ANNEX XI-D:

GLYCAEMIC RESPONSE DETERMINATION STUDIES FOR SNACK BARS MADE WITH RS4)

WORK SUPPORTED BY MGP INGREDIENTS FOR KANSAS STATE UNIVERSITY, MANHATTAN KS, USA

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August 15, 2007

Ody Maningat, Ph.D, Vice President
Applications Technology and Technical Services
MGP Ingredients, Inc.
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Dear Dr. Maningat:

Thank you again for the opportunity and support to conduct research using your resistant starch products. Attached you should find a copy of the approval letter from the Institutional Review Board and our recently submitted article to the Journal of Food Science (in review). Shortly, I will be sending a report of the most recent clinical trial completed this summer.

Thank you again for your support and interest in conducting clinical research.

Appreciatively,

Mark D. Haub, Ph.D.
Assistant Department Head

Full Title

Glycemic Response of Energy Bars Containing Resistant Starch Type 4 in Healthy Older Adults

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Short version of title Resistant Starch in Older Adults…

Choice of journal section

Sensory and Nutritive Qualities of Food

ABSTRACT:

Background: Decreased postprandial glycemia decreases risk for several chronic diseases.

Aim: The purpose of this study was to determine the glycemic response of energy bars containing resistant starch (type 4; RS4) in healthy older adults (age = 68±6 yr).

Methods: Volunteers (n=9; BMI=25±4 kg/m^2) consumed a glucose reference beverage (GLUC), a wheat bar control (CB), and a RS4 bar (RSB) in portions containing 50 g of carbohydrates, with the only differing ingredient between bars being RS4 or puffed wheat. Volunteers consumed each item twice. Blood samples were collected at baseline and at 15, 30, 45, 60, 90, and 120 min after consumption and measured for glucose concentration. Area under the curve (AUC) for blood glucose was calculated and used to determine the glycemic index (GI).

Results: The two bars had significantly ($p < 0.05$) lower AUC values (151±58, and 102±26 for WB and RSB, respectively) than the GLUC (284±95). The AUC for RSB was significantly ($p < 0.05$) lower than WB.

Conclusion: Bars made from RS4 had a low GI and this was less than a bar made from the same conventional carbohydrate ingredients with only one ingredient differing. These data suggest that foods made from RS4 may be an appropriate dietary means to help control blood glucose and improve glycemia-related health outcomes.

Keywords: Low glycemic foods, resistant starch type 4, postprandial glucose response, older adults, glycemic load

Introduction

There are strong efforts by the scientific community, public health organizations, media, and companies to emphasize living a healthier lifestyle. Yet, the culmination of a poor diet, an unhealthy lifestyle, and the aging process tends to lower glycemic tolerance and, thus, place older adults at a higher risk for developing type 2 diabetes mellitus (T2DM) and other health metabolic complications [12]. Discovering foods that better attenuate blood glucose and reduce insulin secretion are in high demand in this population. In addition, these glucose-regulating foods may also prevent, delay, or control other metabolic disorders [4].

A compound gaining scientific interest in the clinical realm is resistant starch (RS). Resistant starch is the part of starch that escapes the small intestine without digestion, and subsequently fermented by anaerobic intestinal bacterial with short chain fatty acids (SCFA) as major byproducts [9]. There are four classifications of RS: RS1, RS2, RS3, and RS4. RS1 refers to resistant starch that is physically enclosed by whole grains. While RS2 is a granular resistant starch. RS3 refers to non-granular, retrograded or crystalline resistant starch, and RS4 is a manufactured or modified resistant starch by means of cross-linking the starch granules [9, 13]. To date, the majority of human clinical trials have been conducted using only RS2 or RS3, which reported decreased blood glucose following consumption of foods with these starches added (REFS).

Resistant starch is generally considered a low glycemic index (GI) ingredient due to its slow rate of digestion and absorption [6] and a prebiotic by assisting in the cultivation of intestinal bacteria. However, with limited clinical trials using actual RS-containing food items, it is difficult to understand the beneficial capacity of RS to assist with glucose control in a real environment. Some clinical trials (10-11) have used RS, but failed to control the ingredients of those food items (10) or only provided sachets for volunteers to use (11). Thus, there are limited trials assessing the glycemic response of RS in actual food; and, in the one study that did (10), it is difficult to be confident that RS was the ingredient eliciting the improvements in glycemia and not due to differences in other ingredients (e.g., protein and/or fat content). Lastly, there is a paucity, if any, research on the clinical outcomes of RS type 4. Thus, given the paucity of clinical data regarding RS4 and the potential health improvements for older adults, the aim of this study was to determine the glycemic response of two novel nutrition bars in healthy older adults.

Materials and Methods

Subjects: The volunteers (female=5 and male=4; age= 68±6 y, BMI= 25±4 kg/m^2) had to meet the following criteria: have no known chronic diseases, non-smokers, no long-term use of medications known to affect glucose metabolism, and able to make several visits to the Human Metabolism Laboratory. The Institutional Review Board of Kansas State University approved the study, and all volunteers signed a written informed consent form.

Study Design Each volunteer visited the laboratory on six different occasions over a 2-week period (3 visits/ week). Randomization was applied to all of the test foods where each volunteer consumed a different product each time. The test foods were two nutrition bars and a GLUC (Sun-Dex, Fisher Scientific, Houston, TX).

Yellow Springs Instruments, Yellow Springs, OH). All blood samples were measured in duplicate. Analysis was repeated if the difference between duplicate samples was greater than 0.1mmol/L.

Calculations of GI and GL: GI is defined as the ratio of the AUC values of food relative to that of standard food (GLUC) with the dose of available carbohydrate being 50 grams. The average of two AUC of the standard drink was used as the reference value, and each GI was calculated for volunteers as [16]:

$$GI = (AUC\ test\ food / AUC\ reference\ food) \times 100$$

The GL for each food was calculated as:

$$GL = GI/100 * 50$$

Statistical Analysis and Sample size Calculations: Four volunteers were found to be sufficient to detect significant differences among GI values of reference and test foods ($power>0.80$, and $p<0.05$; NCSS and PASS 2004, Kaysville, UT) [8]. To calculate AUC, the trapezoidal method was used via NCSS software (NCSS and PASS 2004, Kaysville, UT). Analysis of variance with repeated measures was used to determine significant main effects and interactions with significance level set at $p= 0.05$. Fisher Least Significant Difference for multiple comparisons was used to determine significant differences between the trials. All statistical calculations were performed using NCSS software (NCSS and PASS 2004, Kaysville, UT).

Results and Discussion

Test Foods: Two nutrition bars (Wheat bar, and RS4 bar) and GLUC were tested. Each item provided 50g of available carbohydrate. All the nutrition bars ingredients were purchased from a local grocery supermarket, except RS4 which was provided by MGP ingredients (MGP, Atchison, KS). The only ingredients that differed in each bar were puffed wheat or RS4 (22g/ serving). The other ingredients were the same type and quality for both bars. The test nutrition bars were served with 180ml of water. A 65g portion of WB provides 237 Kcal energy, 1.0g fat, 7.0g protein, 6.0g dietary fibers, and 56 total CHO, while a 80g portion of RSB provides 243 Kcal as energy, 1.9g fat, 6.4g protein, 22g RS, and 72 total CHO. The choice for RS4 was made based on a lower glucose area under the curve value, compared with RS2, from a pilot study.

Carbohydrate Determination: Proximate analysis for both nutrition bars was performed to determine the crude amount of carbohydrates in each. The amount of each bar needed to yield a 50g of available carbohydrate was calculated according to those results.

Glycemic Index Tests: Volunteers were asked to fast 8-10 h the night before the testing day. In the morning of each test, finger-prick capillary blood samples were collected to determine fasting blood glucose levels. Ten minutes were allowed for the test food to be consumed. Over the 2 h following the start of each test, finger-prick capillary blood samples were collected at 15, 30, 45, 60, 90, and 120 min. The blood samples were taken using Safety Lancets (Fisherbrand, Fisher Sci. Houston, TX), and heparinized Micro-Hematocritcapillary tubes (Fisherbrand, Fisher Sci. Pittsburgh, PA). Blood glucose levels were directly measured using a semi-automatic blood glucose analyzer (YSI 2300, *Glycemic Response:* The mean blood glucose response curves for the GLUC, WB, and RSB are shown in Figure (1). The RSB trial elicited significantly ($p < 0.05$) lower blood glucose levels at 30, 45, and 60 min (Fig 1), and a decrease AUC response compared to GLUC and WB (Table 1). Both bars (WB= 5.77±0.4mmol/L, RSB= 5.42± 0.4mmol/L) produced lower mean glycemic responses compared to the GLUC (6.76± mmol/L), with RSB also being decreased relative to WB.

The mean GI value of WB (GI=53±24) was higher than the mean GI value for the RSB (GI=36±16), with both being different from GLUC (GI=100) (Table 2). Both are consider low GI foods (<55). The GL for RSB was lower (GL=18±8) than the GL for WB (GL= 27±12).

Discussion

This study demonstrates, for the first time clinically, that RS4 elicits decreased postprandial blood glucose levels compared to a dextrose reference and a bar made from puffed wheat in healthy older adults. Also, these data demonstrate that the WB and RSB had GI value less than 55 (53, and 36 for WB, and RSB respectively). The AUC values indicate that the RSB attenuated elevations in blood glucose, likely due to deceased absorption of this RS [17]. It is likely that the starch from RS4 was digested over a longer period of time leading to more steady blood glucose levels. This result agrees with the other studies which confirm that foods with higher dietary fiber have lower GI. Since RS by definition of Association of American Cereal Chemists (AACC) is a dietary fiber, RS4 would likely have a low GI. Also of note, based on the results from the pilot study, RS4 appears to lower blood glucose to a greater extent than RS2.

In studies using other types, RS decreased fasting and postprandial blood glucose levels acutely and chronically [11, 17]. Yamada et al. investigated the inhibitory effect of a single meal of bread containing 6 g of RS3 on postprandial blood glucose levels in male and female adults with impaired fasting glucose. They observed that both blood glucose and insulin significantly decreased following the RS3 meal compared to the control bread. Compared their observations, the RSB elicited decreased glucose levels as soon as 15 minutes post-ingestion, while their bread slices did not achieve glucose differences until 60 minutes. The results from the present study appear to elicit a greater effect, but the dose of RS was greater in the RSB than in their bread. Similar results have also been reported following the consumption of muffins with high B-glucan (up to 2.3 g B-glucan/100g muffin) and high-amylose RS (up to 5.06 g/100 g muffin) as postprandial glucose and insulin decreased in obese and normal weight women [2].

Robertson et al. [11] investigated whether 30 g RS3 consumed daily for four weeks by healthy young adults improved insulin sensitivity. Based on their results, they suggested RS might be a promising nutritional therapy to improve insulin sensitivity. However, they recommended that research using a longer duration and an at-risk population is needed to make a more definitive case for including RS our daily diets.

Reader et al. [10] studied the acute effects of consumption of 50g of carbohydrate from RS containing snack bar, an energy bar, and a popular candy bar on the glycemic and insulinemic response in T2DM volunteers. The snack bars were matched according to their macronutrient. The result of this study showed that both glycemic and insulinemic response improved after consumption the snack bar that contains RS (4.75g/bar).

Lately, health impacts of low GI and/or GL values of foods have been tremendously studied. Low GI foods have been shown to improve glycemic control in diabetic and non-diabetic individuals [3, 15]. According to MaKeown et al. [7] there is a positive association between low GI and insulin sensitivity, and reduction prevalence of metabolic syndrome. Influence of different types of dietary fibers and food processing on the glycemic response was also studied [3]. It was found that cookies and crackers made from enriched whole wheat flour with barley β-glucan have lower GI values than the ones made from whole wheat flour ($p<0.01$), but food processing has no effect on GI. On contrary, food processing has a significant ($p<0.01$) influence on the glucose response curves, but not on the source of dietary fibers. This result emphasizes that food process influence glucose responses.

Several studies suggest that consumption of high GI/GL foods increase the risk of CVD [5, 14]. These studies showed that consumption of low GI foods was associated with decreased levels of blood LDL-cholesterol, C-reactive protein, triglycerides, and adipopectin, and improve HDL-cholesterol.

Other researchers studied the impact of low GI foods on weight loss and satiety. Some studies were be able to show the positive effect of low GI foods on body weight and satiety versus high GI foods [5], but another could not find a relation between low GI foods and body weight loss [1].

Conclusion

With interest in low GI diets for T2DM, weight management and other health outcomes, it is important to obtain more information about glycemic responses of new foods and food components that are produced and sold with the intent of improving health. The results of this study demonstrate that the RSB had a decreased glycemic response compared to the WB and GLUC. It is important to note that the only difference between the two bars was the exchange (by weight) of RS4 for puffed wheat. This is a novel aspect, as others [10] have used completely different ingredients in each bar; thereby, making direct comparisons difficult. The low glycemic response of the present RSB appears to be beneficial for human health and suggests that RS4 is a safe means of improving or controlling blood glucose levels.

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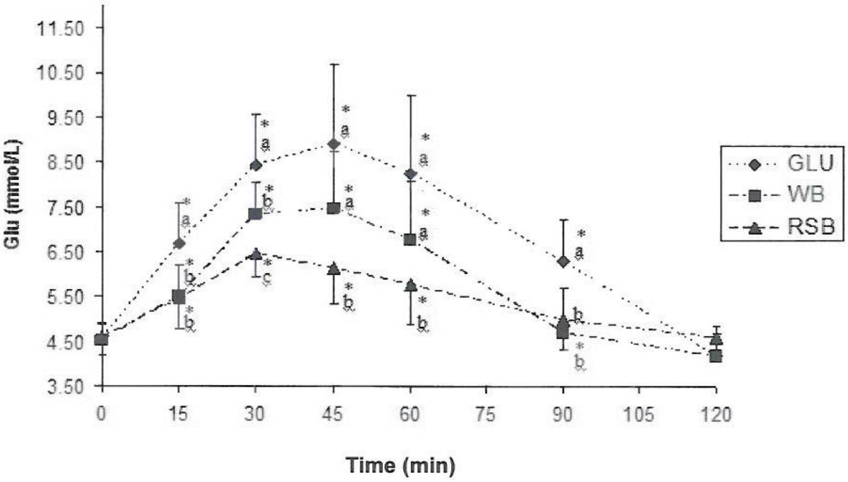

Figure 1: Mean blood glucose response for 9 healthy older adults after consumption the reference drink (GLU; ♦), WB (■), and RSB (▲). Means blood glucose at the same time with different letters differ significantly ($p<0.007$); * =values from the same treatment are significantly different than at pre-ingestion (minute 0) .

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TO: Mark Haub
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Proposal Number: 4076

FROM: Rick Scheidt, Chair
Committee on Research Involving Human Subjects

DATE: November 8, 2006

RE: Approval of Proposal Entitled, "Acute effect of Resistant Starch on Glucose Absorption and Insulin Sensitivity."

The Committee on Research Involving Human Subjects has reviewed your proposal and has granted full approval. **This proposal is approved until** November 8, 2009.

In giving its approval, the Committee has determined that:

- ☒ There is no more than minimal risk to the subjects.
- ☐ There is greater than minimal risk to the subjects.

This approval applies only to the proposal currently on file. Any change affecting human subjects must be approved by the Committee prior to implementation. All approved proposals are subject to continuing review at least annually, which may include the examination of records connected with the project. Announced in-progress reviews will be performed during the course of this approval period by a member of the University Research Compliance Office staff. Injuries or any unanticipated problems involving risk to subjects or to others must be reported immediately to the Chair of the Committee on Research Involving Human Subjects, the University Research Compliance Office, and if appropriate and if the subjects are KSU students, to the Director of the Student Health Center.

When deemed appropriate by the IRB and prior to involving human subjects, properly executed informed consent must be obtained from each subject or from an authorized representative, and documentation of informed consent must be kept on file for at least three years after the project ends. Each subject must be furnished with a copy of the informed consent document for his or her personal records. The identification of particular human subjects in any publication is an invasion of privacy and requires a separately executed informed consent.

It is important that your human subjects project is consistent with submissions to funding/contract entities. It is your responsibility to initiate notification procedures to any funding/contract entity of any changes in your project that affects the use of human subjects.