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 associated with slow digestion and release of 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 glycemnic response, stable isotopes can be used. By labeling the glucose in the food products with 13C and infusing the volunteer with a tracer amount of D-[6,6-2H2]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 glycemnic 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 glycemnic. In general, the consumption of wheat bread results in high postprandial glucose concentrations (high GI) 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. 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, 13C-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 13C-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 starch food product by comparing different 13C-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 13C-enrichment of glucose in plasma obtained with GC/C/IRMS, a comparison was made with LC/IRMS data.

**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 13C-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-[6,6-2H2]glucose (98% 2H APE) (Isotec). In the morning (t = −120), 26.7 mL D-[6,6-2H4]glucose solution (80 × 0.07 mg/kg body weight) was infused and a continuous infusion of 0.07 mg D-[6,6-2H2]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.

**Participants and Methods.** Ten healthy men [age 21 ± 0.5 yr, 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.

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**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).

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**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 GSA$_{fr}$ fraction of the meals (Table 1). The percentage of GSA$_{fr}$ 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 ($144 \pm 15.9$ mmol/L · h), PBB ($168 \pm 32.0$ mmol/L · h), or PA ($115 \pm 20.6$ mmol/L · 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 ($37 \pm 1.5\%$ dose · h) was less than after they consumed CB ($54 \pm 2.9\%$ dose · h) ($P < 0.0001$).

**$^{13}$C-isotopic enrichment measured with GC/IRMS and LC/IRMS.** Comparison of APE obtained with LC/IRMS and GC/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, unless...
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 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-
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/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 13C 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 2H-glucose determination by GC/MS, making the use of GC/IRMS for determination of the 13C:12C 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 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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Literature Cited


