

Hindawi Publishing Corporation
Journal of Nutrition and Metabolism
Volume 2010, Article ID 651063, 6 pages
doi:10.1155/2010/651063

Clinical Study

Consumption of Cross-Linked Resistant Starch (RS_{4XL}) on Glucose and Insulin Responses in Humans

Enas K. Al-Tamimi,¹ Paul A. Seib,² Brian S. Snyder,¹ and Mark D. Haub¹

¹Department of Human Nutrition, Kansas State University, Manhattan, KS 66506, USA

²Department of Grain Science and Industry, Kansas State University, Manhattan, KS 66506, USA

Correspondence should be addressed to Mark D. Haub, haub@ksu.edu

Received 19 May 2009; Revised 26 June 2009; Accepted 9 July 2009

Academic Editor: Michael Pagliassotti

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Objective. The objective was to compare the postprandial glycemic and insulinemic responses to nutrition bars containing either cross-linked RS type 4 (RS_{4XL}) or standard wheat starch in normoglycemic adults ($n = 13$; age = 27 ± 5 years; BMI = 25 ± 3 kg/m²). **Methods.** Volunteers completed three trials during which they consumed a glucose beverage (GLU), a puffed wheat control bar (PWB), and a bar containing cross-linked RS4 (RS_{4XL}) matched for available carbohydrate content. Serial blood samples were collected over two hours and glucose and insulin concentrations were determined and the incremental area under the curve (iAUC) was calculated. **Results.** The RS_{4XL} peak glucose and insulin concentrations were lower than the GLU and PWB ($P < .05$). The iAUC for glucose and insulin were lower following ingestion of RS4 compared with the GLU and PWB trials. **Conclusions.** These data illustrate, for the first time, that directly substituting standard starch with RS_{4XL}, while matched for available carbohydrates, attenuated postprandial glucose and insulin levels in humans. It remains to be determined whether this response was due to the dietary fiber and/or resistant starch aspects of the RS_{4XL} bar.

1. Introduction

Consumption of whole grains has been recommended to improve insulin sensitivity and lower serum glucose and insulin concentrations. Whole grain consumption of three servings or more per day was among changes that were included in the 2005 dietary guidelines to reduce the risk of acquiring chronic diseases [1]. Whole grains are major sources of dietary fiber (DF), yet typical DF consumption patterns do not meet the recommended 25–35 g per day. Therefore, eating more grain-based fiber-rich foods is warranted to help optimize health and potentially manage some chronic metabolic conditions.

At present, resistant starches (RSs) have drawn broad interest for their health benefits and functional properties [2, 3]. Initial clinical studies demonstrated that RS has properties similar to soluble fiber, shows promising physiological benefits in humans, and may prevent disease. Several potential physiological benefits ascribed to RS include attenuation of blood glucose and insulin levels in both healthy and

diabetic individuals, positive effects on large bowel health and prevention of colonic cancer, increased absorption of minerals, serving as a prebiotic, and increased fat oxidation [3–9]. There are four basic “types” of RS. Type 1 (RS1) is composed of starch granules embedded in indigestible plant material. Type 2 (RS2) is native granular starch with a B-type x-ray pattern, such as found in potato and high-amylose maize. Type 3 (RS3) is crystallized starch and maltodextrins made by alternate cooking/cooling processes on starchy materials. Type 4 (RS4) is chemically modified starch typically through esterification, crosslinking (RS_{4XL}), or transglycosylation.

The majority of human clinical trials have been conducted using only RS2 or RS3, which tend to illustrate decreased blood glucose following consumption of foods with these starches added [3, 6, 10–15]. It is difficult to fully understand the beneficial capacity of RS due to the methods used in the human clinical trials that tested the efficacy of RS. For example, one clinical trial failed to control both the amount and source of all the ingredients [16].

In a study investigating the effects of esterified RS type 4 (RS4_{OSA}), Heacock et al. [17] reported that RS4_{OSA} decreased peak glucose and insulin levels when it was administered in water, but RS is typically consumed in foods. Also, in studies by Behall et al. [18, 19], the amount of available carbohydrate differed, which limits the capacity to determine if the attenuation of glucose and insulin was due to the RS or the fact there was less available carbohydrate. Furthermore, Robertson et al. [6] provided packets containing RS2 for volunteers to sprinkle on their food thereby not illustrating the effects that might be achieved when provided in the food supply. Taken together, the available data illustrate that RS has the potential to lower blood glucose. However, few clinical trials testing the effects of RS have controlled ingredients and the amount of available carbohydrates to better delineate the role of RS in affecting the insulin and glucose responses, and no published clinical trials investigating the glucose lowering potential of RS4_{XL} exist.

Therefore, the aim of this study was to investigate the acute effects of consuming RS4_{XL} incorporated into a nutrition bar, while controlling for nonstarch ingredients and available carbohydrates, on postprandial glucose and insulin responses in young adults with a randomized clinical trial (NCT00687960, clinicaltrials.gov).

2. Materials and Methods

2.1. Subjects. The Institutional Review Board of Kansas State University approved the study, and written informed consent was obtained from all volunteers prior of the study. Inclusion criteria were no diagnosis of acute or chronic metabolic diseases, free of gastrointestinal disorders, body mass index of 23–30 kg/m², and nonsmokers. Volunteers were screened for glucose tolerance using a two hour 75 g glucose tolerance test prior to participation. Eighteen healthy younger adults were recruited and 13 participated in and completed the study, while the other five did not meet the criteria. Of the 13 volunteers (age = 27±5 years, BMI = 25±3 kg/m², HOMA = 0.94±0.34), 7 were females and 6 were males. Based on BMI values, these volunteers all have similar risks for all-cause and obesity-related mortality [20]. Also, an oral glucose tolerance test was performed prior to enrollment to ensure each volunteer had normal glucose tolerance.

2.2. Oral Glucose Tolerance Test. Prior to enrollment, volunteers arrived after a 12 hours overnight fast. Blood samples were drawn by finger stick at baseline and 120 minutes after ingesting 75 g of glucose in solution (296 mL; Sun-Dex 75 g, Fisher Scientific, Houston, Tex, USA). Samples were analyzed in duplicate for glucose concentration (YSI 2300 STAT, Yellow Springs, Ohio, USA). The oral glucose tolerance test was used to confirm the absence of prediabetes or diabetes.

2.3. Study Design. All trials were performed at the Human Metabolism Laboratory at Kansas State University. Volunteers completed three trials via a controlled randomized crossover design. During each trial, volunteers consumed

TABLE 1: Ingredients and their concentrations by relative weight (% total) in the test bars.

	PWB	RS4 _{XL}
Puffed Wheat ^a	34	—
Resistant Starch type 4 ^b	—	34
Corn Syrup ^c	20	20
Wheat Germ ^d	18	18
Brown Sugar ^e	11	11
Water ^f	10	10
Gum Acacia ^g	6	6
Panodan 150K ^h	1	1

^a Quaker Oats

^b Fibersym RW; MGP Ingredients, Inc.

^c Karo light corn syrup

^d Kretschmer Original Toasted

^e C&H Pure Cane Sugar, golden brown

^f Tap water (Manhattan, Kan)

^g TIC Gums

^h Danisco

one of the following: dextrose solution (198 mL of a standard 75 g oral glucose tolerance beverage; GLU), a control bar containing puffed wheat (65 g; PWB), and a bar containing cross-linked RS4_{XL} bar (80 g; RS4_{XL}) (Table 1). All treatments were designed to provide 50 g of available carbohydrate (Table 2). All trials were completed by each volunteer with at least a seven-day washout between testing days. This was a quasiblinded experiment in that one treatment was a beverage and the other two were in bar form, which were randomly administered using a Latin Square design. Some female volunteers did not use oral contraceptives, but others used either contraceptive pills or progesterone injections. Regardless, the females were scheduled to perform all the trials during the follicular phase of their menstrual cycle and each served as their own control.

2.4. Experimental Bars. The only altered ingredients between bars were either puffed wheat or RS4_{XL} (Table 1). Briefly, the nutrition bars were prepared by adding puffed wheat or cross-linked RS4 to wheat germ. The dose of the RS4_{XL} (27.2 g) was intended to be close to the dose used previously [6], whereby a significant improvement in insulin sensitivity was observed. The remaining ingredients (water, corn syrup, brown sugar, gum acacia, and Panodan 150 K) were heated to 85°C over 4 minutes, poured over the dry ingredients, and then manually mixed quickly until dry ingredients were evenly distributed throughout the mixture. The mixture was scooped into a metal pan, pressed evenly throughout the pan, and allowed to cool for 20 minutes before cutting into bars. Crude nutrient analysis was determined by proximate analysis, while total dietary fiber was assessed independently (Medallion Labs, Minneapolis, Minn) (Table 2). Available carbohydrate was calculated as the difference between total carbohydrate and dietary fiber as used previously [9]. Controlling for available carbohydrate in this fashion has been shown to affect the glycemic response, while controlling for RS content does not necessarily affect the glycemic response [21].

TABLE 2: Nutrient composition of each treatment per dose (GLU = 198 mL; PWB = 65 g; RS4 = 80 g).

	GLU ^a	PWB ^b	RS4 _{XL} ^b	Δ^c
Total Energy (kcal)	200	261	326	(65 kcal, 125%)
Carbohydrate (g)				
Total	50	56	71	(15 g, 127%)
Available ^c	50	51	51	(0 g, 0%)
Total Dietary Fiber (g) ^d	—	5	20	(15 g, 400%)
Fat (g)	—	1	2	(1 g, 200%)
Protein (g)	—	7	6	(1 g, 86%)

^a glucose tolerance test beverage (Sun-Dex, Fisher Scientific, Houston, Tex)

^b Crude nutrient composition was determined by proximate analysis (total energy, total fat, total protein, total carbohydrate).

^c derived by subtracting total dietary fiber from total carbohydrate.

^d dietary fiber analysis was performed by Medallion Laboratory (Minneapolis, Minn).

^e difference (subtraction value, % value) between bars.

2.5. Food Tolerance Test. The postprandial test (two-hours with seven blood samples) was modified from Flammang et al. [22]. During each test, volunteers arrived to the laboratory after a 10–12 hour overnight fast. An indwelling catheter (Terumo, 22gx1, Terumo Medical Corporation, Elekton, Md) was inserted into a forearm vein. The line was kept patent with 0.9% isotonic saline solution (0.9% sodium chloride USP, B. Braun Medical Inc., Irvine, Calif). Ten minutes after inserting the IV catheter, the fasting blood sample was collected. Thereafter, the volunteers consumed the assigned food item for that day. The solution (GLU) or bar was consumed within 10 minutes. The treatment bars were served with 198 mL of water (to match the fluid that was consumed during the GLU trial). Relative to taking the first bite of food, blood samples were collected at –10, 10, 20, 30, 60, 90, and 120 minutes.

2.6. Blood Samples. After collection, blood samples were centrifuged at 2,500 rpm for 15 minutes at 4°C. The plasma was extracted from the vacutainer and immediately analyzed in duplicate for glucose (YSI 2300, Yellow Springs Instruments, Yellow Springs, Ohio) with the remainder stored at –80°C. The frozen samples were analyzed in duplicate for insulin using an endocrine assay (LINCoplex kit, St. Charles, Mo), and measured by Luminex100 (Austin, Tex) instrumentation. Once the samples were analyzed, the highest value attained over the 120 minutes was determined to be the peak value, with the difference between baseline and peak determined to measure the change from fasting to peak.

Glucose and insulin areas under the curve (iAUC) were determined using the trapezoid method (GraphPad Prism v 5.02, La Jolla, Calif). This approach was adapted from a previous RS feeding study [18]. Fasting insulin and glucose values were also used to calculate the homeostasis model assessment (HOMA) [23].

2.7. Diet and Physical Activity Records. A diet record was completed by the volunteers prior the first test of the study. Volunteers were instructed on how to record their intakes.

They were requested to then eat the same foods from that diet recorded the day before the second and third trials. This procedure was used previously [24]. Also, volunteers were requested to record their physical activity for the day before testing and perform that same activity (or inactivity) the day prior to the subsequent tests.

3. Data Analysis

Sample size estimation was calculated using PASS software (NCSS 2007 and PASS 2005, Kaysville, Utah). Based on results from previously published data using RS2 [6], six volunteers were determined necessary (power > 0.80, and $P < .05$) to detect significant differences in glucose and insulin responses. A repeated measures analysis of variance (SPSS version 11.5, Chicago, Ill) was used to determine significant main effects with significance set at $P = .05$. Paired t -tests (SPSS version 11.5, Chicago, Ill) were used to determine differences between and among trials for peak, change from baseline to peak and iAUC values for glucose and insulin. The comparisons of interest were primarily between the two bars, with the glucose treatment providing a standard point of reference.

4. Results

The three meals provided practically the same amount (50–51 g) of available carbohydrate (Table 2). Both nutrition bars contained similar amounts of protein and were low in fat, but the RS4_{XL} bar contained four times the dietary fiber (20 g versus 5 g) compared to the PWB. Approximately one-fifth of the total dietary fiber in the RS4_{XL} bar was from gum arabic (~4.4 g) with the remainder (~15.6 g) being from RS4_{XL}. The meals differed in their calculated food-energy contents (200–326 kcal, Table 2) because their compositions differed and because their weights to deliver 50 g of available carbohydrates differed.

The commercially available RS4_{XL} used in this study contains 0.4% phosphors, 10.6% moisture, 91.9% total dietary fiber by AOAC-International Method 991.43, and 83.3% RS by a modified Englyst method [25]. At the time of preparing the 80 g bar of RS4_{XL}, the amount of RS added was ~20 g (dry solids) according to the formula in Table 1 and the composition of Fibersym RW, while the amount of gum arabic added was 4.4 g (dry solids). At the time of analyzing the finished 80 g bar, the bar contained 20 g of total dietary fiber (Table 2). Assuming no loss of gum arabic in the preparation of the RS4_{XL} bar, the 4.4 g loss of dietary fiber could be attributed to some damage to the RS, which increased its digestibility. Assuming the ratio of RS to dietary fiber remains constant at 0.9 in partially damaged RS4_{XL}, then the 80 g bar of RS4_{XL} fed to the subjects contained ~14 g RS, implying ~70% was maintained following preparation of the bar.

The RS4 bar elicited decreased glucose and insulin concentrations at several time points (Figure 1) during the 120 minutes postprandial period as compared with GLU and PWB. Also, consumption of the RS4 bar led to an attenuation

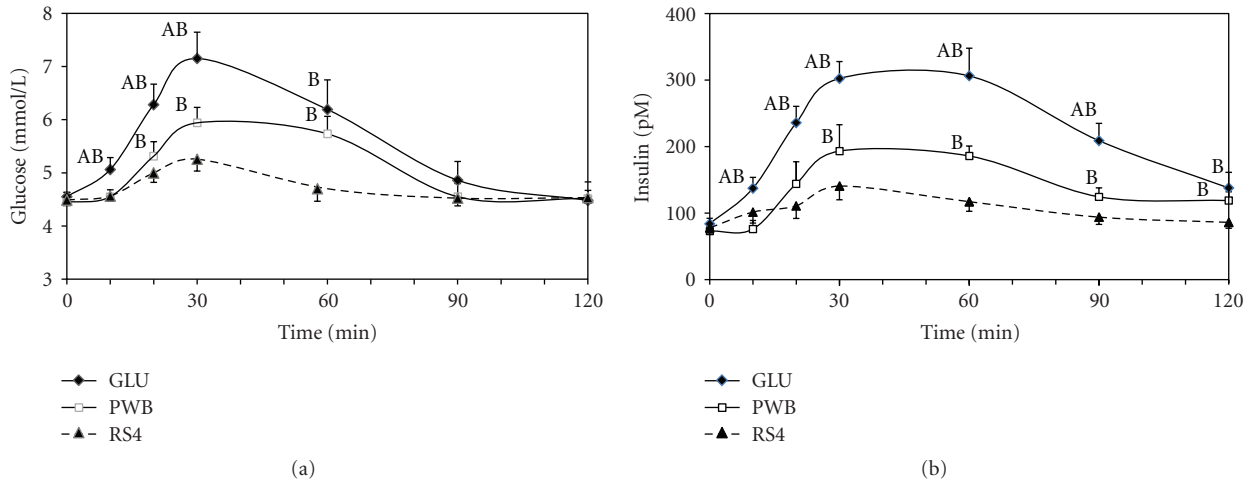


FIGURE 1: Depiction the glucose (a) and insulin (b) responses over two hours following the consumption of each (Glu, PWB, and RS4) treatment. Values represent each mean \pm SE. A: significant difference with PWB; B: significant difference with RS4.

of peak glucose and insulin, and significant differences from baseline to peak and iAUC values glucose and insulin when compared with GLU and PWB (Table 3). The PWB bar attenuated the peak glucose and insulin responses and the iAUC for glucose and insulin compared with GLU. The percent increase from baseline to peak was not different ($P = .068$) from GLU for glucose, while it was for insulin.

5. Discussion

These data, for the first time, indicate that eating RS4_{XL} from wheat in place of standard wheat starch significantly decreased postprandial insulin and glucose responses. These results are in line with others investigating the insulin and/or glucose lowering effects when RS (typically RS2) is added to foods or incorporated in the diet [6, 16, 18, 26, 27], while a few reported no effect of RS2 or RS3 on glycemia [7, 9]. Results from several other clinical trials reported RS decreased the glycemic response, but those studies had volunteers sprinkle RS onto the food instead of it being an ingredient in the food, mixed only with water, ate large (up to 388 g) portion sizes, failed to control for available carbohydrate, and/or the food eaten contained different ingredients with varying amounts of available carbohydrate [6, 16, 18, 26, 27].

One of the more surprising outcomes was that RS4_{XL} significantly attenuated the glycemic and insulinemic responses even when high glycemic ingredients were eaten. This observation is critical for consumers and food scientists when looking for or creating foods to control blood glucose levels in that they may not need to avoid foods containing corn syrup or sugar for the purpose of regulating their blood glucose levels. That is, these data clearly indicate that even though two foods contain identical concentrations of glycemic ingredients, the presence of RS4_{XL} may significantly lower glucose and insulin responses. In the case of the bars that were tested, the volunteers actually consumed more corn syrup and brown sugar in the RS4_{XL} bar since they consumed

15 g more of that bar than the PWB bar. Yet, the glycemic and insulinemic responses for the RS4_{XL} bar were significantly attenuated compared with the PWB bar that was matched for available carbohydrate yet contained less (by weight) sugar and corn syrup (Table 3). This does not imply that eating sugar and corn syrup is healthy, but that foods high in RS4_{XL} attenuate glycemia and insulinemia compared with those with standard whole wheat starch when sugar and corn syrup are present and account for \sim 30% of the energy. This is of interest as sugar and corn syrup have been the target of criticism for potentially contributing to the obesity and diabetes epidemics [28–30].

The observed attenuation of the glucose and insulin responses might be a result of high concentrations of DF and/or RS in the RS4_{XL} bar. In separate in vitro analyses for DF and RS, this version of cross-linked RS4 has been shown to contain 91.9% dietary fiber and 83% resistant starch [25]. Relative to clinical trials that used RS2, Le Leu et al. [31] recently reported that several varieties of RS2 only contain 18–60% dietary fiber and 46–53% RS. Thus, this cross-linked form of RS4 likely elicited the significant difference due to a relatively high content of fiber and RS.

This study is not without limitations. While we did observe a significant attenuation in glucose and insulin responses when RS4_{XL} was incorporated into the bar, it is not possible to know whether the same effects would occur in individuals with insulin resistance or other metabolic conditions. It could be suggested that the current dose used for the RS4_{XL} bar (80 g) might be greater than one would choose to consume at one sitting. A more realistic approach might be to base the doses on average serving sizes, but that would only offer 15–20 g of available carbohydrate in the RS4_{XL} bar. That said a previous study [26] had volunteers consume treatment foods that were nearly five-fold greater. Likewise, we were trying to match the amount of available carbohydrate at 50 g, which is recommended for glycemic index testing [32]. Another potential confounding issue, especially when comparing the bars with the solution, is the difference in gastric emptying [33]. Also, the total energy

TABLE 3: Values for the incremental areas under the curves of glucose and insulin concentrations during each trial. Mean \pm SE; different letters within a row indicates significant difference ($P < .05$).

	GLUC	PWB	RS4 _{XL}
Glucose			
iAUC (mmol/L • 2 hr)	140 \pm 31 ^A	84 \pm 17 ^B	28 \pm 11 ^C
Peak (mmol/L)	7.30 \pm 0.5 ^A	6.33 \pm 0.3 ^B	5.40 \pm 0.2 ^C
Increase (%)	60.5 \pm 10 ^A	42.7 \pm 6 ^A	20.4 \pm 3 ^B
iAUC (pM • 2 hr)	17,575 \pm 2,236 ^A	8,758 \pm 1,132 ^B	3,659 \pm 974 ^C
Peak (pM)	344 \pm 36.7 ^A	211.5 \pm 20.1 ^B	162.3 \pm 22.6 ^C
Increase (%)	335 \pm 53.2 ^A	243.0 \pm 49.3 ^B	126.3 \pm 45.8 ^C

contained within the RS4_{XL} bar did not take into account the energy provided via fermentation of the RS_{XL} into short chain fatty acids. Lastly, we cannot determine the mechanism for the effect observed. It is reasonable to conclude that the RS4_{XL} caused the effects, as it was the only ingredient difference, but it is not possible to determine how much of the change was attributable to the dietary fiber or the RS that is contained within RS4_{XL}.

In conclusion, this is the first published randomized clinical trial to investigate the glucose and insulin lowering potential of RS4_{XL}. Additionally, this is one of a few clinical studies where the treatments were matched for available carbohydrate and the RS was substituted directly for standard starch in the tested food, while all other ingredients were identical. This ingredient and nutrient control minimizes confounding factors that are present when treatments are matched instead for total carbohydrate [18] or when different ingredients are used in clinical trials [16]. While it is unknown what component (dietary fiber or resistant starch) of RS4_{XL} lowered glucose and insulin, it is clear that RS4_{XL} was responsible for the observed differences between bars.

Abbreviations

DF:	Dietary fiber
GLU:	Glucose control solution
iAUC:	Incremental area under the curve
HOMA:	Homeostasis Model Assessment of insulin sensitivity
PWB:	Control bar made with puffed wheat
RS:	Resistant starch
RS4 _{XL} :	Treatment bar made with cross-linked resistant starch type 4.

Acknowledgments

Support for the completion of the project was provided by the American Heart Association (Beginning Grant-in-Aid, 0465357Z), the United States Department of Agriculture (KS347), and MGP Ingredients, Inc. Contribution no. 09-266-J from the Kansas Agricultural Experiment Station. Financial Interests: Dr. Seib invented RS4_{XL} and has been a Consultant with MGP Ingredients, Inc. for 20 years. MGP Ingredients, Inc. helped support the study by providing RS4_{XL} and funding part of the data collection.

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