The Resistant Starch Level of Heat Moisture–Treated High Amylose Cornstarch Is Much Lower When Measured in the Human Terminal Ileum than When Estimated In Vitro

Kazuma Danjo, Shigeyuki Nakaji, Shinsaku Fukuda,* Tadashi Shimoyama,* Juichi Sakamoto* and Kazuo Sugawara

Department of Hygiene and *First Department of Internal Medicine, Hirosaki University School of Medicine, Hirosaki, 036-8562 Aomori, Japan

ABSTRACT According to the definition of resistant starch (RS), the true value of foodstuff-derived RS can be assessed only from that found in the contents of the terminal ileum. To date, a few methods exist for in vivo measurement of RS in the terminal ileum, but their accuracy is questionable. The aim of this study was to quantify the level of RS in the terminal ileum to determine its true value as dietary fiber (DF). Volunteers (n = 7 men) were given a test meal containing 10 g of heat moisture–treated high amylose cornstarch (HMT-HAS) containing 8.8 g of RS as measured by Englyst’s method. A double-lumen tube was positioned in the terminal ileum using the endoscopic retrograde bowel insertion method (ERBI). Intestinal contents were aspirated, and the amount of RS was measured as the glucose concentration (Englyst’s method), and compared with the values for RS administrated orally using the same method. The mean amount of HMT-HAS–derived RS collected in the terminal ileum was 3.37 ± 0.95 g (mean ± SD), which was 34.5 ± 9.7% of the in vitro RS value. Furthermore, there were large individual differences in recoveries, ranging from 22.2 to 47.5%. The measured amount of HMT-HAS–derived RS was much smaller in our in vivo study than that measured in vitro, suggesting that in vitro measurement may inaccurately estimate the RS and DF levels of foodstuffs. The problem is further compounded by the large individual in vivo variations in RS values from subjects consuming identical diets. J. Nutr. 133: 2218–2221, 2003.

KEY WORDS: resistant starch • heat moisture–treated high amylose cornstarch • dietary fiber • absorption • endoscopic retrograde bowel insertion method

The physiologic roles of dietary fiber (DF) have been of interest since Burkitt initially presented his hypothesis associating DF depletion with the etiology of so–called fiber-deficiency diseases (1). Therefore, recommended levels of daily DF intake have been proposed in many countries, such as 25–35 g by the U.S. FDA (2), and 20–25 g by the Ministry of Health and Welfare in Japan (3). However, the average daily DF intake (total DF intake) is only 15–18 g in Japan (4), and similar amounts have been reported in most Western countries (4). Insufficient intake of DF is thus a major problem in the overall promotion of health in the general population today.

The different definitions of “dietary fiber” have several common points such as: “foods not absorbed in the small intestine of humans,” “polysaccharides,” and “the skeletal remains of plant cells that are resistant to hydrolysis by the enzymes of man” (2,3,5). Although starch is assumed to be digested and absorbed completely in the small intestine, the existence of a particular starch that is resistant to digestion, namely, resistant starch (RS), has been recognized in recent years. RS was first defined by Englyst as starch that remains undigested together with the nonstarch polysaccharides (6). A similar definition was adopted in a recent major European collaboration: “RS is the sum of starch and products of starch degradation not absorbed in the small intestine of healthy humans” (7). It is clear that RS must be classified as a dietary fiber according to the definitions outlined above.

The important problem lies in deciding how much RS any given diet contains. According to the definition of dietary fiber, the true value of the RS of food can be derived only from that found in the contents of the terminal ileum. However, to date, two methods have been utilized for in vivo measurement of RS in the terminal ileum. One method requires individuals to be subjected to an ileostomy (8–10), and the other requires oral or nasal intubation of the ileum through the gut (11,12). However, these methods present physiologic problems. In the former approach, the ileostomy subjects have a history of ileostomy, and the other requires oral or nasal intubation of the ileum through the gut. Therefore, these methods present physiologic problems. In the former approach, the ileostomy subjects may have a history of ileostomy subjects, and the other requires oral or nasal intubation of the ileum through the gut. Moreover, the intestinal flora of ileostomy subjects may be quite different than that of healthy subjects. In the oral or nasal intubation studies, accurate physiologic measurement may be prevented by the existence of a tube in the small intestine where foodstuffs are digested and absorbed.

It is important to know the accurate RS value of foodstuffs because RS should be taken into account in recommending the optimum level of daily dietary fiber. If a food contains larger amounts of RS, it has a greater influence on the recommended level of daily intake of dietary fiber. In addition, the RS content may affect the daily energy intake and consequent obesity, which is an important problem in human nutrition.

1 Supported by the Grant-in-Aid for Scientific Research from the Ministry of Culture and Science of Japan (Contract No. 11307005).
2 To whom correspondence should be addressed.
E-mail: nakaji@cc.hirosaki-u.ac.jp.
In this study, we measured in vivo the amount of RS in the orally administrated RS in heat moisture–treated high amylose cornstarch (HMT-HAS) in the terminal ileum of healthy human subjects using the endoscopic retrograde bowel insertion method (ERBI) (13) and compared the values with those measured using an in vitro method (Englyst's method).

**SUBJECTS AND METHODS**

**Subjects.** Healthy men (n = 7), with no history of gastrointestinal disease, 19–26 y old, volunteered to take part in this study. Written informed consent was obtained from each subject before participation in the study. This study was approved by the Ethics Committee of Hirosaki University School of Medicine.

**Test meals.** Test meals contained 100 mL of a basic diet (Table 1, 10 g HMT-HAS (Lodestar, Nihon Shokuhin Kako, Tokyo, Japan) (14) and 5 g of polyethylene glycol-4000 (PEG). Each test meal was made up to 500 mL with distilled water. Because the basic diet contains no fiber, all glucose generated by hydrolysis of the ileal contents must originate from the HMT-HAS, which was made according to the method of Kurahashi and Yoshino (15).

**Colonic intubation.** In preparation for the trial, subjects ate a low residue diet on the day before the procedure. They fasted overnight and consumed the test meal in the morning. Colonoscopy was started 1 h after intake of the test meals, and an experimental double-lumen tube with a balloon was positioned in the terminal ileum using ERBI (13).

**Experimental technique.** After placement of the double-lumen tube, with the subject lying on a bed, a solution of 0.3 g/L phenol-sulfophthalein (PSP) in distilled water was infused continuously at the rate of 1.0 mL/min as a nonabsorbable marker to calculate ileal

RS

Measurement of RS in the ileal contents and in HMT-HAS. RS in the ileal contents was measured by a modification of the method developed by Englyst et al. (16). In brief, starch in the ileal contents was precipitated by the addition of 95% ethanol heated to 60°C, and the sample was kept at room temperature for 1 h. After centrifugation at 1500×g for 10 min, as much supernatant as possible of was removed without disturbing the residue. The residue was washed twice using 5 mL of 80% ethanol and kept at 65°C until it appeared dry. Then 1 mL of 12 mol/L sulfuric acid was added to the dried residue and kept at 35°C for 1 h to disperse the RS. After the addition of 11 mL distilled water, samples were kept at 100°C for hydrolyzation for 2 h. The hydrolyzed sample was cooled to room temperature and the volume was adjusted to 25 mL with distilled water.

Each sample (1 mL) was placed into a separate test tube, 0.5 mL of glucose solution in distilled water (0.5 g/L) and 0.5 mL of 3.9 mol/L sodium hydroxide were added. After mixing, 2 mL of 3,5-dinitroso-licyclate solution was added to each sample; the color was developed at 100°C for 10 min and cooled in water to room temperature. After the addition of 20 mL of denatured water, light absorbance was measured spectrophotometrically at 530 nm, and the concentration of glucose was measured by reference to the standards. To generate a standard, 1 mL of a blank solution (1:1, v/v 50% saturated benzoic acid and 2 mol sulfuric acid), and 1-mL aliquots of each standard glucose solution were tested. To determine the amount of starch in HMT-HAS, 100-, 200- and 300-mg aliquots of HMT-HAS were also treated and measured in the same way. The concentration of PEG was measured by Hyden’s method (17) and that of PSP by spectrophotometry (13,18).

**Ileal flow volume.** Dilution of the constantly perfused ileal marker (PSP) was used to calculate the proportion of ileal contents recovered by aspiration. Thus, the ileal flow volume for 30 min, the PSP dilution rate (concentration of PSP instilled, divided by the concentration of PSP aspirated) and the amount of glucose in RS in the ileal contents were determined by the following formulae (13,18):

\[
TV' = 1.0 \times \frac{\text{concentration of PSP instilled}}{\text{concentration of PSP aspirated}} \times 30 \text{ (for 30 min)}
\]

where TV' represents the total volume of ileal flow in mL, and 1.0 is the PSP infusion rate in mL/min.

\[
TGl = \left(CGl \times 1.0 \times \frac{\text{concentration of PSP instilled}}{\text{concentration of PSP aspirated}} \right) \times 30
\]

where TGl is the total amount of glucose in mg, CGl is the concentration of glucose in g/L and 1.0 is the PSP infusion rate in mL/min.

Amount of RS (mg) = 0.9 × amount of glucose (mg)

The amount of glucose in each sample was determined from the calculated ileal flow volume for each 30 min, and the concentration of glucose present. In addition, two men were given a test meal not containing HMT-HAS to measure the amount of RS in the test meal.

**Statistical analysis.** We used the Pearson’s correlation coefficient to investigate the relationship between RS and PEG concentrations. The level of significance was set at P < 0.05. Values in the text are means ± SD.

**RESULTS**

Glucose concentration was under the detection limit in the ileal contents obtained from the two men who ingested the test meal without HMT-HAS; thus, all of the glucose present in the ileal contents was generated by hydrolysis of the HMT-HAS.

The measured amount of RS in 10 g of HMT-HAS was 8.8 g. When the ileal contents contained RS, this could be confirmed visually. In all subjects, RS appeared in the ileal contents after the start of the PSP marker perfusion, which was continued for at least 1.5 h after the disappearance of RS. The amounts of PEG and glucose increased slowly and peaked at 5.5–6.0 h and 7.5–8.0 h, respectively, after the start of the perfusion. Both PEG and

<table>
<thead>
<tr>
<th>TABLE 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy, nutrients and foodstuffs of the basic diet</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Energy and nutrients</th>
<th>Food stuff</th>
</tr>
</thead>
<tbody>
<tr>
<td>unit/100 g dry diet</td>
<td></td>
</tr>
<tr>
<td>Energy, kJ</td>
<td>1833</td>
</tr>
<tr>
<td>Protein, g</td>
<td>14.6</td>
</tr>
<tr>
<td>Carbohydrate, g</td>
<td>73.1</td>
</tr>
<tr>
<td>Fat, g</td>
<td>9.7</td>
</tr>
<tr>
<td>Vitamin A, µg</td>
<td>486</td>
</tr>
<tr>
<td>Vitamin B-1, mg</td>
<td>0.23</td>
</tr>
<tr>
<td>Vitamin B-2, mg</td>
<td>0.27</td>
</tr>
<tr>
<td>Vitamin B-3, mg</td>
<td>0.05</td>
</tr>
<tr>
<td>Vitamin B-6, mg</td>
<td>0.12</td>
</tr>
<tr>
<td>Vitamin B-5, mg</td>
<td>0.27</td>
</tr>
<tr>
<td>Vitamin B-7, mg</td>
<td>0.17</td>
</tr>
<tr>
<td>Vitamin B-9, mg</td>
<td>0.05</td>
</tr>
</tbody>
</table>

1 HINEX-R, Otsuka Pharmaceuticals, Tokyo, Japan.
glucose decreased slowly thereafter and could not be detected for at least 1.5 h before the end of the perfusion.

The amount of RS recovered within 3 h after the test meal intake was only 3.3 ± 0.6% of the total RS that reached the terminal ileum. The percentage of RS recovered after the RS could no longer be detected by the naked eye was also 3.3 ± 0.5%. In all cases, the concentration of glucose generated by hydrolysis was below the detection limit, i.e., <3.1 g/L, when the perfusion was finished. Therefore, most of the HMT-HAS–derived RS reached the terminal ileum during the procedure.

The amount of RS in the human terminal ileum was measured as 3.37 ± 0.95 g, which was an estimated 34.5 ± 9.7% (ranging from 22.2 to 47.5%) of the in vitro measured level of RS in the same amount of HMT-HAS as ingested by the subjects. The amount of PEG collected in the human terminal ileum was 4.51 ± 0.68 g, and the recovery was 90.1 ± 13.6%. The times required to collect half of the recovered RS and PEG were 5.5 ± 2.0 and 4.8 ± 1.6 h, respectively. RS and PEG concentrations at each time point were correlated (r = 0.98, P < 0.001, n = 21).

**DISCUSSION**

RS analysis confirmed the huge variability in RS measurement depending on the method used (11). For example, when Champ et al. (11) measured RS contents of identical samples using different methods, the method described by Siljestrom and Asp (19), which was derived from the procedure of the Association of Official Analytical Chemists for fiber analysis, resulted in the lowest values, whereas those described by Champ (20) and Englyst et al. (21) gave similar RS contents that were greater than those from the AOAC procedure. In the current study, we used a modified Englyst method (16). Although this method has been modified frequently (21,22), our adaptation is a well-known and well-understood protocol.

Cornstarch has an amylose content of ~25% is a form of nonglutinous starch. High amylose cornstarch, developed by a breeding technique, contains 50–70% amylose. Because of its high amylose content and the highly crystalline structure of the starch granule, high amylose cornstarch is markedly difficult to gelatinize and hydrolyze by amylase (23,24). HAS contains type-2 RS (25), ungelatinized starch granules that are highly resistant to digestion by α-amylase until gelatinized. It has been reported that HAS reduces plasma cholesterol and triacylglycerol concentrations (26–28). HAS and modified HAS such as HMT-HAS (29,30) are used by the food industry to improve the physical properties of various food items. Their consumption is increasing as the consumption of processed foods increases. The heat moisture treatment decreased the susceptibility of HAS to pancreatic α-amylase, whereas it increased the formation of RS (30,31).

The heat moisture treatment (HMT) was created by Sair and Fetzer in 1944 (32). HMT is carried out by heating starch at 100–125°C under 100% relative humidity (30,33–35). However, with conventional methods, it was difficult to obtain a uniform product using partial gelatinization (36–38). Kurahashi and Yoshino (15) solved this problem by introducing a new device suitable for both laboratory and industrial use. The device consists of a combination of vacuum evacuation of a vessel containing starch and subsequent heating of the starch by introducing live steam. This device provides a good uniform product in less time than by conventional methods. Lodestar was made by this method (15).

Although a nonabsorbable marker is usually used to confirm the recovery of ingested target material, such a marker was not necessary in this study. One reason was that the presence of RS could be confirmed by the naked eye. Another reason is demonstrated by the results. The time to collect half of the recovered PEG was shorter than that of RS, suggesting that the transit time of PEG, a water-soluble material, was shorter than that of RS, a water-insoluble material. Because the recovery of PEG was nearly 100%, little RS could reach the terminal ileum before the start of the perfusion. In addition, when RS was no longer visible to the naked eye, it accounted for only 3.3% of the amount of RS recovered in total. Furthermore, Silvester et al. (8) reported that the mouth-to-stoma transit time of RS in solid food was 7.4 h in subjects with an ileostomy. In the present study, foods were suspended in water, which passes through the digestive tract faster than solid foods. Therefore, 10 h of perfusion should give sufficient time to recover all RS at the terminal ileum. Actually, in all cases, the concentrations of glucose were almost 0 during the last 1.5–2 h before the end of the perfusion. Thus, most of the HMT-HAS–derived RS reached the terminal ileum during the procedure, making the use of a nonabsorbable marker unnecessary.

This study has two major findings. The first is that the percentage of RS measured in vitro compared with that in vitro in HMT-HAS was only 34.9%. Previous studies have also measured RS both in vivo and in vitro. Silvester et al. and Englyst et al. were in good agreement between data obtained in vivo and in vitro (7–10,21). They compared the RS contents of foods, as measured by the analytical technique (Englyst's method), with the recovery of starch from these foods when fed to healthy ileostomates. However, persons with ileostomies do not have normal intestines and may therefore have modified digestive processes. Major differences in water flow rate and electrolyte absorption exist between the normal ileum and that in patients with an ileostomy (39). Moreover, it is possible that the flora that inhabit the terminal ileum (40) might break down carbohydrates by fermentation, thus invalidating the ileostomy-based observations.

On the other hand, Champ et al. (11) demonstrated that the percentage of RS measured in vivo was higher than in vitro. They used the intubation method. This method, however, may influence gastrointestinal function (41), slowing gastric emptying by shortening small intestine transit time; this might lead to an inhibition of RS digestion in the small intestine, although this was not proved (11). They reported that the percentages of RS (on a total starch basis) from retrograded high amylose starch and complex starch in their study were 51 and 21%, respectively. This difference can be attributed to the fraction of starch theoretically digestible but in fact truly recovered at the end of the ileum. Finally, they concluded that the ileal digestibility of starches depends strongly on technological treatments that can be applied during the processing of the food (including grinding, cooking, cooling and storage). Furthermore, they said: “Effluents from patients with ileostomies were shown to contain potentially digestible starch and dextrins (42), which are considered by none of the in vitro analytic methods (43). Therefore, RS measured in vivo and in vitro by Englyst et al. (21) is not, qualitatively, the same starch.”

In the current study, the amount of RS from HMT-HAS was much smaller in the human terminal ileum than in the in vitro measurements. This result does not agree with the results in previous studies. We utilized ERBI to measure in vivo the amounts of RS from HMT-HAS in the terminal ileum of healthy humans in this study. This method also has some physiological problems such as the insertion of the colonoscope and overnight fasting that might account for differences. However, the present results are likely to be more accurate...
than those obtained by earlier methods (8–12) because the small intestine remained intact.

Another major finding is that the recoveries varied from 22.2 to 47.5%. The high variability suggests that the in vivo value of RS varies among subjects, even though they may be consuming the same diet. RS digestion may depend on factors that control digestion in the small intestine as has been mentioned previously. These include starch structure, the presence of other food components (nonstarch polysaccharides, lipids), cooking styles, industrial processing and individual physiologic influences such as chewing and transit time (44).

The large differences in recovery rate in the terminal ileum between high amylose starch and complexed high amylose starch [Champ et al. (11)] and between retrograded and fresh maize [Ahmed et al. (45)] support this. On the other hand, the effect of bacterial flora in the small intestine as influx from the large intestine in the ERBI method cannot be denied. However, its effect is likely to be very small, if any, because the collected sample was frozen within 30 min at –30°C. These varying factors that control digestion may affect the differences in the true amount of RS, something that must be considered in studies of its role in experiments attempting to clarify the effect of RS on large bowel carcinogenesis.

Our study demonstrated that the true amount of HMT-HAS-derived RS (in vitro value) was 34.5% of the in vitro value measured by Englyst’s method (16). This suggests that it is difficult to predict the RS content of foodstuffs by in vitro measurements.

ACKNOWLEDGMENT

The authors thank Bunpei Mori for his useful scientific advice.

LITERATURE CITED