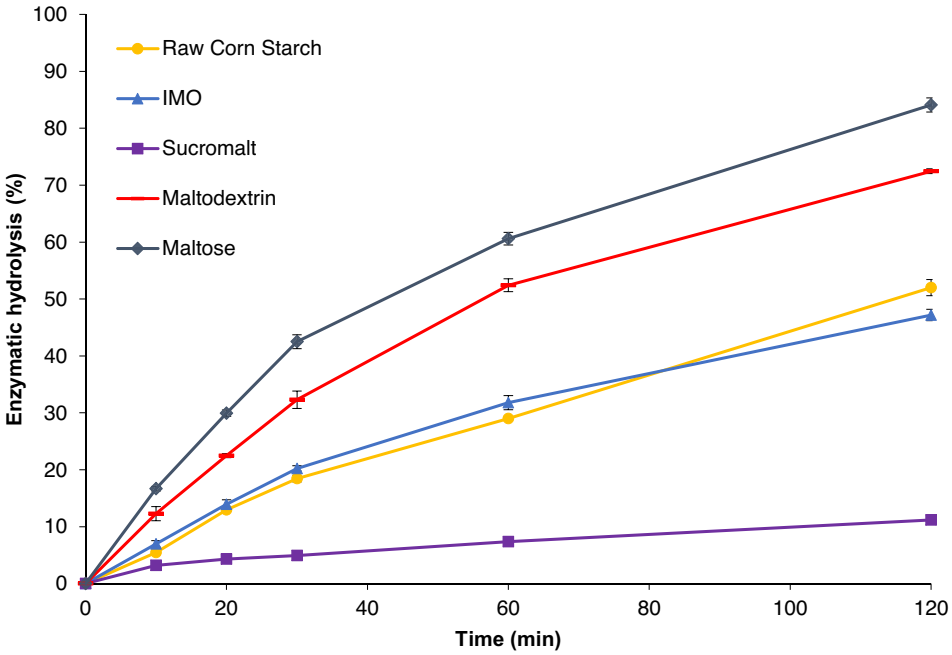

Fig. 2 Digestion profiles of carbohydrate ingredients as measured by in vitro kinetic enzymatic hydrolysis. Maltose is a sugar standard. Means are shown with ± standard error of the mean. *IMO* isomaltooligosaccharide

maltodextrin RDC control would not activate the ileal brake due to its proximal digestion. The findings from this study may provide key insights into designing carbohydrate-based foods with the ability to trigger the ileal brake and possibly control food intake.

Materials and methods

Materials

Five different treatments were made using four carbohydrate ingredients: raw corn starch (Melojel®, Ingredion, Westchester, IL, USA), isomaltooligosaccharide (IMO; VitaFiber®, BioNeutra, Edmonton, Alberta, Canada), sucromalt (Xtend® sucromalt, Cargill, Wayzata, MN, USA), a combination of IMOs and Xtend® sucromalt, and a maltodextrin (M100, Tate and Lyle, Decatur, IL, USA) control. These carbohydrates possess varying digestion rates (Fig. 2) and were incorporated individually or in combination into a yogurt (General Mills, Inc., Golden Valley, MN, USA). Yogurt was selected as a carrier for these carbohydrates, as it is a semi-solid food for which viscosity could be adjusted, yielding similar thickness across the samples, and in which the carbohydrates could be easily incorporated without imparting negative textural attributes.

In vitro digestibility of carbohydrate ingredients

A 120 min kinetic digestion assay was used to determine in vitro digestion profiles of the SDC and RDC carbohydrate ingredients following the method used by Lim et al. [18], with slight modification. Briefly, the substrate (30 µL) was subjected to enzymatic digestion using rat intestinal powder (1 g/10 mL; Sigma-Aldrich Co., St. Louis, MO, USA) in sodium phosphate buffer (20 mM, pH 6.8; Sigma-Aldrich Co., St. Louis, MO, USA) at 37 °C. Samples were collected at 0, 10, 20, 30, 60, and 120 min, at which point the reaction was stopped by boiling the sample tubes in water for 5 min. The samples were then reacted with glucose oxidase–peroxidase (GOPOD) solution (300 µL) and absorbance was read at 510 nm to determine glucose released. Results of the assay indicated all samples were digested at least partially with the following rank in digestibility (least digestible to most digestible): sucromalt < raw corn starch ≤ IMO < maltodextrin < maltose (sugar standard) (Fig. 2).

Yogurt preparation

The yogurts were formulated and custom prepared at General Mills, Inc. (Golden Valley, MN, USA) and later shipped under refrigeration to Purdue University (West Lafayette, IN, USA) to be used in the study. Each yogurt was closely matched in contents of carbohydrate (~ 77 g total, including all carbohydrate ingredients either individually or in combination), fat (~ 0.2 g), and protein (~ 9 g), and each had closely matched energy content (345.8 ± 1.6 kcal) and viscosity (30,961 ± 1,948 cP; measured upon stirring 30 times with a Brookfield Viscometer fitted with a Helipath Stand) (Table 1). The carbohydrate treatment ingredients comprised 26 g (8.7% of the total formulation) for each yogurt. Each treatment type was assigned and labeled with a three-digit

Table 1 Nutritional composition and viscosity of yogurt test meal treatments (per 300 g serving)

	Raw corn Starch	IMO	Sucromalt	IMO + Sucromalt	Maltodextrin
Total carbohydrate (g)	77.8	77.4	77.6	75.3	76.2
Carbohydrate treatment (g)	26.0	26.0	26.0	26.0	26.0
Total fat (g)	0.17	0.19	0.13	0.11	0.16
Protein (g)	8.5	9.2	9.5	9.3	9.5
Calories (kcal)	347	348	349	340	345
Viscosity (cP)[a]	27,675	27,925	38,388	30,650	30,169

IMO isomaltooligosaccharide

[a]Viscosity measured upon stirring 30 times with a Brookfield Viscometer fitted with a Helipath Stand

number. To facilitate double blinding, the treatment number assignments were not made known to the researchers until after the study had been conducted and the data had been analyzed. Immediately prior to serving, ^{13}C-octanoic acid (100 mg) was mixed into each test meal as a tracer for the assessment of gastric emptying. The protein and fat contents of the yogurt allowed ^{13}C-octanoic acid to be readily soluble and evenly distributed in the semi-solid matrix.

Study design

The study was a randomized, double-blind, crossover-controlled trial with five treatments. The randomization scheme (computerized random numbers) for treatment order per participant was devised by the study statistician (NMH) with balancing across treatment positions. One study researcher (MC) enrolled and assigned participants to the treatment orders. All procedures were conducted in accordance with the ethical standards of the Purdue University Institutional Review Board (Protocol #1502015807) and the study was registered at ClinicalTrials.gov (NCT03630445). Gastric emptying, glycemic response, and appetitive response were primary outcome measures, and breath hydrogen content was a secondary outcome measure.

Participant recruitment and inclusion criteria

Healthy normal weight participants were recruited using flyers placed around the Purdue University campus (West Lafayette, IN, USA). The inclusion and exclusion criteria are listed in Table 2. All potential participants completed a prescreening process and gave their written informed consent before being formally enrolled in the study. Menstrual cycle was not controlled for in female participants.

Table 2 Participant inclusion and exclusion criteria

	Criteria
Inclusion	
	BMI 18.5–25.0 kg/m^2
	Age 18–50 years
	Stable weight for the past 3 months (i.e. ± 2.5 kg)
	Regular eating pattern, including breakfast consumption
Exclusion	
	Gastrointestinal disease
	Smokers
	Peri- or post-menopausal women
	Celiac disease (yogurts may contain ingredients with wheat origin)
	Allergies, including dairy, lactose, and gluten
	Pregnant and lactating women
	Following a weight reduction program or having followed one during the last 3 months
	Acute or chronic disease
	Alcohol consumption > 30 units/week
	Hypertension
	Diabetes
	Previous bariatric surgery

Test day procedures and breath test methods

Testing took place 1 day per week for 5 weeks. Participants were instructed to maintain their normal level of exercise throughout the duration of the trial and diet was not controlled. Participants fasted the night before test days (> 10 h), which was confirmed by calibration of each participant's continuous glucose monitor via finger-prick measurement of blood glucose prior to consuming the test meal each test day (i.e., fasting blood glucose < 100 mg/dL). Participants arrived at the testing room at 8:00 AM on each test day. One treatment was given per week, and participants were assigned test days on the same day of the week throughout the study to ensure a 1-week washout period between treatments. On the morning of each test day, after collecting two baseline breath samples for gastric emptying assessment (1.5 L bags, Cambridge Isotope Laboratories, Tewksbury, MA, USA) and one baseline breath sample for breath hydrogen assessment (300 mL; Quintron Instrument Company, Milwaukee, WI, USA), participants consumed a yogurt test meal containing SDC (300 g, 77 g available carbohydrate, including carbohydrate ingredients) in its entirety, and thereafter breath samples were collected into 300 mL bags for gastric emptying (Cambridge Isotope Laboratories, Tewksbury, MA, USA) and for breath hydrogen assessment (300 mL; same type of bag as baseline sample) every 15 min for 4 h. Participants remained seated in the testing room during each test session (though getting up to use the restroom was allowed), and no other foods or drinks were allowed for the duration of the test session.

Breath samples used to calculate gastric half-emptying times were analyzed the same day as collected using a $^{13}CO_2$ urea breath analyzer (POCone, Otsuka Electronics Co., Ltd., Osaka, Japan) and calculations were done as described below. Breath hydrogen production was also assessed as an indication of any potential resistant starch or fiber fermentation. For the assessment of breath hydrogen, 20 mL aliquots of breath sample from each time point were injected into a calibrated BreathTracker Digital Microlyzer (Quintron Instrument Company, Milwaukee, WI, USA).

Calculation of gastric emptying parameters

Gastric half-emptying time and lag phase are parameters used to describe the gastric emptying rates of a food, and they can be obtained using breath tests [19–21]. Briefly, the $^{13}CO_2/^{12}CO_2$ ratio of a sample breath to the corresponding ratio of baseline breath was given as $^{13}CO_2$ delta over baseline (DOB, ‰) output from the breath analyzer. Values for the percent dose ^{13}C recovery (PDR) per hour and cumulative percentage dose recovery (CPDR) over time were calculated [20] and used with each individual's body surface area [22] to model half-emptying times and lag phases for each participant using the following two equations:

$$y = at^b c^{-ct}, \tag{1}$$

where y = PDR per hour (%), t = time (h), and a, b, and c = constants.

$$y = m(1 - e^{-kt})^{\beta}, \tag{2}$$

where y = CPDR over time (%), t = time (h), and m, k, and β = constants (where m = total cumulative dose recovery when time is infinite).

Gastric emptying profiles were modeled using a macro program in Excel (Microsoft Corporation, Redmond, WA, USA) used previously by our group [23–26]. Gastric half-emptying time ($T_{1/2}$), the time necessary for half of the ^{13}C dose to be metabolized, and lag phase (T_{lag}), the time required for the $^{13}CO_2$ excretion rate to attain its maximal level as an indicator of the time it takes for a food to break down within the stomach [20, 27], were then calculated using the following formulas:

$$T_{lag} = (\ln \beta / k), \tag{3}$$

$$(T_{1/2}) = \left(\frac{-1}{k}\right) \times \ln\left(1 - 2^{-\frac{1}{\beta}}\right), \tag{4}$$

where β and k are constants calculated from Eq. 2.

Following modeling, the modeled PDR for one gastric emptying profile for the IMO plus sucromalt treatment plateaued without decreasing by more than 1% of its peak value, as is otherwise typical, which resulted in an overestimation of gastric emptying. We excluded the corresponding gastric emptying value from our analyses, as we have done and described in detail previously [26]. The value also was deemed an outlier according to the extreme studentized deviate test for outliers ($P < 0.05$). The other 99 gastric emptying profiles did not have such an issue.

Glycemic response

Continuous glucose monitors (CGMs; G4 Platinum, Dexcom, San Diego, CA, USA) were used to measure interstitial glucose over time, which represents glycemic response. The day prior to each test day, a trained study personnel inserted a CGM sensor subcutaneously in the abdominal area of each participant at least 5 cm from the waist. Participants were masked to their CGM readings for the duration of the study. Calibration of the CGM systems was performed by two fingersticks 2 h after CGM installation and one fingerstick at

the beginning of the test session each test day. CGMs were removed from each participant at the end of each test session (~4 h after consumption of the test meals). Glycemic response was expressed as change in glucose measured by the CGM. Area under the curve during the 0–120 min and 120–240 min postprandial periods was calculated ($AUC_{0\text{-}120\ min}$ and $AUC_{120\text{-}240\ min}$, respectively), as was the maximum peak glucose value (mg/dl) and time for glucose to return to baseline (min, referred to as 'glucose time to return to baseline' throughout the rest of this paper).

Appetite questionnaires

Participants rated their levels of subjective hunger, fullness, desire to eat, and prospective consumption before consuming the yogurt test meals and every 30 min for the 4 h postprandial period using visual analog scale (VAS) questionnaires (printed format, non-electronic) [28, 29].

Relationship between gastric emptying and glycemic response

Analyses were conducted to probe the relationships between gastric emptying parameters (gastric half-emptying time and lag phase) and the four different glycemic response characteristics (glucose $AUC_{0\text{-}120\ min}$, glucose $AUC_{120\text{-}240\ min}$, maximum peak glucose, glucose time to return to baseline). For such analyses, one-way analysis of variance (ANOVA, PROC MIXED) with repeated measures per participant was conducted, with gastric emptying parameters split into classes (gastric half-emptying time: 0–1.5 h, 1.5–2.5 h, 2.5–3.5 h, 3.5–4.5 h, > 4.5 h; lag phase: 0–0.4 h, 0.4–0.8 h, 1.2–1.6 h, > 1.6 h).

Statistical analysis

Data are reported as mean ± standard error of the mean unless otherwise indicated. Statistical analyses were conducted using SAS 9.3 (SAS Institute, Cary, NC, USA). For primary outcomes, gastric emptying and glycemic response parameters (gastric emptying: gastric half-emptying time, lag phase; glycemic response: glucose $AUC_{0\text{–}120\ min}$, glucose $AUC_{120\text{–}240\ min}$, maximum peak glucose, glucose time to return to baseline) were compared using two-way ANOVA (PROC MIXED), with treatment as a fixed effect and subject/participant as a random effect. Appetitive response (another primary outcome) and breath hydrogen (secondary outcome) were analyzed using two-way ANOVA (PROC MIXED) with repeated measures over time (within subject/participant), baseline values as a covariate (within subject/participant), treatment as a fixed effect (between subjects/participants), and subject/participant as a random effect. Glycemic response was not analyzed using a repeated measures model due to the multiple time points of the CGM. Post hoc secondary exploratory analyses were performed with subject/participant sex as an additional factor for the gastric emptying and glycemic response analyses; such analyses included sex as an additional fixed effect (between subjects/participants) in three-way ANOVAs. Residuals of all models were plotted and visually assessed for homoscedasticity and normality using histograms and quantile–quantile plots. Significance was considered at $P < 0.05$, and Tukey's post hoc tests for multiple comparisons were performed when the overall model was significant ($P < 0.05$ for F value).

A power calculation for five treatments was based on appetitive response (hunger and fullness VAS ratings, as this was the outcome that resulted in a larger sample size than gastric emptying [primary outcome of greatest interest]) with an estimated minimum detectable difference of 24 mm (VAS rating) and standard deviation of 19 mm (VAS rating) as indicated for different carbohydrate-based foods by Alfenas and Mattes [30]. With a power of 0.8, 5 treatments, and α of 0.05, it was determined that a sample size of $n = 20$ was sufficient.

Results

Participants

Thirty-eight (38) males and females completed prescreening for the study. Twenty participants (9 females, 11 males) met the inclusion/exclusion criteria and were enrolled in the study upon obtaining their informed consent to the study procedures. Figure 3 depicts the flowchart for participant recruitment, enrollment, and participation in the study. The main reasons that screened individuals did not meet the inclusion criteria were having a body mass index (BMI) that was out of the desired range (BMI 18.5–25.0 kg/m^2) or not regularly consuming breakfast (self-reported). Participant demographics are shown in Table 3.

Gastric emptying

Overall, gastric half-emptying times were significantly different among treatments ($P < 0.05$; Fig. 4), but lag phases were not ($P > 0.05$; Table 4). Mean gastric half-emptying times were higher for the raw corn starch and IMO SDCs (both means ~ 2.8 h) compared to the maltodextrin RDC control (2.3 h; $P < 0.05$), while sucromalt and the combination of IMO and sucromalt were not different from the control (means for all ~ 2.3 h) (Fig. 4). Times for raw corn starch and IMO did not differ from each other ($P > 0.05$). There was a large range of gastric half-emptying times and, when separated into female vs. male groupings, the data revealed that four of the nine female

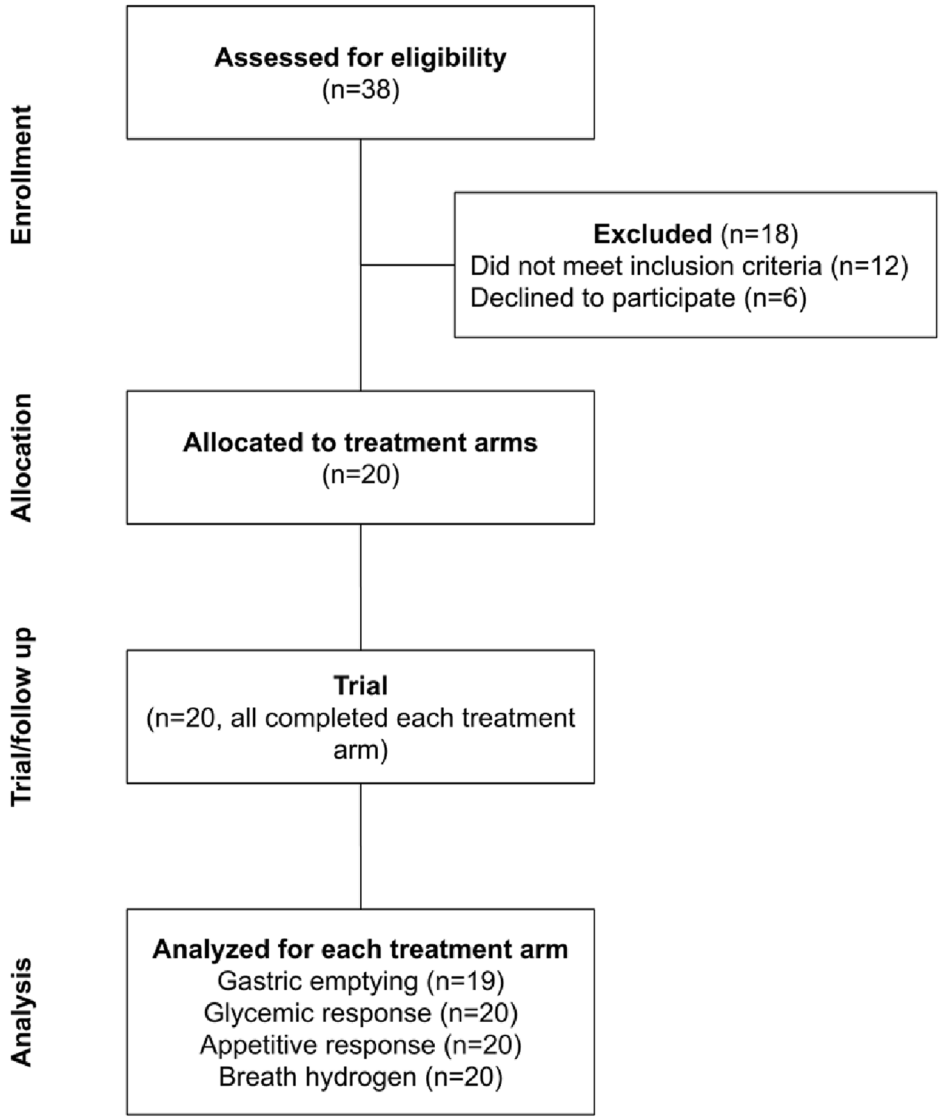

Fig. 3 Participant recruitment, enrollment, allocation, and analysis for the study. The sample size for gastric emptying of the IMO + Sucromalt treatment was 19 ($n = 19$) due to an outlier value for one participant (see Methods for description)

Table 3 Demographics of enrolled participants ($n = 20$)

Demographic characteristic	Range	Mean ± SD
Age (years)	22–47	28.7 ± 5.7
Body weight (kg)	48.1–88.5	68.9 ± 9.4
Height (m)	1.5–1.9	1.7 ± 0.1
BMI (kg/m^2)	18.7–24.8	22.9 ± 1.7

SD, standard deviation

participants (#'s 534, 573, 827, 265) had much higher half-emptying times for raw corn starch and IMO than the rest of the participants for these treatments (Fig. 5). There were similar pronounced responses for the same individuals to these two SDC treatments. Sucromalt, the other SDC, did not increase gastric half-emptying time as much as raw corn starch and IMO, though still some individuals responded (#'s 265, 477, 256, 573). Given that some participants appeared to have pronounced responses to some SDCs while others did not, in a post hoc exploratory sub-analysis we classified participants as "responders" and "non-responders" to the SDC treatments. Responders were designated as participants that had a gastric half-emptying time that was > 30 min longer than the mean gastric half-emptying time for maltodextrin, split by females and males. We have used this 30-min cutoff for alignment with the "minimum detectable difference" value for gastric emptying-based power calculations in previous studies that involved gastric emptying [23, 24]. For females, gastric half-emptying times exceeding 3.0 h indicated the responder classification, whereas for males gastric half-emptying times exceeding 2.5 h indicated the responder classification. Using these criteria, there were 11 total responders to at least one of the SDCs: 6 out of

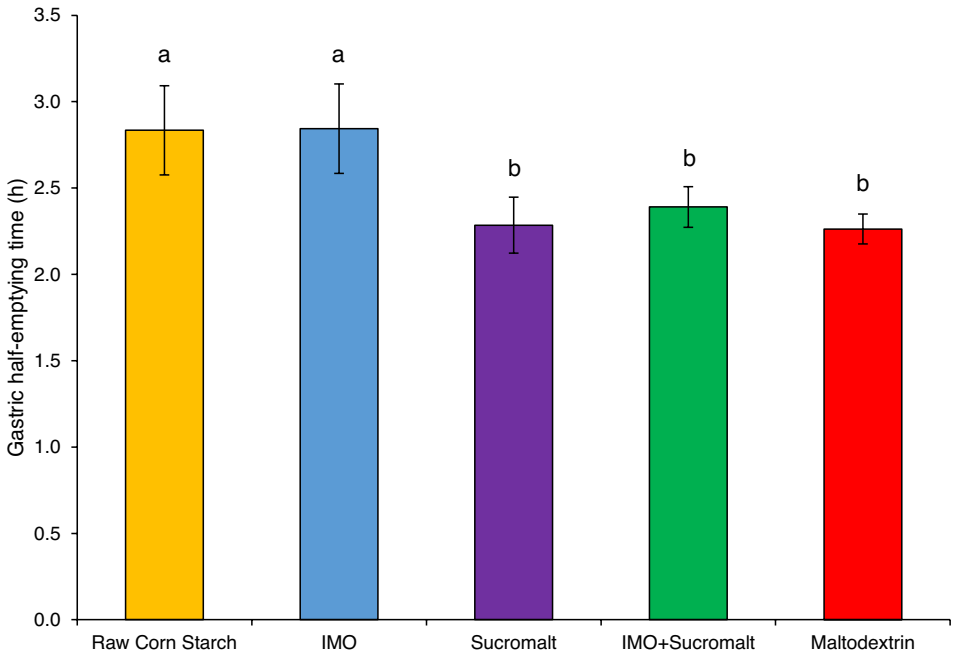

Fig. 4 Average overall gastric half-emptying times after consumption of yogurts containing five different carbohydrate ingredients ($n=20$). Different letters indicate statistically significant differences between treatments ($P<0.05$). Error bars represent ± standard error of the mean (SEM). IMO, isomaltooligosaccharide

Table 4 Average gastric lag phase and glycemic response characteristics ± SEM ($n=20$)

Characteristic	Raw Corn Starch	IMO	Sucromalt	IMO + Sucromalt	Maltodextrin
Lag phase	1.4 ± 0.1^{a}	1.5 ± 0.1^{a}	1.3 ± 0.1^{a}	1.3 ± 0.1^{a}	1.3 ± 0.1^{a}
$AUC_{0\text{-}120\ min}$	1970.0 ± 138.7^{b}	2652.1 ± 187.5^{a}	2805.4 ± 217.2^{a}	2506.4 ± 168.5^{ab}	2517.1 ± 179.2^{ab}
$AUC_{120\text{-}240\ min}$	356.3 ± 205.4^{a}	203.5 ± 143.9^{a}	483.3 ± 339.3^{a}	356.8 ± 245.4^{a}	349.9 ± 190.9^{a}
Maximum peak glucose (mg/dl)	40.1 ± 2.9^{b}	46.8 ± 3.4^{ab}	55.5 ± 3.4^{a}	49.1 ± 3.4^{ab}	50.0 ± 2.4^{a}
Time to return to baseline (min)	144.5 ± 12.4^{a}	117.0 ± 10.2^{a}	126.3 ± 10.3^{a}	134.5 ± 8.5^{a}	129.3 ± 11.6^{a}

AUC area under the curve, *IMO* isomaltooligosaccharide, *SEM* standard error of the mean. Treatment values not sharing the same letter for each characteristic are significantly different ($P<0.05$)

the 9 females and 5 out of the 10 males (Table 5). Among these, eight were responders to the raw corn starch and IMO. Four females, but no males, were responders to sucromalt, and one female and one male were responders to IMO plus sucromalt.

Statistical analysis of gastric emptying data revealed a significant difference between males and females. Overall, females had higher half-emptying times after consuming most of the yogurt test meals compared to males (raw corn starch, IMO, sucromalt, maltodextrin; highest mean value of 3.2 h for IMO in females vs. highest mean value of 2.3 h for raw corn starch in males; Fig. 6). Among females, yogurts containing IMO and raw corn starch exhibited significantly increased half-emptying times compared to IMO plus sucromalt ($P<0.05$). Among males, raw corn starch, IMO, and IMO plus sucromalt had significantly increased half-emptying times compared to sucromalt alone ($P<0.05$). Raw corn starch also had significantly higher gastric half-emptying time than maltodextrin in males ($P<0.05$).

Glycemic response

Glycemic response was measured by CGM and expressed as change in glucose from baseline over time (Fig. 7a). Mean area under the curve (AUC) from 0 to 120 min ($AUC_{0\text{-}120\ min}$) after consumption of yogurt was not different among treatments, with the exception of raw corn starch, which was significantly lower than yogurt with sucromalt and yogurt with IMO ($P<0.05$; Table 4). Peak glucose after consumption of yogurt with raw corn starch was significantly lower than yogurt with sucromalt and yogurt with maltodextrin ($P<0.05$; Table 4). Mean AUC 120–240 min ($AUC_{120\text{-}240\ min}$) and mean time to return to baseline glucose did not differ among treatments ($P>0.05$; Table 4).

When split by sex, glycemic profiles of females showed a "shoulder" of extended higher glucose values that was not observed in males. There was a significant interaction effect between treatments and sex for the postprandial glycemic response ($P<0.05$; Fig. 7b and c). Among male participants,

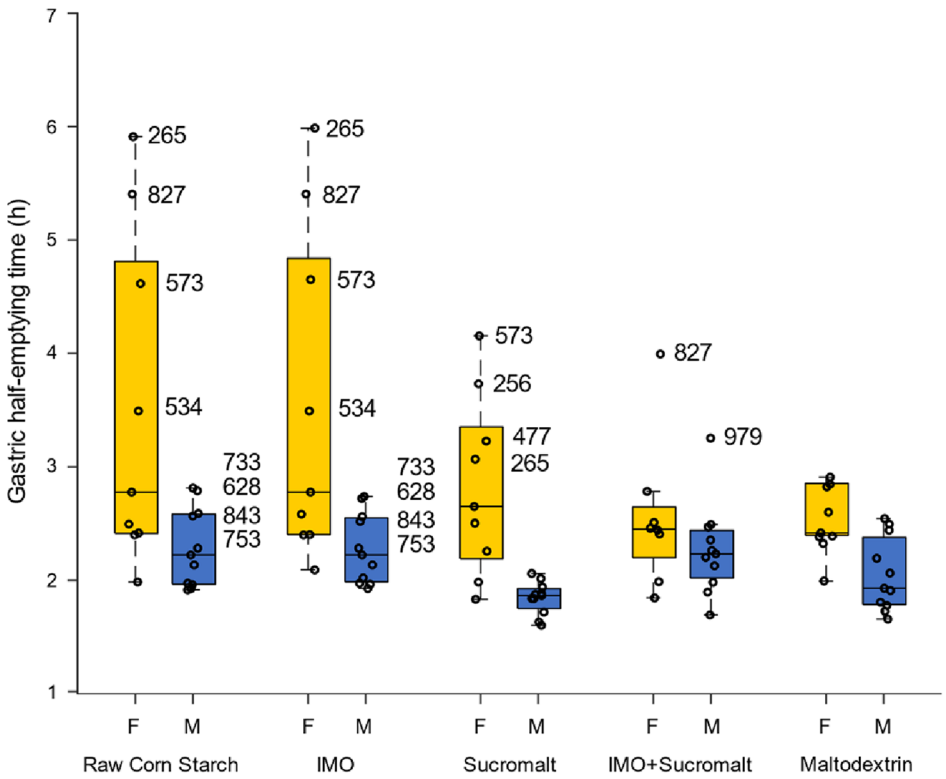

Fig. 5 Range of gastric half-emptying times in females compared to males after consumption of yogurts containing five different carbohydrate ingredients ($n = 20$). Symbols indicate individual participant values. Numbers next to symbols indicate "responder" participant numbers for corresponding half-emptying times. IMO, isomaltooligosaccharide; F, female; M, male

there were no significant differences among yogurt treatments in the overall postprandial glycemic response (change in glucose from baseline over time), though raw corn starch generally had lower initial change in glucose values that remained more stable in the later postprandial period (120–240 min; Fig. 7b). Although there was no significant difference in overall glycemic response among female participants, a lower, more stable glycemic response was visually observed for raw corn starch (Fig. 7c).

Relationship between gastric emptying and glycemic response

To relate carbohydrate digestion to gastric emptying, we examined the relationship between glycemic response characteristics and gastric emptying parameters. There was a trending relationship between glucose time to return to baseline and gastric half-emptying time ($P = 0.08$; Fig. 8a), which was significant for glucose time to return to baseline and lag phase ($P = 0.03$). Because glycemic response represents the complex flux of glucose into and out of the blood with glucose clearance capability increasing with postprandial time [31], glucose time to return to baseline in relation to gastric emptying rate parameters has an upper limit. Therefore, another analysis was done to account for the non-linearity of blood glucose response with postprandial time. Nine instances of gastric half-emptying times exceeding 3.5 h were excluded (out of 99 values total, already excluding the outlier value described above), and the relationship between gastric half-emptying time and glucose time to return to baseline became significant ($P = 0.02$; Fig. 8b). Three of the nine removed gastric half-emptying times were for the raw corn starch treatment, three were for IMO, two were for sucromalt, and one was for the combination of IMO and sucromalt. There was no relationship between either gastric half-emptying time or lag phase and glucose $AUC_{0\text{-}120\ min}$, glucose $AUC_{120\text{-}240\ min}$, and maximum peak glucose ($P > 0.05$).

Appetite questionnaires

The results from the appetitive response questionnaires are presented in Fig. 9. There were no significant differences in appetitive responses among treatments ($P > 0.05$).

Breath hydrogen

Breath hydrogen values did not exceed their baseline values for any of the treatments, indicating that there was no or negligible fermentation of the carbohydrate treatments and that it was not a confounding factor in the study. No statistically significant differences were observed for breath hydrogen among treatments at any time point ($P > 0.05$; Supplementary Information Fig. S1).

Table 5 Participant "responders" to slowly digestible carbohydrates, as indicated by increased gastric half-emptying times compared to mean gastric half-emptying time for maltodextrin[a]

Participant number	Sex	SDC(s) responded to and corresponding gastric half-emptying time (h)
256	Female	Sucromalt (3.7 h)
265	Female	Raw corn starch (5.9 h) IMO (6.0 h) Sucromalt (3.1 h)
477	Female	Sucromalt (3.2 h)
534	Female	Raw corn starch (3.5 h) IMO (3.5 h)
573	Female	Raw corn starch (4.6 h) IMO (4.7 h) Sucromalt (4.2)
628	Male	Raw corn starch (2.8 h) IMO (2.7 h)
733	Male	Raw corn starch (2.8 h) IMO (2.7 h)
753	Male	Raw corn starch (2.6 h) IMO (2.5 h)
827	Female	Raw corn starch (5.4 h) IMO (5.4 h) IMO + sucromalt (4.0 h)
843	Male	Raw corn starch (2.6 h) IMO (2.6 h)
979	Male	IMO + sucromalt (3.3 h)

[a]Calculated as exceeding the mean gastric half-emptying time per sex by more than 30 min. Female cutoff value was 3.0 h and male cutoff value was 2.5 h. Gastric half-emptying times for treatments are shown in parentheses following treatment name. Note that one male participant had a gastric half-emptying time that would fall in the "responder" classification for the rapidly digestible carbohydrate maltodextrin control (Participant 844, 3.3 h). *IMO* isomaltooligosaccharide, *SDC* slowly digestible carbohydrate

Discussion

The objective of this study was to test the hypothesis that SDCs, delivered in a semi-solid yogurt matrix, would delay gastric emptying rate (i.e., increase gastric half-emptying time) through the ileal brake mechanism. Although the exact location that each of the carbohydrate treatments reached in the small intestine could not be directly measured, previous evidence for a dose-dependent relationship between gastric emptying of solid and liquid foods and the ileal brake [10–12] indicates gastric emptying rate (i.e., gastric half-emptying time) can be used as a proxy indicator for the ileal brake, assuming an absence of other factors affecting gastric emptying. Because the yogurt test meals were closely matched in energy content and viscosity, the only difference among treatments was the rate and location of digestion of the carbohydrates. Thus, any differences observed in gastric emptying rate would be due to triggering of the ileal brake.

Overall, gastric half-emptying times were increased to a greater extent by the raw corn starch and IMO SDCs, suggesting activation of the ileal brake; however the response was not consistent among participants, which led us to perform the non-a priori analysis of responders and non-responders. The same four female participants (of nine total) had a substantial increase in gastric half-emptying time for raw corn starch and IMO (mean value of 4.9 h for each vs. 2.5 h for maltodextrin RDC control), and were considered responders. Four male participants were also responders to the raw corn starch and IMO SDCs, although their gastric half-emptying times did not increase as much as females (gastric half-emptying times ranged from 2.5 to 3.3 h for male responders and from 3.0 to 6.0 h for female responders). Responders to sucromalt and IMO plus sucromalt were less consistent, with a subset of four females responding to sucromalt and only one male and female responding to IMO plus sucromalt. This grouping into ileal brake responders and non-responders with SDCs may be due to differences in digestion capacity of individuals (i.e., responders digested the SDCs less efficiently than non-responders). This goes along with the finding that extended glycemic response, which was indicated by longer glucose time to return to baseline ($P = 0.08$ trend in the full group of participants, $P = 0.02$ when nine instances of participant gastric half-emptying times exceeding 3.5 h were excluded), was linked with higher gastric half-emptying time. The response of individuals could also be related to diet histories where responders may have consumed SDCs on a regular basis and developed enterendocrine L-cell responsiveness, while non-responders may consume diets with mainly RDCs. Diet recalls were not done in this study, and could be a part of a future study to understand better the mechanistic underpinnings of these findings.

The results for gastric half-emptying times generally aligned with the hypothesized locational delivery of each of the carbohydrates in the intestine (Fig. 1). Raw corn starch, IMO, and, in some cases, sucromalt, apparently digested into the ileum and triggered the ileal brake as indicated by increased gastric half-emptying time. IMO is a mixture of partially digested gluco-oligosaccharides bound by α-1,6 linkages, including isomaltotriose, panose, and isomaltose [16]. Although IMOs have a prebiotic function, meaning an undigestible fraction reaches the gut microbiota for fermentation, they also can be digested slowly by the host mucosal isomaltase enzyme [16]. In the current study, IMO gave a fairly high glycemic response and was essentially fully digested, as supported by the finding of no increase in breath hydrogen during the postprandial period (Supplementary Information Fig. S1). We speculate that, considering the relatively small dose of treatment carbohydrates in the yogurt

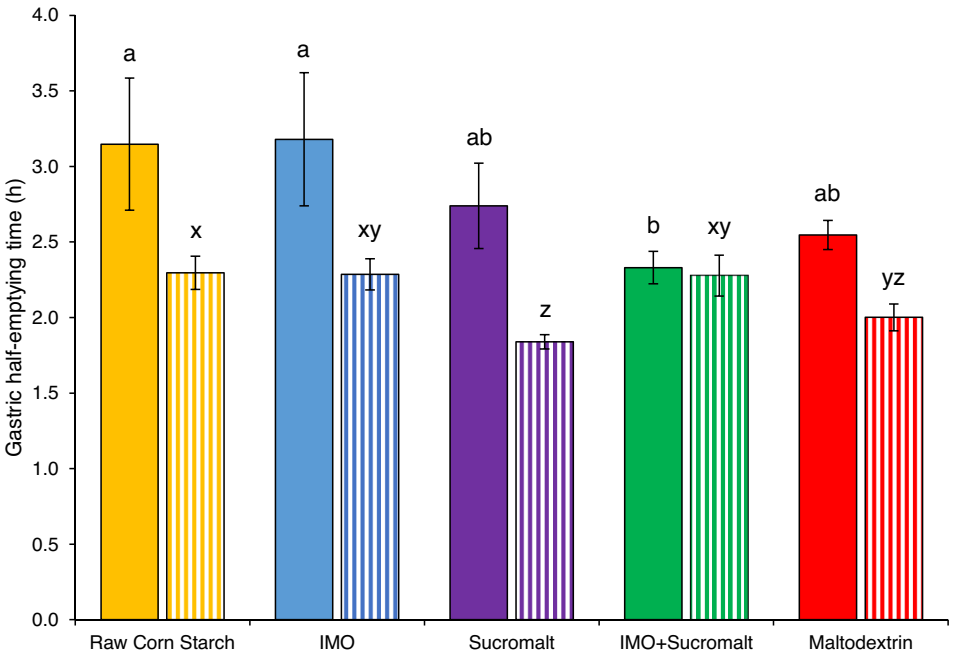

Fig. 6 Average gastric half-emptying times in females compared to males after consumption of yogurts containing five different carbohydrate ingredients ($n = 20$). Females are represented by solid-colored columns, while males are represented by vertical line-patterned columns. Different *ab* letters indicate statistically significant differences between treatments in females ($P < 0.05$). Different *xyz* letters indicate statistically significant differences between treatments in males ($P < 0.05$). Error bars represent ± standard error of the mean (SEM). IMO, isomaltooligosaccharide

(26 g), IMO was mostly digestible and digested slowly and into the ileum. Raw corn starch is considered an ideal source of slowly digestible carbohydrate because its crystalline and amorphous lamellae partially impede the action of digestive enzymes [13, 14]. In this study, the raw starch was fairly digestible as evidenced by the moderate, though still marked glycemic response profile. Given its extended postprandial blood glucose, it appeared to be digested in the ileum, at least in the responder group. Although sucromalt is reported to be slowly yet fully digested and absorbed [32], it did not consistently trigger the ileal brake. In the present study, sucromalt had high peak blood glucose and did not show a reduced glycemic response compared to the maltodextrin control (Fig. 7). The in vitro result for sucromalt (Fig. 2) did not support the in vivo data. Ingestion of sucromalt has been shown to reduce postprandial blood and insulin responses compared to ingestion of high-fructose corn syrup and glucose [17].

The differences in glycemic response between males and females is consistent with results from previous studies indicating that females without diabetes had higher prevalence of impaired glucose tolerance in the postprandial period than men without diabetes [33–36], despite females having higher insulin sensitivity [36, 37]. However, diabetes prevalence is greater among males than females, notably when diagnosis of diabetes is based on fasting plasma glucose and/or hemoglobin A_{1C} measurements but, interestingly, not when based on 2 h plasma glucose after an oral glucose tolerance test [37, 38]. The mechanism underlying this difference is incompletely understood, but it may be tied to differences in sex steroid hormones, height, body composition, body surface area, or physical fitness [37, 39, 40].

The presence of certain macronutrients in the distal small intestine triggers the secretion of gut hormones into the blood that in turn activate the ileal brake [3–5, 41, 42]. Regarding carbohydrates, previous studies have shown that ileal infusions of glucose in canines [43], glucose and hydrolyzed starch in canines [44], and a mixture of starch and maltose in humans [45] slowed gastric emptying and decreased small intestine motility. Additionally, glucose infusion into the distal small intestine in humans increased secretion of the gut hormone GLP-1 [46]. The current goal was to identify or design dietary carbohydrates that trigger this same response when ingested orally. These carbohydrates must be digested sufficiently slow so that they reach the ileum. In a long-term rat feeding study, we showed that a SDC, in the form of starch-entrapped microspheres, activated the gut–brain axis as noted by decreasing gene expression of hypothalamic orexigenic neuropeptides, which was then manifested behaviorally by decreased food intake during meals [1]. Using the same fabricated SDC, designed to be digested in the ileum, gastric emptying rate was modulated in an acute rat study [2]. In humans, a preload of the SDC microspheres increased gastric half-emptying time of a subsequent meal [24], giving further evidence of its ileal brake effect. Our current findings with raw corn starch and IMO in yogurt show that commercially accessible SDCs consumed within a meal have the capacity to trigger the ileal brake in humans, although additional research is required to determine if

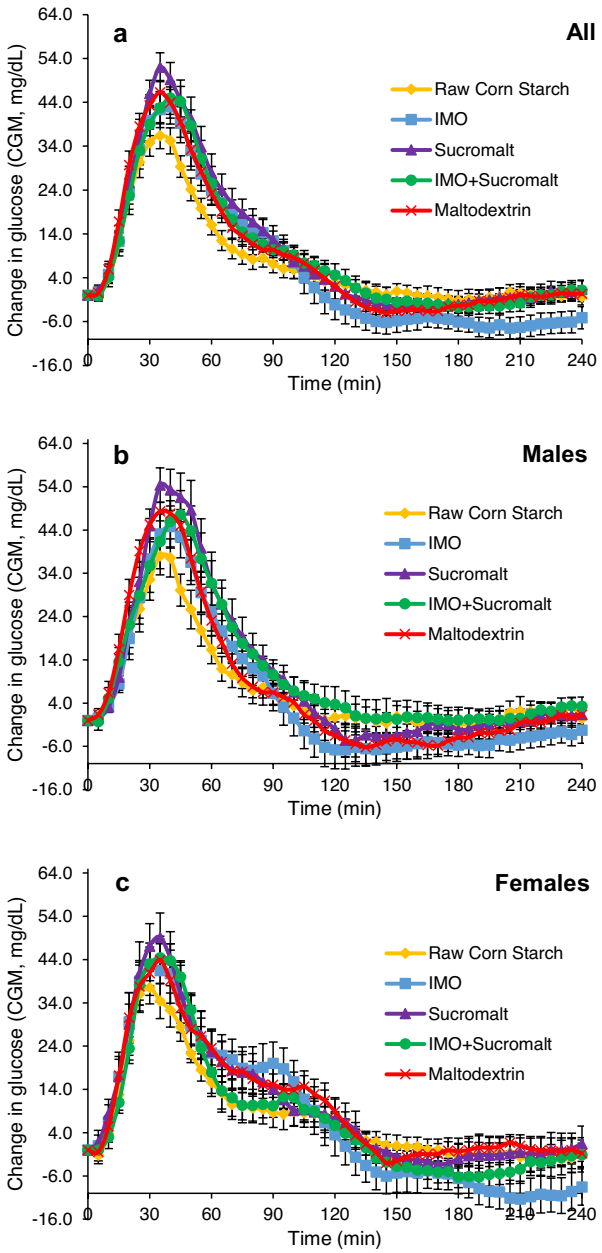

Fig. 7 Postprandial glycemic responses (change in glucose from baseline, mg/dL) measured by continuous glucose monitors 0–240 min after consumption of yogurts with different carbohydrate ingredients ($n = 20$) averaged for all participants as well as split by males and females. The top graph **a** shows the full group of participants, the middle graph **b** depicts males, and the bottom graph **c** shows females. Yellow-orange = raw corn starch, blue = IMO, purple = sucromalt, green = IMO plus sucromalt, red = maltodextrin. Error bars represent ± standard error of the mean (SEM). *CGM* continuous glucose monitor, *IMO* isomaltooligosaccharide

the gastrointestinal effects observed here translate into decreased food intake, especially considering the lack of differences in subjective appetite in the present trial.

The relationship between glucose time to return to baseline and gastric emptying parameters in the exploratory subset of data with removal of gastric half-emptying times exceeding 3.5 h reveals that an extended glycemic response may be related to decreased gastric emptying rate. As mentioned above, glycemic response is reflective of a complex flux of glucose into and out of the blood through the function of food consumed (and how it is digested and absorbed) and the action of insulin and glucagon [31]. Since our exploratory analysis for glucose time to return to baseline and gastric half-emptying time indicated a stronger relationship when only half-emptying times that would be more directly influenced if exogenous glucose influx were included (excluding values > 3.5 h), we formed a hypothesized relationship between time for glucose to return to baseline and gastric half-emptying time according to exogenous glucose influx for times exceeding 3.5 h (Fig. 8c; theoretical values for glucose time to return to baseline are shown for the nine instances of gastric half-emptying times > 3.5 h). Although speculative, this recognizes that overall time for glucose to return to baseline does not indicate that digestion and absorption of glucose from exogenous sources has ceased. In fact, Vonk et al. [31] found that recovery of ^{13}C, indicative of exogenous glucose response, following consumption of 40 g of high-amylose maize starch [Hylon VII; incorporated in 200 mL milk as the vehicle in Vonk et al. [31]] did not return to baseline for 6 h after consumption (which was the termination of their study period) despite a glycemic response return to baseline within 60 min. Because our study involved only 26 g of carbohydrate treatment, our theoretical glucose time to baseline values was set not to exceed 5 h. The association then between glucose time to return to baseline and gastric half-emptying time became more pronounced (Fig. 8c). In any case, in this exploratory approach our data suggest that extended glycemic response is related to decreased gastric emptying rate, which provides support for a triggering of the ileal brake.

The randomization, double-blinding, and crossover design aspects are strengths of the present study. Additionally, closely matching the yogurt test meal treatments for energy content and viscosity allowed us to better discern the impacts of the SDCs tested. Because of the unexpected finding of more pronounced increases in gastric half-emptying time for some SDCs in certain participants, we performed some data analyses showing "responders" and "non-responders" and sex effects that were not planned a priori to the study. Although this suggests that some of the interpretations of these analyses should be made with caution, it is relevant to note that “responders” and “non-responders” in relation to gastric emptying or gastrointestinal motility have been found for vagal nerve stimulation [47], H_2 receptor antagonists [48], lactulose–inulin consumption (as indicated by lack of breath hydrogen response) [49], and eradication

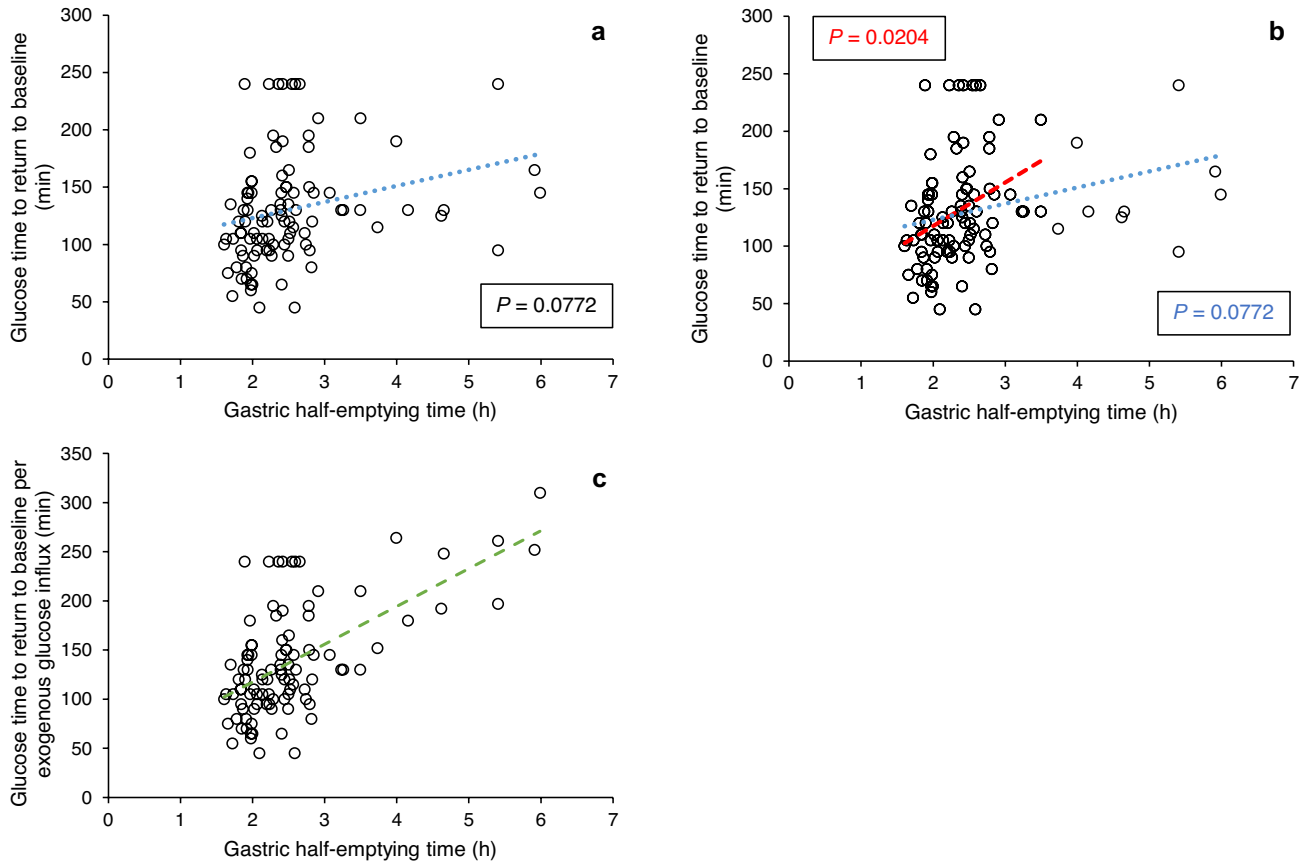

Fig. 8 Longer gastric half-emptying relates to extended glycemic response. Relationship between gastric half-emptying time and time of glycemic response to return to baseline (**a**). Relationship between gastric half-emptying time and time for glucose to return to baseline, analyzed separately with half-emptying times < 3.5 h (red dashed line) compared to all half-emptying times (blue dotted line) (**b**). Hypothesized relationship between gastric half-emptying time and time for glucose to return to baseline according to exogenous glucose flux (**c**); times for glucose to return to baseline have been replaced with hypothesized values for gastric half-emptying values < 3.5 h

therapy to treat *Helicobacter pylori* infection [50]. Furthermore, we have previously observed large differences in gastric half-emptying times of pearl millet-based foods for populations in Mali (mean ~ 5 h half-emptying time) [23] compared to the USA (mean ~ 3 h half-emptying time) [26], with similar gastric half-emptying times in both populations for white rice (mean ~ 3 half-emptying time). Because pearl millet can also be considered an SDC [51–53], this evidence further supports the proposed responder/non-responder dichotomy for gastric emptying of SDCs. Furthermore, the values obtained for gastric half-emptying times in this trial were highly variable, and a larger sample size of participants would have allowed for clearer characterization of responders vs. non-responders, as well as sex effects. The lack of control for menstrual cycle in female participants likely contributed to some of the variability in females, especially considering previous evidence that gastric half-emptying times in females are shorter during the luteal phase of the menstrual cycle than in the follicular phase [54, 55]. However, the magnitude of increase in gastric half-emptying times for the SDCs among responder females in the present trial (0.5–3.0 h) was overall greater than the observed effect due to menstrual cycle in the previous studies (0.3–0.5 h) [54, 55].

Another limitation of the present study was that we did not measure hormones in the blood, such as GLP-1 and insulin, which could potentially help explain the observed glycemic responses and serve as additional indicators of triggering the ileal brake. Furthermore, measurement of exogenous vs. endogenous glucose in the blood would have been a valuable means of differentiating how glucose influx into the blood from SDCs relates to gastric emptying. Measurement of food intake after the 4 h postprandial period could also have been a beneficial means to measure satiety and further characterize another aspect of the ileal brake. As mentioned above, diet recalls would have been useful to understand if diet relates to whether an individual was a responder or non-responder to SDCs, especially considering the evidence that short-term (4–7 days) glucose supplementation [56] or 14-day high-fat diet consumption [57] accelerated gastric

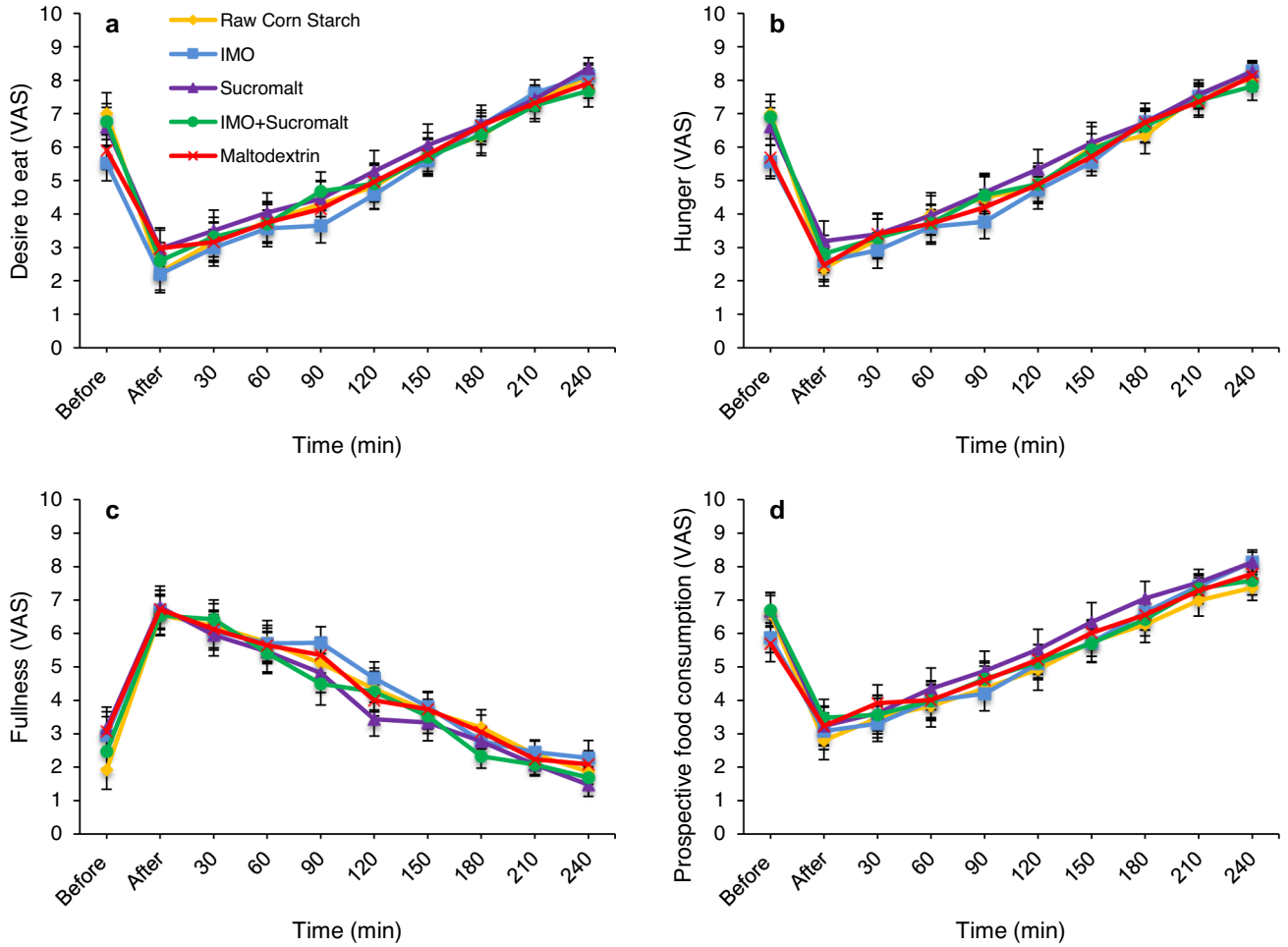

Fig. 9 Average appetite questionnaire ratings immediately prior to and for 4 h (240 min) following consumption of yogurt containing different carbohydrate ingredients ($n=20$). **a** Desire to eat, **b** hunger, **c** fullness, and **d** prospective food consumption. No statistically significant differences were observed among treatments and among participants ($P>0.05$). Yellow-orange = raw corn starch, blue = IMO, purple = sucromalt, green = IMO plus sucromalt, red = maltodextrin. *IMO* isomaltooligosaccharide, *VAS* visual analog scale

emptying of glucose or high-fat meals, respectively. It is also important to note that an assumption made with the ^{13}C octanoic acid breath test, which is an indirect measurement of gastric emptying, is that the tracer is emptying from the stomach at the same rate as the test meal. Using other methods to assess gastric emptying rate of the SDCs tested, such as magnetic resonance imaging or scintigraphy, may be considered.

Conclusion

Our results show that certain SDCs (raw corn starch and IMO) triggered the ileal brake as shown by significantly increased gastric half-emptying times (i.e., decreased gastric emptying rate) in humans, with a more pronounced response in a subset of individuals that we termed "responders" in an exploratory analysis. This was supported by a trending association of extended glycemic response and higher gastric half-emptying time, although no effect was observed on appetitive response. In addition to moderate postprandial glycemic response being a benefit of SDCs, this study shows that some SDCs with apparent ileal digestion also decrease gastric emptying rate through the ileal brake mechanism.

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Author contributions MC: conceptualization, formal analysis, investigation, writing—original draft. AMRH: formal analysis, investigation, writing—original draft, writing—review and editing, visualization. TDG: conceptualization, methodology, formal analysis, resources. MMM: methodology, resources, writing—review and editing. JL: investigation. RSM: conceptualization, writing—review and editing. NMH: methodology, formal analysis. MEH: resources. BRH: conceptualization, methodology, resources, writing—original draft, writing—review and editing, project administration, funding acquisition. All authors read and approved the final manuscript.

Declarations

Conflict of interest TDG, MMM, RSM, NMH, and MEH were employed at General Mills, Inc. when the study was conducted. MC, AMRH, JL, and BRH report no conflict of interest to the study.

Ethical approval All procedures were conducted in accordance with the ethical standards of the Purdue University Institutional Review Board (Protocol #1502015807) and the study was registered at ClinicalTrials.gov (NCT03630445).

Consent to participate Written informed consent was provided by all participants prior to their inclusion in the study.

Consent for publication The authors affirm that human research participants acknowledged consent for publication of the current study.

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