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Carbohydrates designed with different digestion rates modulate gastric emptying response in rats

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Abstract

We sought to determine whether design of carbohydrate-based microspheres to have different digestion rates, while retaining the same material properties, could modulate gastric emptying through the ileal brake. Microspheres made to have three slow digestion rates and a rapidly digested starch analog (maltodextrin) were administered to rats by gavage and starch contents in the stomach, proximal and distal small intestine, and cecum were measured 2 h post-gavage. A stepwise increase in the amount of starch retained in the stomach was found for microspheres with incrementally slower rates of digestion. Postprandial glycaemic and insulinemic responses were incrementally lower for the different microspheres than for the rapidly digestible control. A second-meal effect was observed for slowly digestible starch microspheres compared to glucose. Thus, dietary slowly digestible carbohydrates were designed to elicit incremental significant changes in gastric emptying, glycaemic and insulinemic responses, and they may be a means to trigger the ileal brake.

Keywords

slowly digestible starch; gastric emptying; glycaemic response; insulinemic response; second-meal effect

Introduction

Movement of food in the upper gastrointestinal tract is controlled by gastric emptying rate and small intestinal transit and is regulated through sensors and receptors that trigger nerve and gut hormone responses (Zhang et al. 2015). The “ileal brake” is a feedback mechanism that controls gastric emptying rate and was first recognized by Spiller and Read in 1984 (Read et al. 1984; Spiller et al. 1984). It is associated with the presence of protein

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Disclosure of interest

The authors report no conflict of interest.

hydrolysates, lipid, and glucose and other carbohydrates in the ileum (Zhang et al. 2015). Glucose, cooked rice starch, and maltose perfused into the ileum were shown to slow gastric emptying rate of a meal, and in a dose-dependent manner in human subjects (Miller et al. 1981; Jain, Boivin, Zinsmeister, Brown, J.R. Malagelada, et al. 1989). Starch delivered to the ileum due to delayed starch digestion by starch digestive enzyme inhibitors, acarbose and phaseolamin from Great Northern white beans, triggered the ileal brake (Layer et al. 1986; Enç et al. 2001). Short chain fatty acids produced by dietary fibre fermentation in the colon also reduce gastric emptying rate, which has been called the “colonic brake” (Nightingale et al. 1993; Wen et al. 1995; Ropert et al. 1996). Thus, gastric emptying rate can be affected by multiple feedback signals as well as through stretch sensors in the stomach itself.

Starch, as the main glycaemic carbohydrate in the diet, is nutritionally classified as rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) (Englyst et al. 1992). RDS has been positively correlated with glycaemic index (Englyst et al. 2003). SDS is digested completely in the small intestine at a slower rate than RDS, and RS is the undigested part that reaches the large intestine. Within both RDS and SDS fractions there are variations in digestion profiles. SDS is perhaps the least well understood fraction in terms of such variations and what they might mean physiologically.

Our laboratory reported that SDS, designed to digest slowly and into the ileal region of the small intestine, reduced gastric emptying rate of a non-nutritive paste “meal” in humans, when given as a preload (Cisse et al. 2017), and activated the gut-brain axis of rats to decrease food intake (Hasek et al. 2018). The SDS material was made with defined slow glucose release profiles by entrapment of starch in a natural polymer (gelled alginate) in the form of microspheres that were cooked and dried (Venkatachalam et al. 2009). This SDS functions by allowing α -amylase to enter its porous matrix and digest the starch in a slow manner. By changing the pore size, the rate of digestion of the microsphere *in vitro* was changed from moderately slow to very slow (Venkatachalam et al. 2009). In the current study, alginate-entrapped starch microspheres of similar hardness were designed with varying starch digestion rates as model food materials to test the hypothesis that SDS reduces gastric emptying in a dose dependent manner coinciding with increased amount of starch in the ileum, resulting in reduced glycaemic and insulinemic responses.

Methods and Materials

Test materials

Starch-entrapped microspheres with different digestion rate profiles were prepared by a modification of the method of Venkatachalam et al. (2009). Sodium alginate (a gift from the FMC Corporation, Philadelphia, PA, USA) was dissolved in water at 0.5%, 1.0%, and 1.5% (w/v) levels, mixed with waxy corn starch (10%, w/v), and thereafter dropped into a 2% calcium chloride bath using a 21 gauge hypodermic needle to form microspheres. Increasing sodium alginate concentration produced microspheres with smaller pores. The *in vitro* Englyst assay (Englyst et al. 1992) was conducted on cooked microspheres and showed 62.6, 44.0, and 21.9% for RDS; 33.3, 44.9, and 38.0% for SDS; and 4.2, 11.0, and 40.1% for RS for the 0.5, 1.0, and 1.5% microspheres, respectively (Venkatachalam et al. 2009) (note that Englyst values of RDS, SDS and RS are not absolute, though in relative terms they

showed digestion rate differences suitable for this study). The 0.5% microspheres are referred henceforth as “SDS-1” (slowly digesting microspheres), the 1.0% microspheres as “SDS-2” (more slowly digesting microspheres), and the 1.5% microsphere as SDS-3 (most slowly digesting microspheres). For total starch content analysis, microspheres were pulverized using a ball mill, and content was determined by the Total Starch Kit (Megazyme International Ireland Ltd, Wicklow, Ireland). Microsphere densities, as an indicator of hardness, were measured by a gas pycnometer (AccuPyc II 1340, Micromeritics, Norcross, GA, USA).

Test materials were administered by gavage in two boluses in which a total equivalent amount of 0.48 g starch was divided into two portions containing 0.24 g starch each. The prepared dried microspheres were dispersed in purified water, cooked in a pressure cooker for 10 min, and washed with water three times prior to being used. After the third wash and discarding of the supernatant, 3 ml water and 1 ml 2% guar gum were added to the cooked microspheres. Polycose® (Abbott Nutrition, Columbus, OH, USA), a rapidly digestible maltodextrin, was used as the RDS analog in the same guar gum solution to facilitate the gavage process. The volume of each of the two boluses for each treatment was 4 ml.

Animal study

Twelve-week old Sprague Dawley male rats were obtained from Harlan Laboratories (Indianapolis, IN, USA). Rats were maintained in a temperature-controlled room at 24°C with 12-h light and 12-h dark cycles. Rats had ad libitum access to a pelletized standard chow diet (5001 Rodent Diet, Purina LabDiet, St. Louis, MO, USA) and water. The animal protocol was approved by the Purdue Animal Care and Use Committee (PACUC). Rats were fasted overnight prior to each test day. On the test days, animals were administered RDS and SDS materials by oral gavage: blank control (n=6), RDS (n=4), SDS-1 (n=7), SDS-2 (n=7), and SDS-3 (n=8). At 2 h postprandial time, animals were anesthetized with ether and euthanized, and the stomach, small intestine, and cecum were dissected and removed for further analysis. The small intestine was divided into two segments of equal length, designated as the proximal and distal small intestine. Tissue sections were cut open, put in a tube containing water, and placed in a boiling water bath. Luminal content was collected from the water in the tube. Tissue sections were also scraped to ensure complete removal of the luminal contents and placed in the tube.

Postprandial starch content in different locations of the gastrointestinal tract

—Gastrointestinal luminal contents from the stomach as well as proximal and distal small intestine and cecum of each animal were initially freeze-dried and later pulverized using a ball mill. Total starch content was determined using the same type of Total Starch Kit as above. Starch contents recovered from the gastrointestinal sections were expressed as percentage of the total amount of starch gavaged. The amount of starch retained in the stomach was used as an indicator of gastric emptying.

Postprandial blood glucose and insulin tests—Blood samples were collected from the tail vein at 0, 15, 30, 60, and 120 min following ingestion of the test materials and blood glucose was measured using a Contour glucose meter (Bayer Healthcare LLC, Mishawaka,

IN, USA). Tail blood was also collected at the same time points for insulin analysis. Insulin was measured using a rat insulin ELISA kit (Crystal Chem Inc., Downers Grove, IL, USA) with upper and lower detection limits of 0.1 ng/ml and 12.8 ng/ml, respectively.

Second-meal effect—Rats (5/group) were fasted overnight, and treatments of glucose, SDS-1, and SDS-2 were administered to rats through oral gavage for the first meal in the same manner as above. For the second meal (4 h later), glucose (0.48 g in 2 ml water) was administered by oral gavage. Tail blood glucose was measured by a glucose meter at 0, 15, 30, 60, 120, and 180 min after gavage.

Statistical analysis

Data were expressed as mean $\pm$ standard error of the mean (SEM). One-way ANOVA was used to determine the significance of treatment effect. Tukey's multiple comparisons test was performed when the model was significant. All tests were 2-sided and difference was considered significant when $P < 0.05$. GraphPad Prism (Prism 5.0) was used to perform the statistical analyses and power analysis

Results

Postprandial starch content in different locations of the gastrointestinal tract

To determine the effect of SDS of differing digestion rates on gastric emptying, starch contents were measured in the stomach 2 h after gavage. Starch contents were also measured in the proximal and distal small intestine sections and cecum, because the presence of starch in these regions could indicate triggering of the ileal or colonic brakes. At 2 h post-gavage of SDS-1, SDS-2, and SDS-3 microspheres, the starch contents retained in the stomach were 5.1, 11.7, and 17.4% of total gavaged (Fig. 1A), respectively. The microsphere treatment animals had a stepwise increasing amount of starch retained in the stomach with incrementally slower rates of starch digestion [differences between SDS-3 and SDS-1 and RDS were significant ($P < 0.05$)]. The RDS treatment animals had negligible starch in the stomach. Combined starch contents in the proximal small intestine (Fig. 1B), distal small intestine (Fig. 1C), and cecum (Fig. 1D) were 0.8, 1.1, and 4.1%, respectively. In the proximal and distal small intestine, consumption of SDS-3 resulted in significantly higher starch contents than in the other treatment groups ($P < 0.05$). In the cecum, there was little starch recovered and there were no statistically significant differences among the treatment groups.

Glycaemic and insulinemic responses

Reduction in starch digestion rates decreased postprandial glycaemic responses (Fig. 2). The peak values of blood glucose for the SDS-1 and SDS-2 microspheres (146.9 and 124.3 mg/dl, respectively) were lower and at a later time (60 min) compared with RDS (153.5 mg/dl at 30 min). At 60 min, both RDS and SDS-1 treatment groups (144.8 and 146.9 mg/dl, respectively) showed a higher blood glucose than SDS-3 (116.5 mg/dl) ($P < 0.05$). The glucose response of the SDS-1 group (146.9 mg/dl) was also higher than that of the SDS-2 group (124.3 mg/dl) at 60 min ($P < 0.05$). Glucose responses for all three microspheres trended higher compared to RDS at 120 min, though only the difference between SDS-1 and

RDS groups was significant ($P < 0.05$). Overall, microspheres demonstrated a slower and more sustained glucose release compared to RDS.

Similarly, the response profiles of insulin were lower for the SDS treatments compared to RDS (Fig. 3). Insulin response among the SDS microsphere groups trended downward incrementally for SDS-1, SDS-2, and SDS-3 groups. Insulin response for SDS-1 from 60 min to 120 min trended higher compared to RDS. However, over the time points, there were no significant differences between RDS and the microsphere groups, except for a higher insulin level in the RDS group compared to SDS-3 group at 15 min ($P < 0.05$).

Second-meal effect

The SDS-1 and SDS-2 microspheres were tested compared to glucose for a second-meal effect in glycaemic response. When glucose was administered for a second meal, a significant decrease in peak blood glucose was found for the SDS-2 treatment compared to the glucose control ($P < 0.05$) (Fig. 4).

Discussion

In this study, slowly digestible carbohydrates of differing digestion rates were used as a dietary approach to delay gastric emptying in a stepwise manner. At 2 h postprandial time, the slowest digesting SDS-3 had a higher amount of starch retained in the stomach, which indicates slower gastric emptying. Furthermore, a greater amount of SDS-3 was present in the ileum 2 h postprandially, which suggests that the ileal brake was being activated. A number of studies have shown that carbohydrates perfused into the distal region of the small intestine trigger the ileal brake to slow gastric emptying (Layer et al. 1986; Jain, Boivin, Zinsmeister, Brown, J.R. Malagelada, et al. 1989; Tohno et al. 1995; Enç et al. 2001). However, it was previously unknown if ingested carbohydrates with different degrees of slow digestion would affect gastric emptying and transit of gastrointestinal contents from a meal, and in a dose-dependent manner. We also previously reported a long-term feeding study where the same slowly digestible microspheres reduced food intake and decreased hypothalamic gene expression of appetite-stimulating neuropeptides, suggesting ileal activation of the gut-brain axis through gut hormone/vagal nerve stimulation (Hasek et al. 2018), and thus supporting a feedback control response to slowly digestible carbohydrates. This evidence indicates that consumption of carbohydrates which are digested incrementally more slowly will more potently trigger physiological responses that are beneficial for control of food intake and weight management.

This study was empirical in nature, as outcomes directly indicating the mechanism underlying the ileal brake, such as level of plasma glucagon-like peptide-1 (GLP-1), were not investigated. However, given the previously described relationship between presence of carbohydrates in the ileum and triggering of the ileal brake (Layer et al. 1986; Jain, Boivin, Zinsmeister, Brown, J.-R. Malagelada, et al. 1989; Tohno et al. 1995; Enç et al. 2001), we find our results to be supportive that the ileal brake was being activated to reduce gastric emptying rate. We attempted to make microspheres without starch to test whether they alone had an effect on gastric emptying, but were unable to make the spheres because the collapsed structures formed aggregates that could not be used.

Size and density of nondigestible solids in spherical shape have been demonstrated to influence gastric emptying (Meyer et al. 1985; Sirois et al. 1990). Size and density of the three types of microspheres were measured and found to be similar. The range of size for each of the three types of microspheres was 300–800 μm (Venkatachalam et al. 2009). The densities of SDS-1, SDS-2, and SDS-3 microspheres, as an indicator of hardness, were similar at 1.06, 1.07, 1.08 g/cm^3 , respectively. Moreover, the microspheres were not broken down in the stomach so their integrity and size in the stomach was maintained (visual observation). Thus, the material properties of the microspheres were not a confounding factor to the observed differences in gastric emptying.

Presence of starch in the cecum could trigger the colonic brake through production of short chain fatty acids by bacterial fermentation (Nightingale et al. 1996; Wen et al. 1998). However, in this study only small amounts of starch were found in the cecum for the SDS microsphere treatments. This starch was present in the core of the microspheres (Venkatachalam et al. 2009) making it difficult for bacteria to access (Kaur et al. 2019), particularly in the 2 h post-gavage time period of the study. Also, the second-meal effect has been suggested to be caused by a carbohydrate fermentation feedback mechanism (Brighenti et al. 2006), however, this also seems unlikely in the present study for the same reasons.

Conclusions

Slowly digestible carbohydrates designed in the form of three SDS microspheres of differing slow digestion rates incrementally decreased gastric emptying rates as measured by starch retention in the stomach at 2 h post-gavage in rats. Significantly higher starch in the stomach coincided with more starch recovered in the ileal region of the small intestine. These results suggest that consumer-desirable slowly digestible carbohydrates that digest into the ileum not only have beneficial effects of moderating glycaemic and insulinemic responses, but also slow gastric emptying effects associated with satiety and appetite.

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Figure 1.

Starch content (in % of the total amount ingested) in the stomach (A), proximal small intestine (B), distal small intestine (C) and cecum (D,) of rats 2 h after postprandial oral gavage of 5 treatments: blank control, Polycose® (rapidly digestible maltodextrin, RDS), slowly digesting microspheres (SDS-1), more slowly digesting microspheres (SDS-2), and most slowly digesting microspheres (SDS-3). Different letters indicate treatments were significantly different ($P < 0.05$).

Figure 2.

Glucose response at baseline, 15, 30, 60, and 120 min after oral gavage of 5 treatments: blank control, Polycose® (rapidly digestible maltodextrin, RDS), slowly digesting microspheres (SDS-1), more slowly digesting microspheres (SDS-2), and most slowly digesting microspheres (SDS-3). Peak glucose responses to the SDS-1 and SDS-2 occurred at a later time (60 min) compared with RDS (30 min). At 60 min, both RDS and SDS-1 led to a higher glucose response than SDS-3 ($P < 0.01$ and $P < 0.001$, respectively). SDS-1 group also had higher glucose response than SDS-2 at 60 min ($P < 0.05$). At 120 min, SDS-1 group had higher glucose response than RDS group ($P < 0.01$).

Figure 3.

Insulin response at baseline, 15, 30, 60, and 120 min after oral gavage of 5 treatments: blank control, Polycose® (rapidly digestible maltodextrin, RDS), slowly digesting microspheres (SDS-1), more slowly digesting microspheres (SDS-2), and most slowly digesting microspheres (SDS-3). At each time point, there was no significant difference among RDS and the microsphere groups except for a higher insulin response in RDS group compared to SDS-3 at 15 min ($P < 0.05$).

Figure 4.

Glycaemic response to a glucose load at 15, 30, 60, and 120 min measured 4 h after ingestion of glucose, slowly digesting microspheres (SDS-1), and more slowly digesting microspheres (SDS-2) to examine the second-meal effect. A significant decrease in the second meal peak blood glucose was found for the SDS-2 treatment compared to the glucose control ($P < 0.05$).