The Glycemic Response Does Not Reflect the In Vivo Starch Digestibility of Fiber-Rich Wheat Products in Healthy Men[1–4]

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Abstract

Starchy food products differ in the rate of starch digestion, which can affect their metabolic impact. In this study, we examined how the in vivo starch digestibility is reflected by the glycemic response, because this response is often used to predict starch digestibility. Ten healthy male volunteers [age 21 ± 0.5 y, BMI 23 ± 0.6 kg/m^2 (mean $\pm$ SEM)] participated in a cross-over study, receiving three different meals: pasta with normal wheat bran (PA) and bread with normal (CB) or purple wheat bran (PBB). Purple wheat bran was added in an attempt to decrease the rate of starch digestion. The meals were enriched in ^{13}C and the dual isotope technique was applied to calculate the rate of appearance of exogenous glucose (RaE). The ^{13}C-isotopic enrichment of glucose in plasma was measured with GC/combustion/isotope ratio MS (IRMS) and liquid chromatography/IRMS. Both IRMS techniques gave similar results. Plasma glucose concentrations [2-h incremental AUC (iAUC)] did not differ between the test meals. The RaE was similar after consumption of CB and PBB, showing that purple wheat bran in bread does not affect in vivo starch digestibility. However, the iAUC of RaE after men consumed PA was less than after they consumed CB ($P < 0.0001$) despite the similar glucose response. To conclude, the glycemic response does not always reflect the in vivo starch digestibility. This could have implications for intervention studies in which the glycemic response is used to characterize test products. J. Nutr. 142: 258–263, 2012.

Introduction

Starch digestibility, which varies widely between different food products, is implicated in the pathogenesis, treatment, and prevention of chronic diseases. Frequent consumption of rapidly digestible starch, resulting in postprandial hyperglycemia and -insulinemia, might be a risk factor for the development of insulin resistance, T2DM[12], and cardiovascular disease (1–3).

For T2DM patients, it is important to avoid postprandial hyperglycemia, and foods with slowly digestible carbohydrates can contribute to improved glycemic control (4). Slowly digestible starch might also be beneficial due to the increased nutrient delivery to the distal small intestine and the resulting increase in plasma glucagon-like peptide-1 concentrations, which is one of the proposed explanations for the complete remission of T2DM after bariatric surgery (5). In addition, fermentation of starch that reaches the colon increases the production of SCFA, which are associated with several health benefits (6,7).

Information about starch digestibility can be obtained from in vitro assays (8), which, however, might not always predict the in vivo starch digestion, as we have shown before (9). In addition, carbohydrate-rich foods are often classified using the GI, which reflects the effect on postprandial blood glucose concentrations (10). A low GI of starchy foods is generally assumed to be due to slow digestibility of starch. However, the postprandial glucose response is not only determined by the glucose absorbed from the ingested meal. Endogenous glucose production and glucose uptake into tissues contribute to the postprandial glucose concentrations measured. To get insight

into the in vivo digestive behavior of starchy foods and how this relates to the glycemic response, stable isotopes can be used. By labeling the glucose in the food products with ^{13}C and infusing the volunteer with a tracer amount of D-[6,6-$^2H_2$]glucose, the above-mentioned processes contributing to total glucose concentration can be distinguished. So far, few data are available that describe the value of the glycemic response of different starchy foods for predicting starch digestibility. With this study, we aim to extend this knowledge by investigating different wheat products.

One of the most common sources of starch in human nutrition is wheat (*Triticum* spp.) and products made from wheat can be either high or low glycemic. In general, the consumption of wheat bread results in high postprandial glucose concentrations (high GI) (11) and the starch in wheat bread is therefore considered to be rapidly digested. A wheat product such as pasta is considered as more slowly digestible, due to a generally lower, more prolonged elevation of postprandial glucose concentrations (low GI) (11). Considering the negative aspects of a diet rich in rapidly digestible starch, several attempts are being made to diminish the rapid hydrolysis of starch, for instance, by inhibiting the digestive enzymes using natural ingredients (12). Anthocyanins, a subgroup of plant-derived polyphenols, have been shown to inhibit the action of α-amylase and/or α-glucosidase in vitro (13–18). Besides their presence in berries, vegetables, and red wine, anthocyanin concentrations are high in purple wheat (*T. aethiopicum*) pericarp (bran fraction) (19,20). Thus, we hypothesized that the addition of purple wheat bran decreases the digestion rate of rapidly digestible wheat bread.

To calculate the rate of starch digestion and uptake of starch-derived glucose in vivo, various methods can be used to measure isotopic enrichment of plasma glucose. In our studies, ^{13}C-isotopic enrichment is usually determined with GC/C/IRMS, requiring derivatization of plasma samples prior to analysis (21–24). Since the introduction of the LC interface for IRMS by Krummen et al. (25), several studies showed the power and accuracy of this method in many disciplines (26–30). Because the method using GC/C/IRMS is a labor-intensive method that might increase the uncertainty of the measured isotopic composition, Schierbeek et al. (31) developed a more simple method for the simultaneous measurement of ^{13}C-glucose enrichment and glucose concentration in human plasma, using LC/IRMS. In the present study, this method was used in addition to our standard GC/C/IRMS method.

In summary, this study aimed to investigate whether total plasma glucose concentrations reflect the in vivo starch digestibility of a starchy food product by comparing different ^{13}C-labeled wheat products (breads and pasta). In an attempt to decrease the rate of starch digestion, wheat bread was made with added purple wheat bran. To evaluate our results concerning ^{13}C-enrichment of glucose in plasma obtained with GC/C/IRMS, a comparison was made with LC/IRMS data.

Participants and Methods

Participants. Ten healthy men [age 21 ± 0.5 y, BMI 23 ± 0.6 kg/m² (mean ± SEM)] were recruited. Criteria for exclusion were use of medication, blood donation or use of antibiotics in the past 3 mo, gastrointestinal surgery or dysfunction, inflammatory diseases, and diabetes mellitus. Approval was obtained from the Medical Ethics Committee of the BEBO foundation, Assen, The Netherlands. Each participant gave written informed consent for the study.

Experimental design. The study was performed in a crossover manner, with each participant studied on three occasions at least 1 wk apart. The participants refrained from consuming ^{13}C-enriched foods like cane sugar, corn products, and pineapple for 3 d and from alcohol consumption and strenuous exercise for 24 h before each study day. Food intake on the day before each experiment was individually standardized using a diary. A standard evening meal was provided at the research facility, where the men stayed overnight. Participants fasted overnight but were allowed to drink water. A venous catheter was inserted in each forearm for blood collection and for infusion of D-[6,6-$^2H_2$]glucose (98% ^2H APE) (Isotec). In the morning (t = −120), 26.7 mL D-[6,6-$^2H_2$]glucose solution (80 × 0.07 mg/kg body weight) was infused and a continuous infusion of 0.07 mg D-[6,6-$^2H_2$]glucose/(kg body weight · min) was started and maintained for 8 h. Two hours after the start of the infusion (t = 0), the test meal was ingested. During the study period physical activity was limited.

Sample collection. Blood was collected into 2-mL BD Vacutainer Fluoride tubes (BD Diagnostics). Three basal blood samples were collected (t = −60, −30, and −5) and after the test meal, samples were drawn every 15 min for 2 h and every 30 min for an additional 4 h. After centrifugation (1300 × *g* for 10 min at 4°C), sample aliquots were stored at −20°C until analysis.

Test meals and preparation. The three wheat-based test meals were 132 g control bread with normal wheat bran (CB), 132 g bread with purple wheat bran (PBB), and 119 g (uncooked weight) pasta with normal wheat bran (PA), all prepared at TNO Healthy Living, Zeist, The Netherlands. Each test meal consisted of 50 g available carbohydrates and was consumed with 10 g light margarine (4 g fat), 2 slices lean ham (5 g fat, 6 g protein), and 250 mL tap water within 20 min.

Meals were ^{13}C-enriched by the addition of 12% ^{13}C-labeled wheat [*T. aestivum* var *Paragon* (1.359 AP ^{13}C)] cultured in a $^{13}CO_2$-enriched atmosphere, as described before (32). Normal wheat bran (English Biscuit Wheat) and purple wheat bran (Purple Wheat) were purchased from Meneba.

Breads were prepared with 1110 g unlabeled white wheat flour [*T. aestivum* Kolibri/Ibis (ratio 70/30) (1.085 AP ^{13}C)], 210 g wheat bran (normal or purple bran, resulting in 7% dietary fiber in the final product), 180 g ^{13}C-labeled wheat flour, 975 g water, 25 g yeast, and 30 g salt. After kneading, the dough was let to rise for 30 min and was baked for 30 min at 240°C. Portions of 132 g of bread were stored at −20°C until use. PA was freshly prepared with 739 g wheat flour (*T. durum* de Cecco), 141 g normal wheat bran and 120 g ^{13}C-labeled wheat flour, 400 g water, and 20 g salt. Portions of 119 g were stored at 5°C until use and cooked for 6.5 min in 2 L water before consumption.

Determination of total phenolic concentration. Polyphenols were extracted from the bran and the test meals by boiling in water under reflux (30 min). The total phenolic concentration of the extract was determined using the Folin-Ciocalteu method (limit of detection 0.1 g/100 g) as described by Singleton et al. (33).

In vitro analysis of starch fractions. To analyze the starch fractions (G_T, G_{RA}, G_{SA}, total available glucose, and resistant starch) in the test meals, an adapted version of the Englyst method (8) was used as previously described (32).

To determine the ^{13}C abundance of the test meals, necessary for further calculations, the glucose resulting from this method was derivatized (as described later for plasma samples) and measured by GC/C/IRMS.

Measurement of plasma glucose concentrations. Plasma glucose concentrations were measured on a Roche/Hitachi Modular automatic analyzer (Roche Diagnostics, Hitachi) using a glucose hexokinase method. The within- and between-run CV were ≤2%.

Analysis of isotopic enrichment by GC/MS and GC/C/IRMS. Analysis of isotopic enrichment in plasma by GC requires the derivatization of glucose to glucose penta acetate. The sample preparation was described in detail elsewhere (23,24).

[2]H enrichment was measured by GC/MS as previously described (23), with some modifications. The GC/MS system used was an Agilent 5975 MSD quadrupole instrument (Agilent Technologies). The sample was separated on a 30-m × 0.25-mm (0.25-μm film thickness) AT 1701 capillary column (Alltech Associates). The initial column temperature was 150°C for 0.5 min and was increased to 280°C (80°C/min). The MS was used in the chemical ionization mode and selected ion monitoring was performed for the mass ions m/z 331 (M0) and 333 (M2).

The $^{13}C/^{12}C$ isotope ratio was measured using GC/C/IRMS as previously described (21,22), with some modifications. A Delta plus XL HP 6890 series GC system (Finnigan MAT) was used. The sample was injected in the splitless mode onto a 30-m × 0.32-mm (0.25-μm film thickness) J&W Scientific column (Folsom). Eluting compounds were combusted on-line in a platin-catalyzed CuO oxidation reactor operating at 940°C.

Analysis of isotopic enrichment by LC/IRMS. An aliquot of plasma (25 μL) was mixed with 50 μL of 1 mmol/L trehalose (internal standard) and deproteinated by adding 125 μL of 2 mol/L perchloric acid, incubating for 10 min on ice, and centrifugation at $10,000 × g$ for 20 min. The supernatant was filtered through 0.2-μm Nylon membrane filters (Grace Alltech). The injection volume was 20 μL.

High performance ion-exclusion chromatography was carried out on a LC system consisting of two Knauer pumps and a Midas auto sampler (Spark) fitted with a Transgenomic ICSep ION-300 column (300 × 7.8 mm) (Achrom). The LC system was coupled to the IRMS instrument by a LC-Isolink interface (Thermo Fisher), which is based on wet oxidation of organic components with sodium peroxodisulfate under acidic conditions. The flow rates of the acid reagent (1.5 mol/L phosphoric acid) and the oxidant reagent (0.84 mol/L peroxodisulfate) in the LC interface were both 25 μL/min. Isotopic ratio measurements were carried out on a Delta XP IRMS instrument (Thermo Fisher). The ion source pressure was 0.4 mPa and ions were generated by electron impact at 70 eV. The CO_2^+ signals for the three major ions at m/z 44 ($^{12}CO_2$), m/z 45 ($^{13}CO_2$ and $^{12}C^{17}O^{16}O$), and m/z 46 ($^{12}C^{18}O^{16}O$) were monitored.

Calculations. The $^{13}C/^{12}C$ abundance ratio was expressed as δ ^{13}C (‰) value. The delta notation is defined as δ ^{13}C sample = [(R$_s$/R$_{st}$) − 1] × 1000, where R$_s$ is the $^{13}C/^{12}C$ ratio in the sample and R$_{st}$ is the $^{13}C/^{12}C$ ratio of the International Standard of Vienna Pee Dee Belemnite. The δ ^{13}C was transformed to the abundance of ^{13}C in a sample, defined as AP, as follows:

$$AP = Atom\% = \left[\frac{100 × R_{st} × \left((δ^{13}C/1000) + 1\right)}{1 + R_{st} × \left((δ^{13}C/1000) + 1\right)}\right].$$

The AP values retrieved from the GC/C/IRMS measurement represent the ^{13}C abundance of glucose penta acetate. To calculate the AP from glucose, the AP from penta acetate was determined using two reference glucose samples (from corn and potato) as previously described (23). The obtained value was used to correct for derivatization of the plasma glucose. APE, the isotopic enrichment in a sample compared to the basal value, is defined as AP (sample) − AP (natural abundance).

The systemic RaE was calculated as described by Tissot et al. (34). The used molar percentage enrichment of [6,6-$^2H_2$]glucose [calculated as previously described (23)] and the AP curves were first smoothed (35) before further calculations.

To determine differences in plasma glucose concentrations and RaE, the iAUC was calculated as described before (24) and compared for the time period 0–120 min.

Statistics Data are presented as means ± SEM. A mixed model was used to test statistical differences between meals within participants, using test meal as a fixed factor and participant as a random factor. Partial tests were corrected for multiple comparison (Dunnet, with CB as reference). All analyses were performed with the software package SAS (release 9.1; SAS Institute). A P value < 0.05 was considered significant.

260   Eelderink et al.

Results

Characterization of test meals. The ^{13}C abundance of CB and PBB was 1.118 AP ^{13}C and was 1.119 AP ^{13}C in the PA.

A clear difference in total polyphenol concentration between normal (0.58 g/100 g bran) and purple bran (0.96 g/100 g bran) was measured; however, no difference could be measured between the breads (CB and PBB: 0.10 g/100 g bread), possibly due to the sensitivity of the method.

In vitro analysis of starch fractions showed only minor differences in the G$_{RA}$ fraction of the meals (**Table 1**). The percentage of G$_{SA}$ found in PA was almost double that determined for both breads.

Postprandial plasma glucose concentrations. PA data were available from nine participants, because one did not consume the entire meal. Fasting plasma glucose concentrations did not differ between study days (P = 0.68), nor did the peak values (P = 0.35) (Fig. 1A). The iAUC did not differ after men consumed CB (144 ± 15.9 mmol/L · 2 h), PBB (168 ± 32.0 mmol/L · 2 h), or PA (115 ± 20.6 mmol/L · 2 h) (P = 0.11).

RaE. The rate at which glucose from both breads appeared in the systemic circulation showed no difference, whereas it was much slower after the consumption of PA (Fig. 1B). The iAUC of RaE after men consumed PA (37 ± 1.5% dose · 2 h) was less than after they consumed CB (54 ± 2.9% dose · 2 h) (P < 0.0001).

^{13}C-isotopic enrichment measured with GC/C/IRMS and LC/IRMS. Comparison of APE obtained with LC/IRMS and GC/C/IRMS showed only minor deviations (**Supplemental Fig. 1**). The agreement between both methods was also visualized using a Bland-Altman plot (**Fig. 2A**) in which the majority of points were within the 95% limit of agreement (−0.0015– 0.0040%). The mean difference between both methods was 0.0012%, indicating that the APE values calculated from the measurements on the LC/IRMS were slightly higher. This also appeared from the scatter plot (Fig. 2B), where, especially in the higher region, the points were mainly above the line of equality.

Discussion

This study aimed to investigate to what extent the glycemic response reflects the in vivo starch digestibility of a starchy food product by comparing different ^{13}C-labeled wheat products. Comparing the iAUC of postprandial glucose concentrations after men consumed PA and CB, no difference was observed,

TABLE 1   In vitro analysis of starch fractions in the test meals[1]

Starch fraction	Test meal		
	CB	PBB	PA
		%	
G$_T$	100	100	100
G$_{RA}$	91.4	90.3	87.0
G$_{SA}$	6.1	5.4	10.6
G$_{TA}$	97.5	95.7	97.6
RS	2.3	3.9	2.2

[1] CB, control bread with normal wheat bran; G$_{RA}$, rapidly available glucose (20 min); G$_{SA}$, slowly available glucose (20–120 min); G$_T$, total glucose (indicated as 100%); G$_{TA}$, total available glucose (120 min); PA, pasta with normal wheat bran; PBB, bread with purple wheat bran; RS, resistant starch.
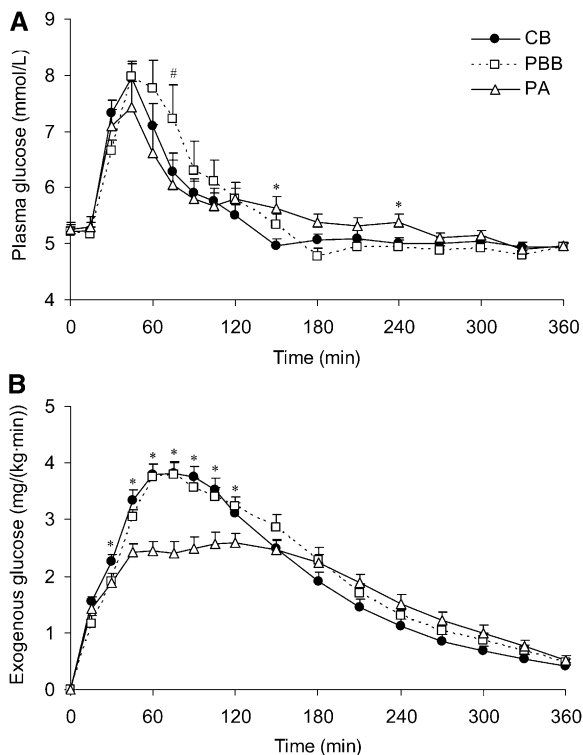
FIGURE 1 Postprandial plasma glucose concentrations (*A*) and RaE (*B*) in healthy men after ingestion of 132 g ^{13}C-enriched CB, 132 g ^{13}C-enriched PBB, and 119 g (uncooked weight) ^{13}C-enriched PA. Values are means ± SEM, *n* = 10 or 9 (PA). *PA differs from CB, *P* < 0.05; #PBB differs from CB, *P* < 0.05. CB, control bread with normal wheat bran; PA, pasta with normal wheat bran; PBB, bread with purple wheat bran; RaE, rate of appearance of exogenous glucose.

wheat bran in the wheat bread had no effect on the rate of starch digestion. Recently, the addition of polyphenol-rich berries to a starch-based food product was also reported to have no effect on the glycemic response (37).

Total polyphenol concentrations in the purple wheat bran were higher than in normal wheat bran, as expected due to higher anthocyanin concentrations in the pericarp of purple wheat (19,20). However, this difference could not be measured in CB and PBB, possibly because the polyphenol concentrations in the complete breads were around the limit of detection of the method due to dilution of the bran fraction with other ingredients. Baking of the bread might also have had an effect on polyphenol concentrations, because anthocyanin pigments readily degrade during thermal processing (38). Li et al. (39) showed that the antioxidant capacity of heat-treated purple wheat bran was not diminished, but whether this holds true for the amylase-inhibiting properties of the anthocyanins after baking is not known.

The ^{13}C-glucose measurement with GC/C/IRMS requires the derivatization of glucose to glucose penta acetate, which is a laborious procedure. To obtain the degree of isotopic enrichment of glucose without the penta acetate ester, a correction is needed, which might increase the uncertainty of the measured isotopic composition. Therefore, the ^{13}C enrichment in post-
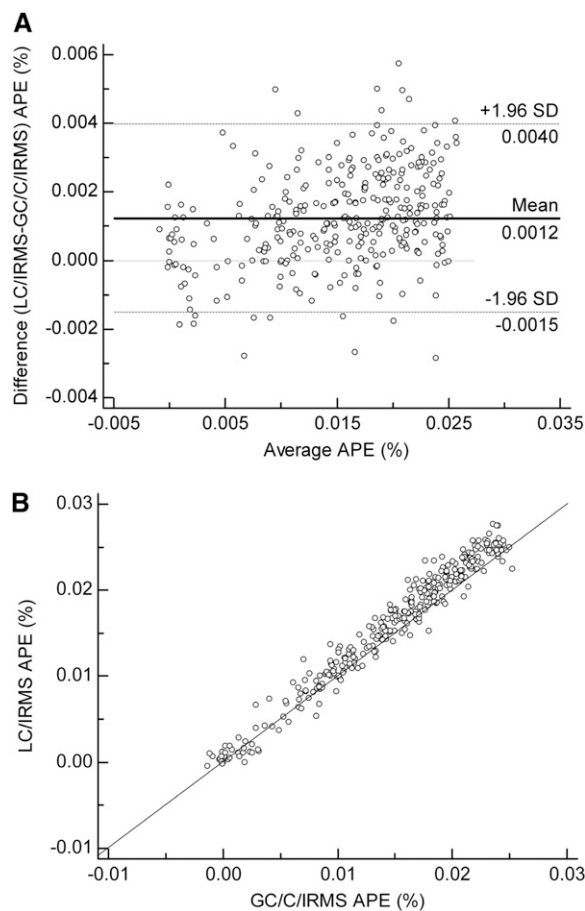
whereas a pronounced difference was found between the RaE after consumption of these test meals. Whereas the RaE after men consumed both breads was comparable, this was much slower after they consumed PA, with the iAUC being 30% smaller than for the CB. Because the glycemic response was unable to show the slowly digestible properties of PA, these results indicate that total postprandial glucose concentrations do not always predict the in vivo digestion rate of a starchy food product. This could be explained by various factors as, e.g., a difference in endogenous glucose production or glucose clearance rate, which will be examined in detail in future work. In a similar study, Schenk et al. (36) investigated the underlying glucose kinetics of breakfasts with a low and a high GI. Despite a pronounced difference in total blood glucose response, the RaE was similar for both test meals. The observed low glycemic response was explained by a difference in tissue glucose uptake. The discrepancy between the glycemic response and RaE observed by both Schenk et al. (36) and us emphasize the importance of determining RaE by using stable isotope technology. This is especially relevant in mechanistic studies relating intestinal absorption of starch-derived glucose with metabolic effects. Also, intervention studies investigating the effect of a low-compared to high-GI diet might give more consistent results when the test products are characterized based on the RaE rather than on the glycemic response.

PBB was made in an attempt to slow starch digestion, because the principal anthocyanin present in purple wheat, cyanidin-3-glucoside (19,20), has shown inhibitory activity toward pancreatic α-amylase in vitro (18). However, in our study, the purple

FIGURE 2 Comparison of ^{13}C APE values determined with GC/C/IRMS and LC/IRMS in 11 postprandial plasma samples from 10 healthy men after ingestion of 3 ^{13}C-enriched test meals visualized in a Bland-Altman plot (*A*) and a scatter plot (*B*) [*n* = 328 (33 in each man, 2 missing values)]. APE, atom percent excess; GC/C/IRMS, GC/combustion/isotope ratio MS; LC/IRMS, liquid chromatography/isotope ratio MS.

prandial plasma samples was also determined using a recently developed LC/IRMS method (31) that does not need derivatization of glucose. Comparison of all data indicated that LC/IRMS measurements result in a slightly higher APE, mainly in the higher APE region. Because in this study the GC/C/IRMS data were used for RaE calculation, no overestimation of differences between bread and pasta was made. Taken together, the comparison of both analytical methods confirmed and strengthened our findings. Determination of ^{13}C enrichment in plasma using LC/IRMS is, however, more convenient due to less laborious sample preparation and is therefore recommended as method of choice. In our dual label studies, derivatization is also required for ^{2}H-glucose determination by GC/MS, making the use of GC/C/IRMS for determination of the ^{13}C:^{12}C ratio more practical. Thus, the use of stable isotopes is a robust method providing good insight in the in vivo rate of starch digestion. Both GC/C/IRMS and LC/IRMS techniques provided reliable results enabling the calculation of RaE from each test meal.

In conclusion, this study showed that the in vivo rate of starch digestion cannot always be predicted by measuring the glycemic response. Despite a similar glycemic response after men consumed CB and PA, the RaE was much slower after they consumed PA. To generate reliable and comparable results in intervention studies investigating low- compared to high-GI diets, care should be taken when characterizing test meals based on their GI. Ingestion of two products with the same GI can, due to different glucose kinetics, result in different postprandial metabolic processes, which consequently can lead to great variances in the outcome measures under study.

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