ORIGINAL RESEARCH

Protein, Carbohydrate, and Fat Metabolism

CURRENT DEVELOPMENTS IN NUTRITION

Metabolic Responses to Native Wheat Starch (Midsol™ 50) versus Resistant Wheat Starch Type 4 (Fibersym® RW): Standard versus Marketplace Testing Protocols

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ABSTRACT

Background: To investigate the effect of resistant starch (RS) on acute glycemic or insulinemic responses, the FDA indicates that control and RS-enriched foods must contain equivalent amounts of digestible carbohydrate. However, RS-containing foods typically contain less digestible carbohydrate per serving than control foods. Thus, controlling for digestible carbohydrate may yield different responses as compared with controlling for serving size.

Objective: The aim was to compare the postprandial metabolic responses to native wheat starch (NWS) versus RS type 4 (RS4) using digestible carbohydrate–matched portions compared with weight-matched portions.

Methods: A single-blind, randomized-controlled crossover trial examined glycemic and insulinemic responses over 2 h following consumption of 4 cracker conditions and a dextrose beverage in apparently healthy participants ($n = 14$). Crackers provided 50 g of digestible carbohydrate using the FDA's meal-intervention protocol or 35 g of carbohydrate by weight for the marketplace substitution method. Crackers differed only by the type of starch additive: NWS (Midsol™ 50; MGP Ingredient, Inc.) or RS4 (Fibersym® RW; MGP Ingredients, Inc.). Glucose concentrations were assessed at baseline and at 15, 30, 45, 60, 90, and 120 min; insulin concentrations were measured at baseline and 30, 60, and 120 min.

Results: There were no significant differences between 50 g digestible carbohydrate cracker conditions for glucose or insulin incremental AUC (iAUC). The 35 g carbohydrate by weight conditions were not different for glucose iAUC [mean (95% CI): 35 g NWS: 1317 (677, 2169); 35 g RS4: 701 (262, 1351); $P > 0.05$]. However, insulin iAUC was lower following 35 g RS4 compared with 35 g NWS [35 g RS4: 92 (1, 259); 35 g NWS: 697 (397, 1080); $P < 0.01$].

Conclusions: In healthy adults, consumption of RS4 crackers decreased postprandial insulin responses compared with NWS crackers when using the marketplace substitution method compared with the FDA standard testing method, with similar postprandial glucose responses. Comparisons of the FDA standard testing method and the marketplace substitution method should be investigated further to elucidate differential physiological impacts on consumers. *Curr Dev Nutr* 2021;5:nzab011.

Keywords: resistant starch type 4, RS4, postprandial glycemia, postprandial insulinemia, fiber, substitution method, FDA standard testing method

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Abbreviations used: iAUC, incremental AUC; IPAQ-SF, International Physical Activity Questionnaire–Short Form; NWS, native wheat starch; RS, resistant starch; RS4, resistant starch type 4; 35RS4, 35 g of RS4 crackers by weight; 35NWS, 35 g of NWS crackers by weight; 50Dex, 50 g dextrose beverage; 50NWS, 50 g of digestible carbohydrate from NWS crackers; 50RS4, 50 g of digestible carbohydrate from RS4 and NWS crackers.

Introduction

Low consumption of dietary fiber, which is principally indigestible carbohydrate, is associated with reduced insulin sensitivity and increased risk for type 2 diabetes (1). The consumption of dietary fiber has shown promising outcomes associated with the reduction of risk for metabolic disease, including improving metabolic response outcomes, promoting satiety, and for obesity prevention and treatment (2). While numerous studies have shown the beneficial health outcomes associated with dietary fiber consumption, national consumption remains at ∼50% of the recommended dietary intake (3–5). Resistant starch (RS) type 4 (RS4) has recently emerged as a fiber (6) and has shown beneficial health outcomes in the postprandial period following acute consumption when substituted for flour in food (7–9, 10, 11–13). However, there is

limited evidence to show an effect of RS4 on glycemic and insulinemic responses when the test and control foods are matched for digestible carbohydrate.

The FDA maintains 2 methods of acceptable protocols to test a fiber ingredient for food-labeling purposes. The test ingredient should either be added on top of all ingredients or substituted in place of an ingredient, while matching for digestible carbohydrates between the test and control foods (14). In contrast to the FDA protocol, the marketplace substitution method simply replaces an ingredient with the fiber. Fiber-fortified foods in the marketplace are not formulated to match the digestible carbohydrate of the original products; rather, the fiber ingredients replace digestible carbohydrates. This typically reduces the total caloric and digestible carbohydrate content of a product, while increasing the fiber content.

The FDA standard testing method often increases the total caloric content of a test food or requires large amounts of that food to be consumed during testing sessions. In comparison, using the marketplace substitution method can decrease the total caloric content for a test food when digestible carbohydrates are not matched. These methods are important for the food industry, as any food that has shown a beneficial health outcome in accordance with FDA regulations can be labeled with a health claim (15). These health claims are tightly regulated by the FDA as a single health claim on a food product has been shown to increase sales substantially (16). Therefore, it is important to compare FDA standard testing protocols with marketplace substitution methods when investigating metabolic responses to different food products to determine differences between testing methods on health outcomes.

The primary aim of this study was to investigate the metabolic responses, specifically glucose and insulin incremental AUC (iAUC), following consumption of 50 g of digestible carbohydrate from native wheat starch (NWS) crackers (Midsol™ 50; MGP Ingredients, Inc.) and RS4 crackers (Fibersym® RW; MGP Ingredients, Inc.). The secondary aim of this study was to investigate the metabolic responses to both crackers using the FDA standard testing protocol, where both crackers were matched for digestible carbohydrate, as well as the marketplace substitution method where RS4 was substituted in place of digestible carbohydrate. The marketplace substitution method was investigated using 35 g of crackers on a final product weight basis, representing an amount that is typically consumed according to the FDA (17). We hypothesized that the Fibersym® RW cracker would elicit a reduced glucose and insulin response compared with the NWS crackers at both the 50-g digestible carbohydrate dose and the 35-g-by-weight condition.

Methods

Participants

Fourteen apparently healthy participants (ages 20–38 y) with no history of diagnosed health conditions completed this study. Participants completed a medical history questionnaire to determine eligibility in the study. Participants were excluded from participation if they had a baseline fasting blood glucose ≥100 mg/dL, consumed a diet high in dietary fiber (>50 g/d), were current smokers or smoked within the last 6 mo, were pregnant or lactating, had an allergy to wheat or gluten, or had any diagnosed health conditions that may affect metabolism. Written and oral consent were obtained from all participants. This study was approved by the Institutional Review Board for Research Involving Human Subjects at Kansas State University (IRB #8740) and conformed to the Declaration of Helsinki.

Experimental design

This study was conducted as a single-blind, randomized-controlled crossover trial, where all participants underwent all testing conditions. Participants were randomly assigned to a series of 5 randomly ordered conditions, blocked in a Latin-square design. The conditions were as follows: *1*) 50 g dextrose beverage (50Dex; Trutol® 50 glucose-tolerance beverage; Thermo Scientific™), *2*) 50 g of digestible carbohydrate from NWS crackers (50NWS), *3*) 50 g of digestible carbohydrate from RS4 and NWS crackers (50RS4), *4*) 35 g of NWS crackers by weight (35NWS), and *5*) 35 g of RS4 crackers by weight (35RS4). Further details for the cracker conditions are outlined in **Table 1**. All testing sessions were performed at the Physical Activity and Nutrition Clinical Research Consortium at Kansas State University, Manhattan, Kansas. All participants completed all 5 conditions, each following a 10–12-h fast, with a minimum of 48 h between sessions. To ensure consistency in responses, participants were asked to maintain their typical dietary and physical activity habits throughout the study. Additionally, participants were asked to write down what they ate the night prior to their first testing session and were reminded to consume this same meal prior to subsequent testing sessions. Inclusion criteria included the following: *1*) apparently healthy adults with no diagnosed health conditions, *2*) nonsmokers, *3*) not consuming a high- or low-fiber diet (>50 g/d or <5 g/d), and *4*) lack of a wheat or gluten allergy. Participants who did not meet all inclusion criteria were excluded from participation. Participants who met the inclusion criteria at the first visit were enrolled in the study and subsequently completed pretrial assessments of height, weight, and waist circumference, followed by an oral-glucose-tolerance test protocol. Next, participants completed a postprandial assessment for 1 of the 5 randomized conditions. The remaining 4 visits were performed using the postprandial assessment only. Satiety was measured via the Holt Satiety Questionnaire at baseline and at 30, 60, 90, and 120 min during each testing session, while adding a DXA scan at the final testing session to measure body composition.

Postprandial assessment protocol

Upon enrollment into the study, and completion of pretrial measurements, at each session an indwelling catheter was inserted in the antecubital vein in the forearm by a researcher trained in phlebotomy. A 24-gauge safelet intravenous catheter (Exel International) was used to maintain an open port throughout the 2-h testing session. A steady infusion of 0.9% NaCl was used to maintain catheter function and was fixed in place via Tegaderm film (3M Healthcare). A 3-mL syringe (BD), attached to a 3-way stopcock (Fisher), was used to clear the intravenous line of saline prior to whole-blood collection. A 5-mL syringe (BD) was then used to collect whole blood at each time point. Once the baseline blood sample was drawn, participants were instructed to consume their cracker condition or glucose tolerance beverage within a 15-min period. The 2-h postprandial assessment started upon completion of the final swallow for every condition to minimize the differences in consumption time and to add control to the start time among all participants. Approximately 500 mL of water was provided

TABLE 1 Nutrient composition of each condition (per 100 g)[1]

	50NWS	50RS4	35NWS	35RS4
Ash, g	1.0	1.7	0.5	0.7
Moisture, g	3.4	4.2	1.7	1.2
Carbohydrate, g	50.3	71.3	25.8	26.1
Digestible carbohydrate, g	50.0	50.0	25.6	5.1
Dietary fiber, g	0.3	21.3	0.2	21.0
Fat, g	7.6	10.8	3.9	4.0
Protein, g	6.0	8.5	3.1	3.1
Total calories, kcal	292.0	331.4	149.6	69.5
Final product weights provided to participants, g	68.3	35.0 RS4 + 61.3 NWS (96.3 total)	35.0	35.0

[1]Digestible carbohydrate was calculated as carbohydrate (g) minus dietary fiber (g). NWS, native wheat starch; RS, resistant starch; 50NWS, 68.33 g native wheat starch cracker (delivering 50 g digestible carbohydrate); 50RS4, 61.27 g resistant starch type 4 + native wheat starch crackers (delivering 50 g digestible carbohydrate). Marketplace substitution conditions: 35NWS, 35 g native wheat starch cracker; 35RS4, 35 g resistant starch type 4 cracker.

at each session and was kept constant throughout the duration of the study.

Analytical procedures

To determine blood glucose, blood samples were taken at baseline and at 15, 30, 45, 60, 90, and 120 min following consumption of the randomized condition. To determine insulin, samples were taken at baseline and at 30, 60, and 120 min. Whole-blood samples were drawn into a 5-mL syringe (BD). A small sample of whole blood was expelled from the 5-mL syringe and whole-blood glucose was measured using a Bayer Contour Glucose Monitoring System. Samples were measured in duplicate or until 2 readings from the glucose monitoring system were within 5 units of agreement (milligrams per deciliter). The final value used for analysis was the mean of 2 measurements that were within 5 units of agreement. Upon completion of the glucose measurement, the remaining whole blood was expelled into a 6-mL Vacutainer test tube coated with EDTA (BD). Blood samples were centrifuged at 1800 × *g* at room temperature for 12 min. The plasma was then pipetted into 3 aliquots in 0.6-mL microcentrifuge tubes (Fisher) and stored at −80°C until study completion (~6 mo). Upon completion of data collection, 1 aliquot of plasma was shipped to the Radioimmunoassay and Biomarkers Core of the University of Pennsylvania Diabetes Research Center to determine plasma insulin using double-antibody radioimmunoassay in duplicate (EMD Millipore, Billerica, MA). The insulin intra-assay CV was 4.99% and inter-assay CV was 11.3%. The remaining 2 aliquots of plasma, intended for insulin analysis, were stored in case of shipment failure or in cases where analysis of samples yielded errors. Where catheter insertion was not possible, or upon failure of the intravenous line once inserted, blood glucose was collected via finger stick, whereas insulin was not collected. Among all instances where catheter insertion was not possible, participants refused additional catheter insertions primarily due to needle discomfort. Blood glucose was not used for data analysis in these situations, as it was determined that these methods were statistically different. Therefore, there were missing data for participants on days where catheter failure occurred.

Analytic parameters

Peak parameters (milligrams per deciliter) were defined as the highest concentration of glucose or insulin observed during the 2-h testing session. Baseline-to-peak (milligrams per deciliter) was defined as the difference between the peak value and the baseline value the participant had at the start of the testing session. Time-to-peak (minutes) was defined as the amount of time it took for the participant to reach the peak value observed during the testing session.

The estimated means and CIs from the models are reported in the text and iAUC, peak, baseline-to-peak, and time-to-peak were analyzed for glucose and insulin. A final sample size of $n = 13$ was determined to be sufficient to detect effects based on data from Al-Tamimi et al. (13). Estimated values were converted back to the original units using the emmeans package in R (R Foundation for Statistical Computing), and raw data are graphed (**Figure 1**) to assist with interpretability.

Body-composition measures and questionnaires

Height, weight, and waist circumference measures were collected in duplicate and the mean values were used for data analysis. Height was measured to the nearest 0.1 cm using a portable stadiometer (Invicta Plastics). Participants were measured with heels, buttocks, and shoulders touching the flat upright surface. Weight was measured to the nearest 0.1 kg with an electronic scale (Pelstar LLC). Waist circumference was measured to the nearest 0.1 cm using a tape measure by locating the top of the iliac crest. Measurements were taken at the end of the participant's normal exhalation. DXA was used to determine body composition (GE Prodigy; Lunar-General Electric). Participants were asked to take jewelry, shoes, and anything with metal off before lying in a supine position for ~6–10 min for the DXA scan. Satiety was measured throughout each trial using the Holt Satiety Scale. This 7-point visual analog scale has equally spaced options that include extremely hungry, hungry, semi-hungry, no particular feeling, semi-satisfied, satisfied, and extremely satisfied. The Holt Satiety Scale was previously used to determine the satiety index of common foods and used in this study to measure satiety to the cracker conditions (18). The International Physical Activity Questionnaire–Short Form (IPAQ-SF) was used to assess physical activity levels for each participant. This questionnaire covers 4 domains of physical activity, including work-related, transportation, household/gardening, and leisure-time physical activity. The questions include frequency factors, number of days per week, and time conducting various activities including sitting, walking, and moderate- and vigorous-intensity physical activity. The primary use of the IPAQ-SF in this study was to determine whether physical activity levels changed during the study.

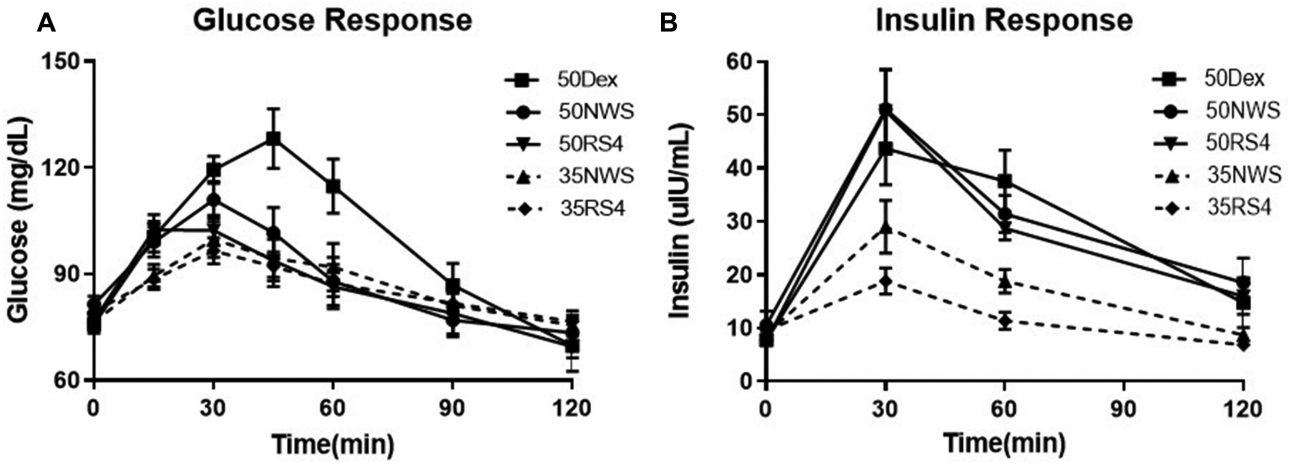

FIGURE 1 Postprandial glycemic and insulinemic responses to dextrose and all cracker conditions during the 2-h test period. Values are means ± SEs. (A) Glucose response (mg/dL). (B) Insulin response (μIU/mL). FDA standard testing conditions: 50Dex, 50 g dextrose; 50NWS, 68.33 g native wheat starch cracker (delivering 50 g digestible carbohydrate); 50RS4, 61.27 g resistant starch type 4 + native wheat starch crackers (delivering 50 g digestible carbohydrate). Marketplace substitution conditions: 35NWS, 35 g native wheat starch cracker; 35RS4, 35 g resistant starch type 4 cracker.

Dextrose beverage and cracker details

The glucose-tolerance test beverage provided 50 g of dextrose (catalog number 401074P; ThermoFischer Scientific). The crackers were produced locally at the American Institute of Baking (Manhattan, KS). The 4 cracker conditions were made using the same ingredients other than the type of starch. The RS4 cracker was made by replacing NWS in a cracker with a type 4–resistant wheat starch, whereas the NWS cracker was made solely from NWS, all the while maintaining protein content constant by adding wheat gluten. The 50NWS cracker condition was tested using 68.33 g NWS providing 50 g of digestible carbohydrate from NWS. The 50RS4 cracker condition was tested using a combination of 35 g RS4 + 61.27 g NWS providing 50 g of digestible carbohydrate. The 35NWS cracker was tested as 35 g of NWS cracker by final product weight, and 35RS4 was tested using 35 g of RS4 cracker by final product weight. All ingredients were food grade and generally recognized as safe (GRAS). Both the NWS (Midsol^{TM} 50) and RS (Fibersym$^{®}$ RW) were provided by MGP Ingredients, Inc. (Atchison, KS), with food-grade documentation (Table 1). Finished NWS and RS4 crackers were nearly identical and could only be distinguished by the label on their designated package.

Statistical analysis

Data analyses were conducted in R, version 3.5.3 (19). Repeated-measures mixed-effects regression models were conducted for each of the outcome variables to account for missing data. Primary outcomes were glucose iAUC and insulin iAUC. Secondary outcomes were glucose and insulin peak, baseline-to-peak, and time-to-peak. Main effects of condition, dose, and condition × dose were included as fixed effects in all primary and secondary analyses. Condition (NWS, RS4) and dose (50 g digestible carbohydrate, 35 g by weight) were effect coded. Subject was included as a random effect to account for individual differences. Two groups of analyses were used to determine effects of condition and dose. Experimental conditions were analyzed in groups based on the method of preparation. The first analysis included the FDA standard testing conditions (50Dex, 50NWS, 50RS4). The second analysis included the marketplace substitution method conditions (35NWS, 35RS4) and the 50-g cracker conditions (50NWS, 50RS4). To determine the effect of condition, 50Dex, 50NWS, and 50RS4 were analyzed together as these conditions were matched for digestible carbohydrate. To determine effects of dose, 50NWS, 50RS4, 35NWS, and 35RS4 were analyzed together and 50Dex was excluded from this analysis because there was not a low dose of dextrose used as a condition in this study. Post hoc comparisons were conducted for significant effects using the emmeans package in R. Outcome variables were transformed for normality according to Box Cox analyses using the MASS package in R. Specifically, a Box Cox analysis was conducted which informed the type of transformation that should be used to transform each variable. Following the transformation of data, a histogram and residual plot were generated to verify the normal distribution of data. If >1 transformation was identified using the Box Cox analysis, the transformation used was aligned according to similar variables. For example, if glucose iAUC was transformed via a square-root transformation and the Box Cox for insulin iAUC showed a log and square-root transformation as potential options, both transformations were performed and histograms and residual plots were generated to observe the normal distribution of data. If one of the transformations appeared drastically in favor of another potential transformation, then that transformation was used. However, if both transformations yielded a similar distribution of data, the square root transformation was used to align the insulin iAUC data with the glucose iAUC transformation. Glucose and insulin iAUCs were obtained using GraphPad Prism version 8.1.0 with the trapezoidal method (GraphPad Software, Inc.). Statistical significance was set at $P < 0.05$. Glucose peak, insulin peak, and insulin baseline-to-peak were log-transformed. Glucose iAUC, insulin iAUC, glucose baseline-to-peak, and insulin time-to-peak were square-root transformed.

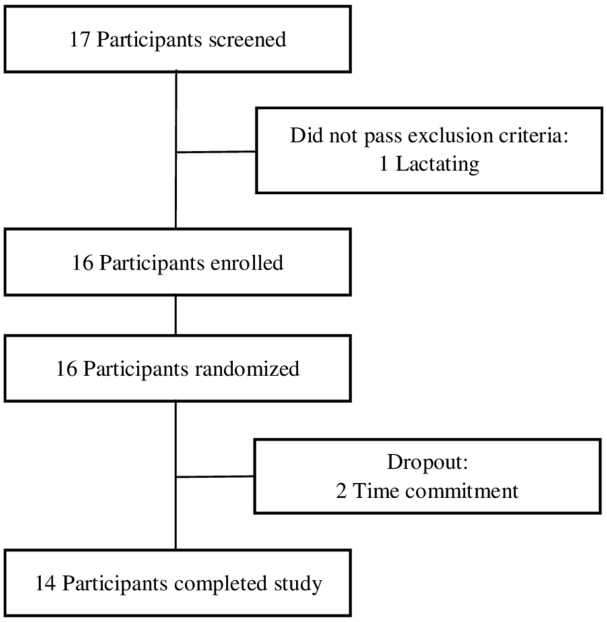

FIGURE 2 CONSORT diagram. CONSORT, Consolidated Standards of Reporting Trials.

Missing values for glucose and insulin were due to intravenous catheter failure with participant refusal for placement of a new catheter and processing issues with insulin analysis of samples. All data collected via the catheter, during visits with catheter failure, were included in data analysis if they had passed a presumed peak following the 1-h time point.

Results

Fourteen apparently healthy adults (9 males, 5 females) between the ages of 20 and 38 y completed the study, with 1 participant withdrawing following the second testing session (**Figure 2**). Demographic, anthropometric, baseline glucose, and baseline insulin results are shown in **Table 2**. Seven subjects were classified as overweight or obese [BMI (kg/m^2) ≥24.9].

TABLE 2 Baseline participant characteristics of individuals who completed the study[1]

	All (n = 14)
Sex (M:F), n	9:5
Age, y	24.6 ± 4.7
Height, cm	175.5 ± 8.8
Weight, kg	76.6 ± 16.8
Waist circumference, cm	87.2 ± 11.0
BMI, kg/m^2	24.8 ± 10.1
Body fat (DXA), %	19.3 ± 10.1
Fasting glucose, mg/dL	79.3 ± 9.1
Fasting insulin, μIU/mL	1.1 ± 0.6

[1]Values are means ± SDs unless otherwise indicated.

Comparison of 50-g FDA standard testing method conditions

When comparing the FDA standard testing protocol 50-g conditions (50Dex, 50NWS, 50RS4) matched for digestible carbohydrate, consumption of both the 50NWS and 50RS4 crackers resulted in a lower glucose iAUC, glucose peak, and glucose baseline-to-peak when compared with 50Dex (all $P < 0.001$) (Figure 1 and **Table 3**). Glucose iAUC was ∼58% lower, glucose peak was ∼15% lower, and glucose baseline-to-peak was ∼45% lower for both cracker conditions when compared with 50Dex. However, no statistically significant differences were observed between the 50NWS and 50RS4 crackers (all $P > 0.05$). No differences were observed for glucose time-to-peak between 50-g conditions (all $P > 0.05$). No differences were observed between 50-g conditions for insulin iAUC, insulin peak, insulin baseline-to-peak, or insulin time-to-peak (all $P > 0.05$). Results for 50-g comparisons are displayed in Table 3.

Comparison of 35-g marketplace substitution method

Marketplace substitution cracker comparisons (35NWS, 35RS4) did not include the comparison with 50Dex. When comparing the 35-g conditions, there was no effect of dose ($P = 0.32$), condition ($P = 0.17$), or dose × condition ($P = 0.22$) for glucose iAUC, glucose peak, glucose baseline-to-peak, or glucose time-to-peak (all $P > 0.05$). However, there was a significant effect of dose ($P < 0.0001$), condition ($P < 0.0001$), and dose × condition ($P < 0.01$) for insulin iAUC. The 35RS4 condition elicited a lower insulin iAUC, insulin peak, and insulin baseline-to-peak compared with the 35NWS condition (all $P < 0.01$). Insulin iAUC was 87% lower for the 35RS4 condition compared with the 35NWS condition. Insulin peak was 39% lower for 35RS4 compared with 35NWS and insulin baseline-to-peak was 66% lower for 35RS4 compared with 35NWS. No differences were observed for insulin time-to-peak ($P > 0.05$). Results for the 2 testing protocols (FDA and marketplace substitution) are compared in Table 3.

Discussion

In light of the prevalence of metabolic disease globally and the promising research on resistant starches thus far, the primary aim of this study was to investigate glycemic and insulinemic responses to Fibersym® RW (RS4) crackers compared with NWS crackers. As part of this aim, we compared RS4 and NWS under 2 different testing protocols: the FDA standard testing protocol and the marketplace substitution method. When comparing the 50-g conditions using the FDA standard testing protocol, glycemic responses to RS4 and NWS crackers were similar over a 2-h testing period but differed considerably from dextrose. Similarly, the insulin responses did not differ between 50-g cracker conditions. We hypothesized that the RS4 cracker would elicit a lower glycemic and insulinemic response compared with the NWS cracker, regardless of the testing protocol used. The null results can be explained given that the RS4 in the 50RS4 cracker condition was substituted for digestible carbohydrate in the 50NWS cracker condition, in accordance with the FDA standard testing method. This addition led to a 50RS4 condition containing a similar amount of digestible carbohydrate compared with the 50NWS condition, which could be expected to elicit a similar glycemic and insulinemic response. We hypothesize that these

TABLE 3 Summary of means by testing method[1]

Method and variable	Dextrose control (50Dex)	FDA standard testing method (50 g matched digestible carbohydrate)			Substitution method (true-to-life) (35 g matched by weight)		
		NWS	RS4	*P*	NWS	RS4	*P*
Glucose							
iAUC, mg/dL × 2 h	3016 (2115, 4076)	1251 (720, 1929)	1276 (739, 1960)	0.99	1317 (677, 2169)	701 (262, 1351)	0.37
Peak, mg/dL	134 (122, 147)	115.4 (106.6, 124)	111.1 (102.3, 120)	0.61	104.3 (94.1, 114)	97.5 (87.4, 108)	0.70
Baseline-to-peak, mg/dL	56.1 (42.5, 71.5)	30.2 (21.7, 40.2)	32.0 (23.2, 42.2)	0.98	28.2 (18.5, 39.9)	16.9 (9.61, 26.2)	0.20
Time-to-peak, min	34.3 (27.0, 41.6)	28.9 (21.9, 36.0)	27.9 (20.8, 34.9)	0.99	46.1 (36.4, 55.8)	36.4 (26.7, 46.1)	0.50
Insulin							
iAUC, μIU/mL × 2 h	2470 (1836, 3199)	2311 (1820, 2861)	2330 (1836, 2882)	0.99	697 (397, 1080)	92 (10, 259)	<0.01
Peak, μIU/mL	42.3 (32.2, 55.6)	47.9 (36.5, 63.0)	45.8 (34.9, 60.2)	0.58	21.7 (16.1, 29.4)	13.3 (9.8, 17.9)	<0.01
Baseline-to-peak, μIU/mL	28.3 (17.1, 42.4)	38.0 (26.8, 53.9)	36.9 (26.0, 52.3)	0.99	13.9 (9.34, 20.8)	4.75 (3.18, 7.09)	<0.01
Time-to-peak, min	49.3 (39.8, 58.8)	36.4 (29.4, 43.4)	34.3 (27.3, 41.3)	0.97	39.3 (29.8, 48.8)	40.0 (30.5, 49.5)	0.99

[1]Values are means (95% CIs). Testing method comparisons with the dextrose control beverage are reported in the Results section. Statistical significance was set at $P < 0.05$. *P* values indicate comparisons between cracker conditions. iAUC, incremental AUC; NWS, native wheat starch; RS4, resistant starch type 4; 50Dex, 50 g dextrose.

results may be due to the baking process used for the crackers, considering previous testing on RS4 in a nutrition bar, in accordance with the FDA standard testing protocol, has shown reduced glucose and insulin responses following preparation of nutrition bars as described previously (13).

When examining crackers made using the marketplace substitution method, without matching for digestible carbohydrate (35NWS, 35RS4), the insulinemic response was 87% lower for the 35RS4 cracker compared with the 35NWS cracker. Although the glycemic response to 35RS4 was ∼45% lower compared with 35NWS, there was not a statistically significant difference between the 35-g cracker conditions. This was primarily due to large within-individual variability within both 35-g cracker conditions (Table 3). The lack of statistical differences in glycemic response between the 35-g cracker conditions was surprising and did not support our original hypothesis. Previous research has reported a reduced glycemic response to RS4 following both the FDA standard testing method and the marketplace substitution method (8, 9, 10, 13). Reduced insulin responses have been previously reported when investigating foods containing higher amounts of fiber (20–23). In addition, RS has been shown to elicit a reduced insulinemic response compared with foods matched for digestible carbohydrate or by weight (13, 24–26). Our results agree with these studies, as we showed reduced insulin responses following acute consumption of an RS4 cracker. Of particular interest, reduced insulin responses for RS4 and other types of RS, even when compared with foods eliciting similar glycemic responses, is a recent finding that indicates a reduced load on the physiological systems of glucose control (7, 27). Additional research is needed to elucidate a potential beneficial effect of RS on insulin sensitivity.

The type of testing method for food products is important to consider, especially when these products are being tested to obtain FDA food-label classifications. The current standard testing methods for products seeking a fiber food-label classification are to match both test and control foods by adding the test ingredient on top of the control food or by substituting the ingredient for a similar ingredient, while matching for digestible carbohydrate. From a physiological perspective, the addition of fiber on top of the 50-g carbohydrate amount may not produce a metabolic response to the test food; it might simply delay that response or have no effect, depending on the mechanism of action. Furthermore, when investigating high-fiber foods, the amount of product needed to elicit a marked change in glycemic or insulinemic response may not be known. This is particularly true when RSs are part of the product formulation, as researchers typically test at the 50-g digestible carbohydrate amount to preserve the power to detect differences where differences might be small. However, the 50-g carbohydrate amount represents a challenge for glucose and insulin testing in high-fiber foods because 50 g of carbohydrate is likely to elicit a large glucose and insulin response, irrespective of fiber content.

The FDA testing methods may not represent how an individual is likely to consume a fiber ingredient in a fortified food. The FDA standard testing method of matching for digestible carbohydrate raises an additional concern for testing high-fiber foods due to the low digestible carbohydrate amount that some products contain. Low amounts of digestible carbohydrate in products lead to testing that may use drastically larger quantities of food than would typically be consumed by individuals. Further, this testing method could lead to multiple treatments that

contain drastically different amounts of food. Theoretically, this could yield results that are primarily impacted by different mechanisms, and results that are potentially irrelevant to consumers if the products are not consumed in large quantities. It is important that the consumer knows how a given product may elicit a beneficial or harmful metabolic response. Consumers may be trying to lose weight, improve metabolic health, or substitute more "healthful" products in place of less healthy options they would normally choose. Accurate information regarding more true-to-life metabolic responses to food products should be provided to consumers so that they can make informed choices regarding which products they would like to consume.

One argument in favor of using the FDA standard testing method is that it uses an approach that matches digestible carbohydrate, such that multiple treatments can be compared while adding a layer of control over the digestible carbohydrate amounts of treatments. If beneficial metabolic outcomes are observed following acute consumption of high-fiber products, then theoretically, they would carry over regardless of how consumers eat the products. However, beneficial metabolic effects may not be seen, compared against competing products, when these products are tested using lower amounts of the matched digestible carbohydrate. There may be a minimum dose of fiber necessary to elicit beneficial metabolic outcomes. According to the FDA Reference Amounts Customarily Consumed Per Eating Occasion, a typical serving size for crackers is ~30 g per eating occasion (28). This reported amount is based on a per-weight basis, meaning the product would be made using the marketplace substitution method and would represent a product on the shelf of a grocery/convenience store. These differences observed between standard testing methods and the more true-to-life marketplace substitution testing method highlight an important concern regarding how foods are tested compared with how they are sold and consumed.

There are several strengths of the current study that should be considered when evaluating the results. Strengths of this study include the novelty of the study design to parse apart differences between the FDA standard testing method and the more true-to-life marketplace substitution testing method where fiber is substituted in place of other carbohydrate ingredients but not matched for digestible carbohydrates. This study design elucidates a need for further evaluation of the current FDA standard testing methods for glucose and insulin responses required for fiber food-label classification. The single-blinded, randomized-controlled crossover trial design is another strength of this study, as it allowed for multiple comparisons of cracker types and the method used to produce the cracker conditions, while minimizing within subject heterogeneity. The primary limitations of this study include the absence of a 35-g dextrose comparison, inhibiting our ability to directly compare the FDA standard testing method with the marketplace substitution method for this carbohydrate amount. This condition was not included in order to limit participant burden. The small sample size of human subjects is also a potential limitation. The use of the Bayer Contour glucometer is another potential limitation as this is a consumer-geared device that may not report the most accurate absolute glucose values. Although this is a potential limitation, this was accounted for with multiple measurements to ensure a more accurate measure of absolute glucose values. A final limitation is the use of multiple comparisons, which inherently increases the potential for type I error. Future studies should investigate differences between FDA standard testing methods and marketplace substitution methods with respect to glycemic and insulinemic responses using different products and different carbohydrate doses. In addition, broader populations should be investigated to determine whether similar effects are observed, including populations with chronic conditions such as diabetes.

Our results indicate differential outcomes using an FDA-approved protocol for testing of the glycemic and insulinemic responses to an RS4 and NWS cracker as opposed to a marketplace substitution protocol. The FDA standard testing method reported no differences between crackers at the 50-g digestible carbohydrate amount. However, there was a significantly lower postprandial insulinemic response for the RS4 cracker using the marketplace substitution method, matched by weight (35 g) and not by carbohydrate amount, even with similar postprandial glycemic responses as compared with the NWS cracker. Given the potential of fiber, and more specifically RS, for improving metabolic responses, current testing for FDA claims on food labels should be evaluated further to determine their appropriateness as compared with using more true-to-life testing amounts.

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