Effects of high-resistant-starch banana flour (RS$_2$) on in vitro fermentation and the small-bowel excretion of energy, nutrients, and sterols: an ileostomy study[1–3]

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ABSTRACT

Background: Resistant starch (RS) has attracted interest because of its effects in the colon and implications for health. Knowledge of how RS influences small-intestinal absorption of nutrients, sterol metabolism, and colonic fermentation is sparse.
Objectives: The objectives of this study were to measure the effect of RS$_2$, a type of RS in banana flour, on the ileal excretion of energy, nutrients, and sterols, and to compare in vivo measurement of RS in the ileostomy model with previously published intubation data. In addition, we sought to estimate a fermentation pattern by using ileal effluents for in vitro fermentation.
Design: The present study was divided into 2 parts. Study A involved 10 ileostomy subjects who were given a controlled diet with the addition of 30 g raw green banana flour (RBF)/d, which contains RS$_2$, or cooked green banana flour (CBF)/d in random order. Study B involved 7 ileostomy subjects who were given a plant-polysaccharide-free diet with the addition of 30 g RBF/d.
Results: In study A, the dry weight of the ileostomy effluents and the ileal excretion of energy, iron, and chenodeoxycholic acid, but not total sterols, were higher after the addition of RBF than of CBF to the diet. In vitro fermentation of the ileal effluents obtained after the addition of RBF to the diet showed higher concentrations of acetate and butyrate. In study B, the ileal excretion of starch was lower than the amount calculated from earlier studies by use of the intubation technique.
Conclusions: The addition of RBF containing RS$_2$ to the diet of ileostomy subjects did not interfere with small-bowel absorption of nutrients or total sterols, except for a small increase in iron excretion. The ileostomy model seems to give reliable results for in vivo measurement of RS.   *Am J Clin Nutr* 2002;75:104–11.

KEY WORDS   Resistant starch, starch digestion, green banana flour, ileostomy, intestinal absorption, energy, iron, sterols, fermentation, butyrate

INTRODUCTION

Resistant starch (RS) is defined as "the sum of starch and products of starch degradation not absorbed in the small intestine of healthy individuals" (1, 2). RS has potential physiologic effects in both the small and large intestine. Postprandial glucose and insulin responses are proportional to the amount of digestible carbohydrate in a meal, and thus are lower after a meal containing high amounts of resistant compared with digestible starch (3–8). The results of studies of the effect of RS on serum lipids in humans are conflicting, however. Two crossover studies showed a decrease in total serum cholesterol concentrations and triacylglycerols, and one study showed an increase in total serum cholesterol concentrations (9–11). Moreover, a study by Heijnen et al (12) found no effect on fasting concentrations of serum lipids.

In vitro fermentation of RS was shown to increase the proportion of butyrate (13–15) and decrease the fecal excretion of secondary bile acids (16, 17). There is, however, a lack of knowledge concerning the effect of RS on the absorption of nutrients, minerals, and sterols in the small intestine. The ileostomy model provides the opportunity to study small-intestinal digestion and absorption of starch and small-intestinal excretion of nutrients after the addition of different types and amounts of RS. It is also possible to use ileal effluents for in vitro fermentation.

Raw green banana flour (RBF) was chosen as a model starch in the present study to achieve 2 products that differ as little as possible in their content of RS$_2$ (ie, the type of starch in banana flour): RBF and the same flour cooked (CBF). Banana is one of the few sources of RS$_2$ that is available in an ordinary meal. The same products were used in a previous intubation study by Faisant et al (18).

The aims of the present study were the following: *1*) to add either RBF (RS$_2$) or CBF to the diets of ileostomy subjects and then compare the dry weight of the ileostomy effluents and the ileal excretion of starch, energy, fat, nitrogen, sodium, potassium, calcium, magnesium, iron, zinc, and sterols (study A); *2*) to evaluate the in vitro fermentation pattern of ileostomy effluents with a high or low content of RS (study A); *3*) to compare in vivo RS in the ileostomy model with results obtained by using the intubation technique reported previously by Faisant et al (18)

TABLE 1
Menus for study A, ordinary diet, and for study B, plant-polysaccharide-free diet

	Study A: ordinary diet	Study B: plant-polysaccharide-free diet
Breakfast (at 0800)	Yogurt	Yogurt
	Cheese sandwiches	Boiled egg
	Orange juice	Ham, mayonnaise
	Coffee or tea	Orange juice
		Coffee or tea
Snack	Sponge cake	
Lunch (at 1200)	Fillet of pork	Fillet of chicken
	Rice	Loin of pork
		Cheese, mayonnaise
Snack	Sponge cake	Meringue
Dinner (at 1800)	Fillet of plaice	Fillet of pork
	Mashed potatoes	Meringue
Evening snack (at 2000)	Cheese sandwiches	Fillet of chicken
	Coffee or tea	Cheese, mayonnaise
		Meringue
		Coffee or tea

(study B); and *4*) to evaluate the effect of different background diets and different doses of RS on starch excretion in ileostomy subjects (studies A and B).

SUBJECTS AND METHODS

Subjects

Ten ileostomy subjects (5 men and 5 women) with a mean age of 45.9 y (range: 28–70 y) and an average body mass index (BMI; in kg/m2) of 24.1 (range: 19–35) volunteered for the study. The subjects had been previously proctocolectomized for ulcerative colitis, with only a minor part (<10 cm) of the distal ileum removed, and all subjects had well-established ileostomies. All subjects were in good health without symptoms or signs of small-bowel inflammation or dysfunction. The wet weight of ileostomy fluid was, on average, 523 g/d (range: 321–866). All the subjects were nonsmokers and no drugs were taken during or 1 wk before the study. The subjects gave their informed consent to participate in the study, which was approved by the Ethics Committee of Sahlgrenska University Hospital, Göteborg, Sweden.

Study design

The present study consisted of 2 parts, study A and study B. Ten subjects participated in study A and 7 of the 10 subjects participated in study B. No washout period was implemented because all carbohydrates ingested during morning hours are excreted within 24 h (19–21), and only short-term (ie, postprandial) effects on sterol metabolism were investigated in the present study (21, 22). The diets were adjusted to each subject's individual needs. The subjects came to the Metabolic Ward at Sahlgrenska University Hospital, Sweden, each morning to eat breakfast and were then provided with meals for the rest of the day.

In study A, subjects were given a controlled, constant, ordinary Swedish diet with a low content of RS for 4 d. After one run-in-day, subjects were given 30 g/d of either CBF or RBF together with breakfast each study day, or a breakfast without the addition of banana flour, in random order.

In study B, subjects were given a controlled, constant, plant-polysaccharide-free diet for 2 d. On the second day, after one run-in-day, subjects were given 30 g RBF/d with breakfast. Five of the subjects also received, at random, the addition of 15 g/RBF for 1 d.

Test products

Two different test products were used in the present study: RBF, which contains RS_2, and banana flour taken from the same batch of RBF and cooked (ie, CBF). This was done to achieve 2 test products that were as similar as possible, apart from the RS content. RBF was prepared from green bananas from Martinique, purchased locally before gas treatment used to induce ripeness. The bananas were peeled, cut into small slices, freeze-dried, and milled (in a 3 mm sieve). Preparation of the CBF was described previously (18). Thirty grams of banana flour contained 23.1 g α-glucans. According to the method of Englyst et al (2), the RS content for 30 g RBF was 542 g/kg, ie, 16.3 g RS, and that for 30 g CBF was 41 g/kg, ie, 1.2 g RS. The test products were mixed with yogurt and eaten immediately.

Diets

The composition of the diets was calculated from analytic data and from the Swedish food-composition tables (23). All food items were purchased in batches before the study and the same batch was used for consumption by all subjects. The food was prepared in advance in the metabolic kitchen; individual portions of the meals were stored at −18 °C and were thawed and heated on the day of consumption. The subjects were carefully instructed not to leave any food or to eat anything other than the food served. Duplicate portions of each diet were taken for complete analysis. The menus for the diets in studies A and B are shown in **Table 1**. The composition of energy-containing macronutrients of the basal diet in study A was 34% of energy from fat, 16% of energy from protein, and 50% of energy from carbohydrate. The mean analytic composition was 9.3 MJ energy/d, 81 g fat/d, 91 g protein/d, 119 g starch/d, and a calculated amount of 14 g dietary fiber/d.

Sampling procedure

To avoid bacterial degradation of the effluents, ileostomy bags were changed every 2 h during the day from 0600 to 2200. The same bag was kept during the night. Each bag was immediately sealed and frozen on dry ice. The frozen bags were delivered every morning to the metabolic laboratory where the bags were weighed, stored at 18 °C, and freeze-dried to a constant weight. In study A, the dry effluents from each 24-h period (from 0800 to 0800 the next day) were then pooled and homogenized for analysis. In study B, the effluents were analyzed in separate portions for each 2-h period, from 0800 to 2200, and in one portion from 2200 to 0800.

Chemical analyses and physical analysis

Freeze-dried food and ileostomy effluents were analyzed with the same methods and all analyses were performed in duplicate. Nitrogen was analyzed with a modified micro-Kjeldahl technique (19) and crude protein was calculated as N × 6.25. Fat was determined according to van der Kamer et al (24). Samples of freeze-dried ileostomy contents and diets were wet-ashed at 300 °C in a microwave sample preparation system (CEM Corp, Matthews, NC) and dissolved in deionized water

before magnesium, calcium, zinc, and iron were analyzed by atomic absorption spectrophotometry (GBC Scientific Equipment Pty Ltd, Dandenong, Australia) and sodium and potassium were determined by flame photometry. Energy from food and ileostomy contents was determined by means of bomb calorimetry. Approximately 1 g freeze-dried sample was formed into a pellet and ignited in a bomb calorimeter (Gallenkamp Automatic Adiabatic Bomb Calorimeter; Gallenkamp, Leicestershire, United Kingdom).

Bile acids and neutral steroids in ileostomy contents were analyzed as previously described (25). Briefly, internal standards 5-alfa-cholestane (Sigma Chemical Co, St Louis) and hyodeoxycholic acid (Serva Feinbiochemika, Heidelberg, Germany) were added, ileostomy excreta saponified, and bile acids deconjugated by alkaline hydrolysis. After extraction and methylation with 2,2-dimethoxypropane, acid and neutral steroids were separated and quantified by gas-liquid chromatography with the use of a Varian 3700 system (Varian Instrument, Palo Alto, CA). A 12.5 m SE-54 capillary column (Hewlett-Packard, Lite Falls, DE) was used, with hydrogen as the carrier gas and with flame-ionization detection. The cholesterol content of the diet was analyzed by use of the same method.

RS in the test products and diets, in addition to total α-glucans in ileal effluents, including free glucose, maltose, and maltooligosaccharides, was determined by a method adapted from Englyst et al (2), as previously described (18). Dietary fiber was analyzed by the enzymatic-gravimetric method according to Asp et al (26). Oligosaccharide extraction and analysis was performed according to Faisant et al (18), as previously described. Size exclusion chromatography on a Superose 12 column (Amersham Pharmacia Biotech AB, Uppsala, Sweden), X-ray diffraction analysis, and differential scanning calorimetry were performed as previously described (18). Transmission electron microscopy was performed as previously described (18).

In vitro fermentation of ileal effluents

The fermentation pattern of ileal effluents collected from 6 subjects in study A after the addition of 30 g CBF or RBF/d to the diet was investigated by using an in vitro system (27). Each sample was run in duplicate and each fermentation was repeated twice. Fresh human feces was anaerobically collected from healthy volunteers (methane producers, stools from 3 subjects) who had been eating an unspecified Western diet and had received no antibiotic treatment for 3 mo. The inoculum was prepared by mixing the feces immediately with carbon dioxide–saturated nutritive buffer (wt:vol; 1:3), then filtered through surgical gauze to remove fine particles. Ileal effluents were incubated in a serum bottle with 15 mL inoculum.

Because the aim of the present study was to compare the fermentation of the intestinal content that would arrive in the colon of healthy subjects after the consumption of 2 different diets, the same proportion of effluent from each diet period was used as a substrate during the in vitro fermentation. The CBF period was used as a reference; 100 mg fermentable substrate (a mean of 232.5 mg dry effluent and 30.2 g starch) was used. The mean amount of sample used from the RBF period was calculated as 326.6 mg, of which 97.9 mg was starch. The amount of other substances in the samples was 202.3 and 228.6 mg for CBF and RBF, respectively.

Each bottle was sealed, placed in a stirred water bath at 37 °C, and kept under a constant flow of nitrogen for 5 min. Every 0, 4, 8, and 24 h, in vitro fermentation was stopped by the addition of 0.15 mL of a solution of saturated mercuric chloride. The pH was measured immediately. The medium was centrifuged (3000 $\times$ *g*) for 10 min at 20 °C. The supernatant fluid was preserved by adding 1:10 vol:vol of a solution of phosphoric acid (5%) and mercuric chloride (1‰ wt:vol‰) and then was frozen for short-chain fatty acid analysis. The pellets were freeze-dried and weighed for further sugar analysis. Two control tests were conducted, 1 containing inoculum without substrates and 1 containing substrates without inoculum. Gas production was measured during the course of fermentation, and hydrogen and methane were analyzed at the end of each fermentation period (at 8 or 24 h). Individual short-chain fatty acid production was determined at 0, 4, 8, and 24 h. The initial (0 h) concentration of each corresponding short-chain fatty acid was deducted from the other values to achieve the increase in concentration.

Calculations and statistical methods

Values are given as means ±SEMs. The mean cholesterol excretion minus dietary cholesterol is expressed as net cholesterol excretion. Student's paired *t* test was used to compare ileal excretion values between dietary periods. Bonferroni's adjustment of *P* values was used and $P < 0.05$ was considered significant. The software package SYSTAT (version 7.0; Systat Inc, Evanston, IL) was used for calculations. A two-factor analysis of variance of repeated measures in time, by using Tukey's correction for comparisons at different time points, was used for the analysis of short-chain fatty acids from the in vitro fermentation. The SAS software package (version 6.12; SAS Institute, Cary, NC) was used for calculations.

The mean transit time (MTT) of starch was calculated as

$$\text{MTT (h)} = [\sum_{0}^{N}(\text{amount of starch in sample})$$
$$\times\ t]/\text{Total starch recovered} \qquad (1)$$

where *t* is the time interval between the test meal and recovery of starch in the effluents from sample 0 to *N*.

RESULTS

Ileal excretion of starch

In study A, the difference in starch excretion between the addition of 30 g/d CBF compared with RBF to the diet was 15.1 ± 0.6 g/d (**Table 2**). The ileal starch excretion during the period when no test product was added to the diet was 5.0 ± 0.4 g/d. There was a positive correlation between the amount of starch fed and recovered when subjects received only the background diet with no extra starch (**Figure 1**). When 30 g RBF/d was added to the diet (RBF period minus period when no extra starch was added), the additional excretion of starch was 16.4 ± 0.4 g/d.

In study B, the ileal excretion of starch after the addition of 15 and 30 g RBF/d to the plant-polysaccharide-free diet of 5 subjects was 7.7 ± 0.4 and 15.8 ± 0.4 g/d, respectively. There was a negative correlation between the total amount of starch excreted for each subject and mean transit time, with a faster transit time corresponding to a higher amount of RS recovered in the effluents (**Figure 2**).

TABLE 2
Mean ileal excretion of starch and other nutrients after the addition of 30 g cooked green banana flour (CBF)/d or 30 g raw green banana flour (RBF)/d to the diets of 10 ileostomy subjects in study A

	Ordinary diet plus 30 g CBF/d	Ordinary diet plus 30 g RBF/d	P
Wet weight (g/d)	540 ± 50[1]	562 ± 55	NS
Dry weight (g/d)	50 ± 3	69 ± 3	<0.001
Energy (kJ/d)	861 ± 56	1164 ± 51	0.001
Starch (g/d)	6.3 ± 0.4	21.4 ± 0.6	<0.001
Dietary fiber (g/d)	15.3 ± 1.0	14.8 ± 1.1	NS
Fat (g/d)	2.1 ± 0.4	2.5 ± 0.2	NS
Nitrogen (g/d)	1.9 ± 0.1	1.8 ± 0.1	NS
Sodium (mmol/d)	59.6 ± 5.7	59.8 ± 6.5	NS
Potassium (mmol/d)	4.8 ± 0.6	4.8 ± 0.5	NS
Calcium (mmol/d)	24.7 ± 2.4	24.2 ± 2.2	NS
Magnesium (mmol/d)	6.8 ± 0.8	7.6 ± 0.6	NS
Iron (μmol/d)	181 ± 22	198 ± 23	0.02
Zinc (μmol/d)	133 ± 12	141 ± 12	NS

[1] $\overline{x}$ ± SEM.

The extra excretion of starch in study A (16.4 ± 0.4 g/d) when 30 g RBF/d was added to an ordinary diet was compared with the starch excretion during the same period in study B (15.8 ± 0.4 g/d), when 30 g RBF/d was added to a plant-polysaccharide-free diet. There was no significant difference in starch excretion between the periods ($P = 0.74$).

In study A, the α-glucans recovered in the ileal effluents were mainly composed of insoluble starch. Glucose and dextrins (1 < degree of polymerization < 10) were <1% and 1.4%, respectively, after the addition of CBF, and 2% and 13.4%, respectively, after the addition of RBF to the diet.

In the effluents obtained after the addition of RBF to the diet, many intact granules were observed by transmission electron microscopy, most with damaged surfaces (**Figure 3**). No intact granules, but large amounts of cell wall material, were observed in the effluents obtained after CBF was added to the diet.

A B-type X-ray diffraction pattern was observed for ileal effluents obtained after ingestion of RBF and for RBF itself. An amorphous diagram was obtained for CBF.

Size exclusion chromatography was performed to assess the chain length of total α-glucans in the ileal samples. The elution profile of the effluents obtained after the addition of RBF to the diet showed one peak that concentrated at Kav 0, indicating a total exclusion of these molecules from the column.

In study B, the ileal effluents obtained after the addition of RBF to a plant-polysaccharide-free diet were composed of 70.9% insoluble starch and 29.1% oligosaccharides, and glucose and dextrins (1 < degree of polymerization < 10) were 4.3% insoluble starch and 25.8% oligosaccharides, respectively.

Transit time

In study B, the transit time was shorter for starch excretion in the intubation study (2–3 h; 18) than it was in the ileostomy study (6 h).

Ileal excretion of other nutrients

In study A, the dry weight of the ileostomy effluents and the excretion of energy and starch were significantly higher during the period that involved the addition of RBF rather than CBF to the diet (Table 2). The differences in dry weight and energy excretion were 19 ± 1 g/d and 303 ± 33 kJ/d, respectively. There
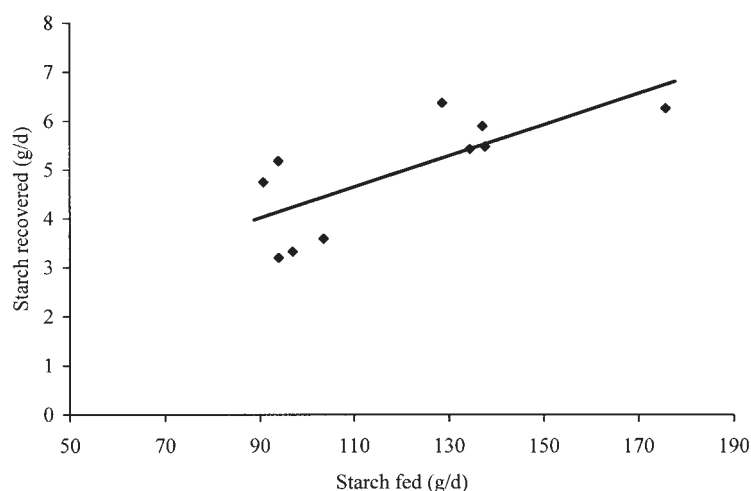

FIGURE 1. Study A. Correlation between starch fed and starch recovered in 10 ileostomy subjects consuming a diet designed to be low in resistant starch and dietary fiber ($r = 0.74$, $P < 0.02$).
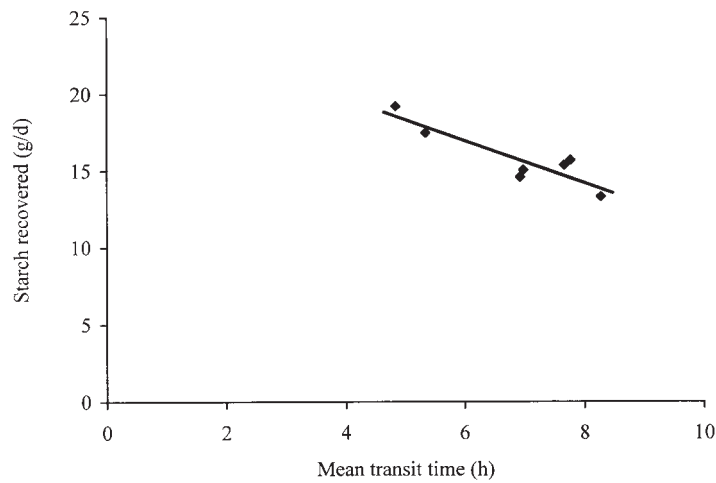
FIGURE 2. Study B. Correlation between starch recovered and mean transit time through the small bowel in 7 ileostomy subjects after the addition of 30 g raw green banana flour/d to a plant-polysaccharide-free diet ($r = -0.90$, $P < 0.01$).

was no significant difference in excretion of fat, nitrogen, sodium, potassium, or zinc between the 2 periods. The ileal excretion of iron, however, was slightly but significantly higher after the addition of RBF compared with CBF to the diet.

Ileal excretion of sterols

In study A, there was no significant difference in the excretion of cholesterol or of total bile acids between the 2 periods (**Table 3**). There was, however, a difference in the excretion of the separate bile acids. The ileal excretion of chenodeoxycholic acid was higher during the RBF period, but there was no significant change in cholic acid excretion. There was no significant difference in the concentration of bile acids in the effluents between the periods.

In vitro fermentation of ileal effluents

In study A, after 24 h of in vitro fermentation, pH was significantly lower and short-chain fatty acid concentrations were significantly higher in the ileal effluents from the RBF period compared with the CBF period because of higher acetate and butyrate concentrations (**Table 4** and **Figure 4**). Additionally, the molar ratio of butyrate was significantly higher whereas the molar ratio of propionate was lower in fermented ileal effluents from the RBF period.

DISCUSSION

In the present study, a linear dose-response relation was found between the amount of starch ingested and the amount recovered in ileal effluents. This is in accordance with the findings of Chapman et al (28) for potato starch in different amounts. It seems that for these types of starches (RS_2), there is a linear dose-response relation up to amounts of ≥ 100 g.

The RS content of RBF found in the present study (52.7%) is close to the in vitro value found by using the Englyst method (54.2%), which is not surprising as the Englyst method was validated on the basis of ileostomy studies (2, 29–31).

The effect of a normal background diet compared with a plant-polysaccharide-free diet has not been investigated previously. In study A, by using an ordinary diet as the background diet, 71% of the α-glucans in the RBF were recovered in the ileal effluents. In study B, with the same subjects and with the use of a plant-polysaccharide-free background diet, 68.4% of the α-glucans from RBF were recovered. Thus, the content of plant polysaccharides in the background diet did not have any major influence on the recovery of α-glucans in the present study.

The ileal excretion of starch from the diet in study A, without any addition of test product, was on average 5 g/d, which is 4% of the amount of starch ingested. This figure is in the same range as the estimation of 4.11 g/d based on in vitro data from European diets (EURESTA; 32), but is lower than the analytic data of 7.16–9.23 g/d from Italy (33). The diet in the present study was designed to be low in RS. Therefore, there is reason to believe that the amount of RS in the common Western diet is high, or that the figures from EURESTA are too low. In other parts of the
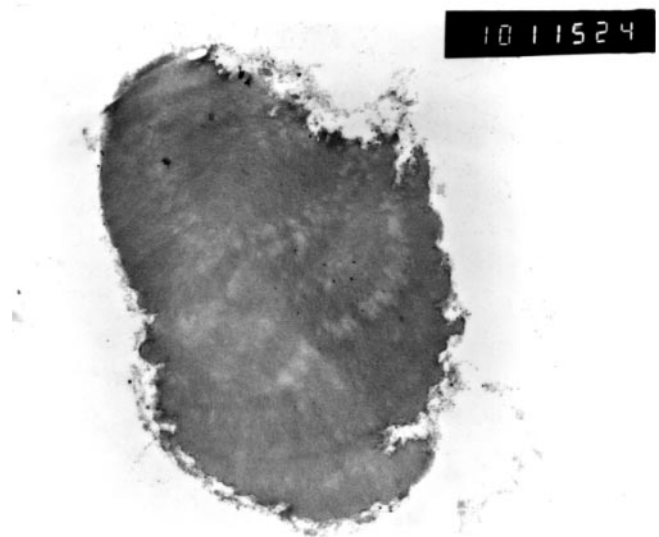

FIGURE 3. Study A. Transmission electron microscopic picture of ileal effluents after the addition of 30 g raw green banana flour/d to the diet in 10 ileostomy subjects. The picture shows an intact starch granule, which is damaged at the surface.

TABLE 3
Mean ileal excretion of sterols after the addition of 30 g cooked green banana flour (CBF)/d or 30 g raw green banana flour (RBF)/d to the diets of 10 ileostomy subjects in study A

	Ordinary diet plus 30 g CBF/d	Ordinary diet plus 30 g RBF/d	P
Net cholesterol (μmol/d)	655 ± 139[1]	604 ± 131	NS
Cholic acid (μmol/d)	763 ± 149	708 ± 113	NS
Chenodeoxycholic acid (μmol/d)	458 ± 67	607 ± 82	0.012
Total bile acids (μmol/d)	1221 ± 215	1314 ± 192	NS

[1] $\overline{x}$ ± SEM.

world, the amount of RS can probably be assumed as being considerably higher in the diet (34). In a study by Platel and Shurpalekar (35) that was based on in vitro measurements of RS, the content of RS in the Indian diet was estimated as 10 g/d.

There was no significant change in fat or nitrogen excretion upon the addition of RBF compared with CBF to the ordinary diet. The difference in energy excretion from the small bowel was 303 ± 33 kJ/d, which is comparable with the difference in starch excretion. Thus, the exchange of CBF for RBF did not change the excretion of energy from other sources than starch.

The effect of RS on the apparent absorption of minerals in the small bowel of humans has not, to our knowledge, been reported before. Supplementation with RS_2 did not significantly change the apparent ileal absorption of magnesium, calcium, zinc, sodium, or potassium. There was, however, a small, but highly significant, reduction in the ileal excretion of iron after the addition of RBF to the diet compared with CBF. Iron availability is highly dependent on the tannin and inositol phosphate content of food products, and there is a possibility that these inhibitors may be broken down, thereby allowing better iron absorption during the CBF period. Analyses of tannin equivalents were performed and showed that the RBF and CBF contained 0.61 and 0.03 mg tannin equivalents, respectively. The higher tannin equivalent content of the RBF could thus explain the higher iron excretion during that period.

The excretion of chenodeoxycholic acid in the present study was significantly higher after the addition of RBF (RS_2). However, total bile acid excretion was not changed and there was no significant change in the excretion of cholesterol. In a previous ileostomy study of retrograded high-amylose cornstarch (RS_3) compared with ordinary cornstarch, we found a lower ileal excretion of cholic acid, but no effect on cholesterol excretion, with the high-amylose cornstarch (21). Our ileostomy data do not support a quantitative change of sterol excretion induced by RS, which is of importance for the sterol balance, but do indicate the possibility of changes in excretion patterns of individual bile acids. The significance of this should be further elucidated.

In a previous study by Faisant et al (18), with the use of the intubation technique, the subjects were given the same breakfast as in the present study B, with the addition of 30 g RBF from the same batch as in the present study. The total mean excretion of α-glucans after the ingestion of 30 g RBF in the present study was 15.8 ± 0.7 g/d (68.4% of ingested α-glucans), which could be compared with the 19.3 ± 0.7 g/d (83.7% of ingested α-glucans) in the earlier intubation study (18). The transit time was shorter in the intubation study than the present ileostomy study. The faster passage and higher total excretion of starch in the intubation study was probably due to the presence of the feeding tube in the small intestine and in the stomach, which increased intestinal motility.

It could, however, be possible that the ileostomy model gives an underestimation of RS because of the loss of the ileal brake or

TABLE 4
Amount of starch in residue, pH, and concentrations and yields of total short-chain fatty acids (SCFA), acetate, propionate, and butyrate after in vitro fermentation of ileal effluents for 24 h in study A[1]

	Ordinary diet plus 30 g CBF/d	Ordinary diet plus 30 g RBF/d	P
Total starch in residue (mg)	0.5 ± 0.5[2]	12 ± 1	0.0004
pH	5.48 ± 0.04	5.12 ± 0.03	0.0001
Total SCFA			
(mmol/L)	107 ± 3.1	120 ± 2.8	<0.0001
(mmol/g ileal substrate)	7 ± 0.2	5.5 ± 0.1	<0.0001
Acetate			
(mmol/L)	54 ± 2	62.3 ± 3	<0.0001
(mmol/g ileal substrate)	3.5 ± 0.1	2.9 ± 0.1	<0.0001
Molar ratio (%)	55 ± 0.8	55 ± 1	NS
Propionate			
(mmol/L)	18.3 ± 0.6	17 ± 0.5	0.0039
(mmol/g ileal substrate)	1.2 ± 0.1	0.8 ± 0.02	<0.0001
Molar ratio (%)	18 ± 0.4	14 ± 0.5	<0.0001
Butyrate			
(mmol/L)	27 ± 0.7	34.8 ± 1.1	<0.0001
(mmol/g ileal substrate)	1.7 ± 0.05	1.6 ± 0.05	NS
Molar ratio (%)	27 ± 0.9	31 ± 0.9	0.0086

[1] CBF, cooked green banana flour; RBF, raw green banana flour.
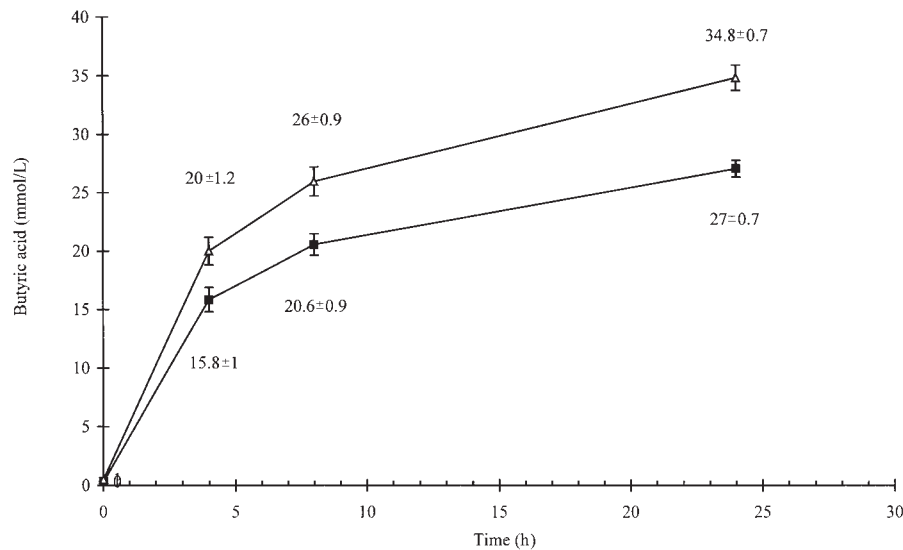[2] $\overline{x}$ ± SEM.

FIGURE 4. Study A. Mean (±SEM) concentrations of butyric acid during in vitro fermentation of ileal effluents obtained from ileostomy subjects after the addition of 30 g cooked green banana flour/d (■) or raw green banana flour/d (△) to the diet. There was a significant difference between the diets at 24 h, $P < 0.0001$.

bacterial fermentation at the end of the ileum as a result of changes in the environment. There is, however, evidence that the loss of the ileal brake is of minor importance (28, 29, 36, 37) and that the production of short-chain fatty acids and lactic acid in ileal effluents corresponds to 2–3 g carbohydrate, of which starch is only a part (38). The underestimation is therefore likely to be small, and it is suggested that the true recovery lies between that of the intubation technique and the ileostomy model, but probably closer to the figures obtained with the ileostomy model.

In vitro fermentation studies that used starch-containing products (13–15) showed increased production of butyrate with RS-containing diets. In the large intestine, starch is fermented together with endogenous products, eg, cells from the small intestine. By using ileal effluents for in vitro fermentation, after the addition of a product high or low in RS to the diet, it is possible to improve the method of in vitro fermentation because the endogenous compounds are present during the fermentation (39–41). In the present study, the mean concentration of total short-chain fatty acids was significantly higher during the fermentation of the effluents obtained after the addition of RBF compared with CBF to an ordinary diet. The increase was attributed to higher concentrations of acetate and, mainly, butyrate. The molar proportion of butyrate was also higher (Table 4 and Figure 4). After fermentation of the high-RS effluents, 58% and 11% of the starch remained after 4 and 24 h of fermentation, respectively, whereas with the low-RS effluents, only 37% and 1% of the starch remained after 4 and 24 h, respectively. Fermentation, especially the production of butyrate, is considered to be of importance for the nutrition of the colonic mucosa (16). It is known that in populations eating a Western diet, fermentation activity decreases at the end of the colon where colon cancer most often occurs (34). Increased intakes of fermentable carbohydrates could be a way of changing the fermentation activity in the colon with possible beneficial effects for the colonic mucosa.

In conclusion, the ileostomy model seems to be the most reliable technique available for measuring RS in vivo. The addition of RBF with a high content of RS_2 to the diet did not influence the excretion of other nutrients or total sterols, except for a small increase in iron excretion, and may have beneficial effects on colonic fermentation. The effects of different carbohydrates on colonic fermentation need to be further studied in vivo.

In the present study, we tested the maximum amount of RS_2 that could be included in the diet. However, practical use of green banana flour would be limited to special functional food products.

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