The Glycemic Response Does Not Reflect the In Vivo Starch Digestibility of Fiber-Rich Wheat Products in Healthy Men1–4

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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/m² (mean ± 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 13C and the dual isotope technique was applied to calculate the rate of appearance of exogenous glucose (RaE). The 13C-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, 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

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6 Abbreviations used: APE, atom percent excess; CB, control bread with normal wheat bran; GC/C/IRMS, GC/combustion/isotope ratio MS; GI, glycemic index; G50, rapidly available glucose; G50S, slowly available glucose; GT, total glucose; iAUC, incremental AUC; LC/IRMS, liquid chromatography/isotope ratio MS; PA, pasta with normal wheat bran; PBB, bread with purple wheat bran; RaE, rate of appearance of exogenous glucose; T2DM, type 2 diabetes mellitus.
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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 $\Delta-[6,6-\text{2H}_2]$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 $\alpha$-amylase and/or $\alpha$-glucosidase in vitro (13–18). Besides their presence in berries, vegetables, and red wine, anthocyanin concentrations are high in purple wheat (T. aestivum) 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 $\Delta-[6,6-\text{2H}_2]$glucose (98% $^{2}$H APE) (Isotec). In the morning ($t = -120$), 26.7 mL $\Delta-[6,6-\text{2H}_2]$glucose solution (80 × 0.07 mg/kg body weight) was infused and a continuous infusion of 0.07 mg $\Delta-[6,6-\text{2H}_2]$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 (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 $^{13}$CO₂-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 ($G_a, G_{RA}, G_{SA}, G_{TA}$, 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).
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 deproteinized 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.

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 13C-labeled wheat products. Comparing the iAUC of postprandial glucose concentrations after men consumed PA and CB, no difference was observed, suggesting similar glycemic responses.

Table 1 summarizes the in vitro analysis of starch fractions in the test meals. The CB bread contained more rapidly available glucose (GRA) than PBB and PA. The system RaE calculated as described by Tissot et al. (34) was 1.5% dose -2h for CB, while it was 1.119 AP 13C in the PA. A mixed model was used to compare the data from both methods (24), where, especially in the higher region, the points were mainly above the line of equality.
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 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 pentacetate, which is a laborious procedure. To obtain the degree of isotopic enrichment of glucose without the pentacetate ester, a correction is needed, which might increase the uncertainty of the measured isotopic composition. Therefore, the $^{13}$C enrichment in post-
prandial plasma samples was also determined using a recently
developed LC/IRMS method (31) that does not need derivati-
zation 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/IRMS
data were used for RaE calculation, no overestimation of dif-
fferences between bread and pasta was made. Taken together,
the comparison of both analytical methods confirmed and strength-
ened our findings. Determination of $^{13}$C enrichment in plasma
using LC/IRMS is, however, more convenient due to less labo-
rious sample preparation and is therefore recommended as
method of choice. In our dual label studies, derivatization is also
required for $^3$H-glucose determination by GC/MS, making the
use of GC/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/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 con-
sumed CB and PA, the RaE was much slower after they con-
sumed 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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