Digested of so-called resistant starch sources in the human small intestine\textsuperscript{1–3}

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ABSTRACT

Background: Resistant starch sources, which are only partially digested in the small intestine, can be used to increase colonic availability of short-chain fatty acids.

Objective: To study the characteristics of the fermentation of resistant starch, the digestion of resistant starch in the small intestine has to be quantified. We compared the metabolic fates of highly digestible cornstarch (DCS), Hylon VII (type 2 resistant starch), and Novelose 330 (type 3 resistant starch), which are of corn origin and, therefore, naturally enriched in \textsuperscript{13}C.

Design: After administration of 40 g starch or glucose to 7 healthy volunteers, glucose and exogenous glucose concentrations in serum and \textsuperscript{13}CO\textsubscript{2} excretion in breath were analyzed for 6 h. \textsuperscript{13}C abundance in carbon dioxide was analyzed by isotope ratio mass spectrometry (IRMS) and \textsuperscript{13}C abundance in glucose by gas chromatography–combustion IRMS.

Results: By comparing the area under the curve (2 h) of exogenous glucose concentration in serum (\textsuperscript{13}C glycemic index) after intake of starch or glucose, \textsuperscript{13}C glycemic indexes for DCS, Hylon VII, and Novelose 330 were calculated to be 82 \pm 23\%, 44 \pm 16\%, and 43 \pm 15\%, respectively. Comparison of 6-h cumulative percentage dose recovery in breath showed that 119 \pm 28\% of DCS, 55 \pm 23\% of Hylon VII, and 50 \pm 26\% of Novelose 330 was digested in the small intestine.

Conclusion: The exogenous glucose response in serum and the \textsuperscript{13}CO\textsubscript{2} excretion in breath can be used to estimate small intestinal digestion of resistant starch, which amounts to \approx 50\%.

INTRODUCTION

To increase the supply of short-chain fatty acids in the colon, resistant starch can be consumed (1, 2). For studying the overall biological effects of fermentation of resistant starch, a quantitative approach is desirable. However, quantitation is difficult because of the partial digestion of the resistant starch source in the small intestine. Several techniques have been used in humans to quantify the amount of starch that resists pancreatic amylase, including direct intubation of the ileum, ileostomy sampling, measurement of the breath-hydrogen response due to colonic fermentation, and in vitro digestion methods (3–11). All of these techniques have intrinsic shortcomings. Therefore, we developed an in vivo approach under physiologic conditions. For this purpose, we used 3 types of starch as model compounds: highly digestible cornstarch (DCS, Custard; AJP, Koog aan de Zaan, Netherlands), Hylon VII (Unilever Research Laboratory, Vlaardingen, Netherlands), and Novelose 330 (Unilever Research Laboratory). DCS and resistant starch vary greatly in the ratio of amylase to amylpectin (26\% amylase:74\% amylpectin and 62\% amylase:38\% amylpectin, respectively). Hylon VII and Novelose 330 differ in amyllose structure: Hylon VII is a type 2 resistant starch and Novelose 330 is a type 3 resistant starch (2). All 3 types of starch are of corn origin and, therefore, naturally enriched in \textsuperscript{13}C (12). After intestinal absorption, exogenous glucose is ultimately oxidized to \textsuperscript{13}CO\textsubscript{2}. This implies that besides the exogenous glucose response in serum, the \textsuperscript{13}CO\textsubscript{2} excretion in breath can be measured after consumption of \textsuperscript{13}C-enriched substrates (13–16). With advanced mass spectrometry technology, we monitored the metabolism of cornstarch–derived glucose in blood and \textsuperscript{13}CO\textsubscript{2} excretion in breath and calculated the small intestinal digestion of each of these starches.

SUBJECTS AND METHODS

Experimental protocol

Three groups of 7 young, healthy volunteers (aged 20–26 y) consumed glucose and \geq 1 of the 3 starches. Glucose was used as an intraindividual standard. Five volunteers participated in >2 tests. The substrates were studied in a parallel group design. For 6 h, the glucose concentration and the \textsuperscript{13}C:12C ratio in serum and the \textsuperscript{13}C:12C ratio in breath carbon dioxide were analyzed. From the serum glucose response, the glycemic index was calculated according to the method of Jenkins et al (17). From the serum exogenous glucose response, the \textsuperscript{13}C glycemic index was...
calculated (see Calculations). A digestion index was also calculated from the $^{13}$CO$_2$ excretion data ($^{13}$CO$_2$ glycemic index). The protocol was approved by the Medical Ethical Committee of the Faculty of Medical Sciences of the University of Groningen.

The subjects were asked to not consume $^{13}$C-rich foods such as corn products, cane sugar, and pineapple for 2 d before the test. Furthermore, the subjects fasted from 2200 the evening preceding the test until the start of the experiment at 0800 the next day. During the test, they refrained from consuming food and energy-containing drinks and remained seated during the 6-h test period. The experiments were performed with intervals of ≥48 h between the tests to wash out residual $^{13}$C from the previous test. Forty grams of each substrate was dissolved in 200 mL low-fat milk ≥1 h before the start of the test: DCS, $^{13}$C enrichment relative to Pee Dee belemnite $\delta (^{13}$C$_{PDB}) = -12.19‰$; Hylon VII, $\delta ^{13}$C$_{PDB} = -12.33‰$; or Novelose 330, $\delta ^{13}$C$_{PDB} = -11.44‰$. Glucose derived from corn had a $\delta ^{13}$C$_{PDB}$ value of -14.93‰. Flavorings and sweeteners (without energy) were added to make the test meal palatable. Finally, gelatin was added to the mixture to stiffen it and to get equal consistencies for raw and cooked starch test meals (not described in this article).

**Blood sampling and breath collection**

Two-ml blood samples were collected before, at 15-min intervals during the first hour, every 30 min during the second and third hour after the meal, and every hour until the end of the experimental period (6 h). For this purpose, an intravenous catheter was placed in the cubital vein of one of each subject's arms. Blood was collected in evacuated containers containing 5 mg sodium fluoride and 4 mg potassium oxalate (Becton Dickinson, Meylan Cedex, France). Blood was centrifuged at 900 g for 10 min and plasma was stored at -20°C until analyzed. Breath samples were collected in triplicate in 10-mL gas collection tubes (Exetainers; Labco, High Wycombe, United Kingdom) at 15-min intervals during the first 2 h and every 30 min thereafter until the end of the experiment. Breath air was blown into the gas collection tubes with a straw.

**Analytic procedures**

**Breath 13CO2 analysis**

The procedure to analyze $^{13}$C enrichment in breath in our laboratory was described previously (18). During the 6-h period, the total carbon dioxide exhalation rate was measured 3 times at 1.5 h intervals with indirect calorimetry (Oxycon; Dräger, Breda, France). The total carbon dioxide exhalation rate was calculated as the percentage dose recovered (PDR)/h. Total recovery after 6 h was calculated as the area under the PDR/h-time curve and expressed as the cumulative percentage dose recovered (cPDR). The $\delta ^{13}$C$_{PDB}$ values of the glucose and starch substrates were determined by total combustion using an on-line coupled elemental analyzer.

**Calculations**

The $\delta ^{13}$C$_{PDB}$ value was converted to the atom % (AP) value. The AP values after ingestion of substrate were corrected for the baseline abundance. The difference [atom % excess (APE)] was used for further calculations. For breath carbon dioxide, the APE value was related to the carbon dioxide excretion measured by indirect calorimetry to calculate the substrate-derived carbon dioxide exhalation rate. This exhalation rate was calculated as the percentage dose recovered (PDR)/h. Total recovery after 6 h was calculated as the area under the PDR/h-time curve and expressed as the cumulative percentage dose recovered (cPDR). The $\delta ^{13}$C$_{PDB}$ of the plasma glucose pentaacetate derivative was converted to the $^{13}$C AP. The concentration of glucose derived from the $[^{13}$C]starch (exogenous glucose) in plasma was calculated as follows:

$$[\text{Exogenous glucose}] = \frac{[\text{total glucose}] \times (\text{AP}^{13}C_i - \text{AP}^{13}C_{\text{substate}})/\text{AP}_{\text{substrate}} - \text{AP}_{\text{body}})}{\times 2.67} \quad (1)$$

where [total glucose] is glucose concentration in plasma (mmol/L), AP $^{13}$C$_i$ is the $^{13}$C AP of plasma glucose pentaacetate at time point $t$ after ingestion of $[^{13}$C]starch, AP $^{13}$C$_{\text{substrate}}$ is the AP $^{13}$C of plasma glucose pentaacetate before ingestion of $[^{13}$C]starch, AP $^{13}$C$_{\text{substrate}}$ is the AP $^{13}$C of the administered starch, AP $^{13}$C$_{\text{body}}$ is the AP $^{13}$C of endogenous glucose, expressed as the basal $^{13}$C abundance in breath, and 2.67 is the factor to correct for the dilution of glucose $^{13}$C abundance by the $^{13}$C abundance of the derivatizing acetate carbon atoms.

**Glycemic indexes**

The classic way to substantiate starch digestion is by measuring the glycemic index as described previously by Jenkins et al (17). This implies measuring the area under the curve (AUC) of the serum glucose concentration over the first 2 h after administering a starch and dividing this by the serum glucose response after consumption of an equal amount of glucose. The $^{13}$C glycemic index was calculated as the ratio of the AUC of the
exogenous glucose curve \( (t = 2 \text{ h}) \) after starch consumption and the equivalent area obtained after consumption of glucose, including only the areas above fasting concentrations. The \(^{13}\text{CO}_2\) glycemic index was calculated as the ratio of the 6-h cPDR in breath of starch to that of glucose.

### Statistics

Statistical evaluation of the differences between group means was made by using Student’s \( t \) test. \( P < 0.05 \) was considered to indicate significance. The statistical analyses were performed by using EXCEL ‘97 (Microsoft Corp, Redmond, WA).

### RESULTS

The 2-h AUC for total glucose concentration in serum derived from the intake of DCS, Hylon VII, and Novelose 330 related to the same measure after glucose intake, leads to the glycemic index values given in Table 1. High SDs for the obtained mean values were found under these experimental conditions.

The \(^{13}\text{C}\) cumulative excretion in breath after consumption of 40 g glucose is presented in Figure 1B. The combination of both values per time point resulted in a time curve of exogenous glucose response (Figure 1B). In Figure 2, the total glucose response in serum after consumption of DCS, Hylon VII, and Novelose 330 is compared with the exogenous glucose response in serum in the same experiments. It is clear that the differences between DCS and Hylon VII and Novelose can be documented better by using the serum exogenous glucose response. Because of its high digestibility, DCS gave a more pronounced rise in exogenous glucose concentration in serum than did Hylon VII or Novelose.

In Figure 3, the cumulative exogenous glucose curve after intake of glucose and the starches is illustrated. In the first period of the curve \( (t < 60 \text{ min}) \), effects of variations in gastric emptying play a relatively major role. In the latter part of the curve \( (t > 3 \text{ h}) \), postabsorptive events have more influence. Therefore, we decided to compare the cumulative exogenous glucose response in serum after glucose and starch intake at \( t = 2 \text{ h} \), which is the same period used in the classic glycemic index calculation method of Jenkins et al. When these calculations were made, \(^{13}\text{C}\) glycemic index values were derived (Table 1). These values more closely resemble the amylose-amylopectin ratio of starch than do those obtained by the classic glycemic index method (Table 1).

The total carbon dioxide excretion in breath after administration of 40 g glucose and the \(^{13}\text{C}\)-\(^{12}\text{C}\) ratio in breath samples determined by IRMS analysis are shown in Figure 4A. By combining these data, the \(^{13}\text{CO}_2\) excretion rate (Figure 4B) and 6-h cPDR (Figure 4C) were calculated. The results when this method was applied after consumption of DCS, Hylon VII, and Novelose 330 are shown in Figure 5. Again, a more pronounced response was found after consumption of the well-digested DCS than after the resistant starch sources. Simultaneous measurement of hydrogen in breath indicated that in the 6-h period, no increase in breath hydrogen occurred, which suggests that the starch was not fermented in this period (data not shown). From this we concluded that the measured \(^{13}\text{CO}_2\) was derived from digested starch and not from fermented starch.

To quantify the differences in the digestion process, the \(^{13}\text{CO}_2\) response to corn-derived glucose was used. In Figure 6, the \(^{13}\text{C}\) cumulative excretion in breath after consumption of glucose, DCS, Hylon VII, and Novelose 330 is shown. Roughly, the differ-

### Table 1

Characterization of digestibility of starch

<table>
<thead>
<tr>
<th>Starch</th>
<th>GI</th>
<th>(^{13}\text{GI})</th>
<th>(^{13}\text{CO}_2)-GI</th>
<th>Percentage amylopectin</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCS</td>
<td>98 ± 138</td>
<td>82 ± 23</td>
<td>119 ± 28</td>
<td>74</td>
</tr>
<tr>
<td>Hylon VII</td>
<td>22 ± 13</td>
<td>44 ± 16(^2)</td>
<td>55 ± 23(^2)</td>
<td>38</td>
</tr>
<tr>
<td>Novelose 330</td>
<td>48 ± 28</td>
<td>43 ± 15(^2)</td>
<td>50 ± 26(^2)</td>
<td>38</td>
</tr>
</tbody>
</table>

\( ^1\) ± SD; \( n = 7 \). GI, glycemic index after consumption of 40 g starch; DCS, digestible corn starch; Hylon VII, type 2 resistant starch; Novelose 330, type 3 resistant starch.

\( ^2\) Significantly different from DCS, \( P < 0.01 \).

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FIGURE 1. After administration of 40 g glucose to 7 healthy volunteers, the mean (±SD) \(^{13}\text{C}\)-\(^{12}\text{C}\) ratio in serum glucose was analyzed by using gas chromatography–combustion isotope ratio mass spectrometry (A; △-△); the mean (±SD) total glucose concentration was measured in the same samples (B; ◆-◆). These 2 values were used to calculate the exogenous glucose concentration in serum (B; □-□). The calculation is described in Methods.
ference in response to corn-derived glucose and cornstarch reflects intestinal hydrolysis. Assuming that glucose is absorbed completely from the small intestine, the partial digestion of starch can be derived by comparing the 6-h cumulative $^{13}$C excretion after corn-derived glucose and cornstarch consumption. These data were calculated for DCS, Hylon VII, and Novelose 330 and are presented in Table 1 as the $^{13}$CO$_2$ glycemic index. In this case, a 6-h period was used to minimize the problem of variable intermediate storage of glucose in tissues before oxidation.

Both techniques of measuring the exogenous glucose concentration in serum and measuring the $^{13}$CO$_2$ excretion in breath are indirect reflections of intestinal digestion. In an attempt to optimize these variables, the kinetics of both techniques were compared. In Figure 7, the values for the exogenous glucose curve in serum and the $^{13}$CO$_2$ excretion rate in breath after consumption of glucose, DCS, and Hylon VII are compared. As can be observed, there is a high degree of similarity; the differences are due to a lag phase in the breath excretion, as would be expected. Furthermore, the differences are larger for the glucose and DCS curves than for the Hylon VII curves.

DISCUSSION

Short-chain fatty acids fulfill important biological functions in the colon (21). Butyrate has a distinct role in epithelial cell proliferation and differentiation (22, 23). It is not known what the physiologic range of butyrate production is in humans, but because of changes in food consumption patterns, daily intakes of substrates for short-chain fatty acids are steadily decreasing. A way to increase the supply of butyrate in the colon could be the consumption of resistant starch sources (24–26). To understand and evaluate the biological effects of consumption of resistant starch, we need to know first to what degree the resistant starch is digested in the small intestine. Information on digestibility is difficult to obtain because of the inaccessibility of the intestinal tract in humans. Several techniques to quantify the amount of starch that is resistant to the action of small intestinal enzymes have been used. The shortcomings of these techniques were discussed elsewhere (3, 4, 9, 11). We based our studies on the pioneering experiments of Normand et al (13), who described the use of $^{13}$C-enriched starch for in vivo starch digestion studies. The hydrolysis product, $[^{13}$C]glucose in serum, was analyzed by GC-CIRMS, and the metabolic end product, $^{13}$CO$_2$ in breath, was analyzed by IRMS. We followed the same approach using cornstarch products, which are naturally enriched in $^{13}$C.

We calculated a $^{13}$C glycemic index based on comparison with the response to exogenous glucose. This $^{13}$C glycemic index was compared with the classic glycemic index; we found that the SDs for the obtained mean values were smaller with the $^{13}$C glycemic index than with the classic glycemic index. This may be because the $^{13}$C-glycemic-index method refers to exogenous glucose only and the classic glycemic index test cannot discriminate between endogenous and exogenous glucose. Furthermore, the values

![Figure 2](image2.png)

**FIGURE 2.** Mean (±SD) glucose response in serum after consumption of 40 g glucose (○-○), digestible cornstarch (•-•), Hylon VII (type 2 resistant starch; □-□), and Novelose 330 (type 3 resistant starch; ▲-▲) in 7 healthy volunteers. Both the total glucose response (A) and the exogenous glucose response (B) are shown.

![Figure 3](image3.png)

**FIGURE 3.** Mean (±SD) cumulative exogenous glucose response in serum (area under the concentration-time curve; AUC) after consumption of 40 g glucose (○-○) or starch in 7 healthy volunteers: digestible cornstarch (•-•), Hylon VII (type 2 resistant starch, □-□), and Novelose 330 (type 3 resistant starch, ▲-▲).
resemble the percentage of amylopectin in the starch more closely. A clear difference between the response to DCS, which is a highly digestible starch, and that to resistant starch can be found. DCS was digested an average of 80% and resistant starch ≈50%. This last value agrees with the data of Champ et al (11), who used retrograded amyllose starch. [13C]glucose is ultimately oxidized to 13CO2 and excreted via the breath. By measuring the excretion rate and cumulative excretion, the intestinal digestion of starch and the subsequent absorption of glucose can also be studied. This approach was used by Hiele et al (14, 27) to study the digestion of raw crystalline cornstarch. Also, with 13CO2 breath tests [13C]glucose is used as a reference substrate. The assumption is that [13C]glucose derived from a glucose or a starch load undergoes similar metabolic events.

By using this approach, data about intestinal digestion of DCS, Hylon VII, and Novelose 330 were obtained (Table 1). These data led to the conclusion that DCS is effectively digested and resis-
tant starch is digested ≈50%. No difference between Novolose 330 and Hylon VII was observed, despite more processing of the first compound. Many intermediate factors, such as differences in insulin response, tissue glucose clearance, and variable glucose oxidation rates, explain the intra- and interindividual differences in the outcome. The overall similarity in final outcomes via the $[^{13}C]$glucose method and the $^{13}CO_2$ method is surprising and indicates that the rate-limiting step in the total process of converting raw starch to carbon dioxide is the hydrolysis step.

To optimize the proposed method for use in future studies to characterize factors involved in small intestinal digestion, the differences between the data obtained with exogenous glucose concentration in serum and $^{13}CO_2$ excretion in breath were highlighted. The results indicate that the differences are smaller when the starch is digested slower. Obviously, with a rapid influx of glucose from the small intestine, the insulin response will be higher and, consequently, there will be storage in tissues. This relation between exogenous glucose, endogenous glucose, and insulin will be studied in detail in future experiments. The results of the present experiment indicate that the kinetics of starch digestion can be derived from the $[^{13}C]$glucose response in serum as well as the $^{13}CO_2$ excretion rate in breath and that these measurements can be used as a first screening of the digestibility of a starch source.

REFERENCES