Fermentation of Resistant Rice Starch Produces Propionate Reducing Serum and Hepatic Cholesterol in Rats

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ABSTRACT This study was designed to investigate the effects of different proportions of rice starch and cornstarch on lipid metabolism in rats fed high dietary cholesterol. Male Wistar rats were fed a 10 g/100 g fat diet containing 1 g/100 g cholesterol with 0 (control diet), 15, 30, 45 or 63 g/100 g rice starch with an enzyme resistant starch concentration of 1.26, 1.39, 1.52, 1.65 or 1.80 g/100 g, respectively, for 4 wk. Groups fed diets with < 63 g/100 g rice starch were supplemented with cornstarch to 63 g/100 g. The two kinds of starch had different structures as seen using scanning electron microscopy (SEM). The rice starch was an aggregation (r = 20–60 μm) of smaller granules (3–8 μm in diameter), whereas the cornstarch was composed of larger (5–15 μm in diameter), single granules. The compound rice starch (0.99 kg/L) was larger in size and denser in structure than cornstarch (0.63 kg/L). Serum total cholesterol concentrations in rats fed both the 45 and 63 g/100 g rice starch diets were significantly lower than in all other groups (P < 0.05). The serum propionate concentration in the rats fed 63 g/100 g rice starch diets was significantly higher than that of other groups. Hepatic triglyceride and total cholesterol concentrations in rats fed 63 g/100 g rice starch diets were significantly lower than in the control group. These results suggest that, because the compound rice starch was an aggregation of smaller granules, larger in size and denser in structure than cornstarch, it was digested more slowly and altered lipid metabolism. Resistant rice starch may be fermented to produce propionate, which reduces serum and hepatic cholesterol. J. Nutr. 130: 1991–1995, 2000.

KEY WORDS: rat • rice starch • resistant starch • propionate • cholesterol

In the past, when scholars studied the metabolic relationship between multiple carbohydrates and lipids, they tended to focus on dietary fiber. Recently, however, researchers have discovered that some starch cannot be digested. This fraction is termed resistant starch because of its resistance to amylase degradation (Englyst et al. 1992). Cereals are not only a rich source of starch but also of dietary fiber, and can reduce the danger of arterial blood diseases (Anderson 1969, Anderson and Gustafson 1988, Cheng and Yu 1997). However, different cereal starches have different properties and structures as well as a range of physical and chemical properties. Rice is the staple food in many Asian cultures and also the main source of carbohydrate. Resistant starch in cornstarch has been shown to reduce serum cholesterol in rats (De Deckere et al. 1992). Englyst et al. (1992) classified starches according to three categories of digestibility, i.e., rapidly digestible starch, slowly digestible starch and resistant starch. The last-mentioned category is further differentiated by whether the starch is inaccessible to enzymes, contains chemically resistant starch granules or is retrograded starch.

Resistant starch lowers plasma lipids in rats, but some types do not do so in humans (Noakes et al. 1996) or pigs (Topping et al. 1997). It appears that rats may differ fundamentally from humans, because resistant starch lowers plasma lipids in that species (Levrat et al. 1996), whereas in humans, resistant starch lowers fecal bile acid excretion (Langkilde et al. 1998).

Topping et al. (1997) published micrographs of starch granule etching, and Marsono et al. (1993) studied resistant starch in the porcine large bowel. Muir et al. (1998) examined a shift to a high rice starch Chinese diet in humans and reported on the unexpected nature of the changes. Chen et al. (1984) reported that dietary propionate reduced cholesterol accumulation in both serum and liver of cholesterol-fed rats.

Very little work has been conducted investigating the effects of resistant rice starch levels with cholesterol supplementation on lipid metabolism, or using scanning electron microscopy (SEM) to observe the physical properties of undigested starch in feces. The objective of this research was to evaluate the effects of resistant rice starch levels with cholesterol supplementation on lipid metabolism, or using scanning electron microscopy (SEM) to observe the physical properties of undigested starch in feces. The objective of this research was to investigate the effects of different resistant starch levels with cholesterol supplementation on lipid metabolism, or using scanning electron microscopy (SEM) to observe the physical properties of undigested starch in feces. The objective of this research was to investigate the effects of different resistant starch levels with cholesterol supplementation on lipid metabolism, or using scanning electron microscopy (SEM) to observe the physical properties of undigested starch in feces.

### MATERIALS AND METHODS

Male Wistar rats (Animal Center of Taiwan University Medical College) weighing ~202 g each, were housed individually in wire-bottomed stainless steel cages in a temperature- and humidity-controlled room (at 22°C), with a 12-h light/dark cycle and free access to food and water. All animal experimental procedures followed the published guidelines (National Science Council 1994). The rats were then divided randomly into five groups, each fed a different diet containing 1 g/100 g high cholesterol. The five different diet treatment groups were divided on the basis of five different kinds of carbohydrate source as follows (Table 1): 1) C, 63% cornstarch (AIN-76 diet); 2) R15, 15% rice starch + 48% cornstarch; 3) R30, 30% rice starch + 33% cornstarch; 4) R45, 45% rice starch + 18% cornstarch; and 5) R63, 63% rice starch.

The cereal husks of Japonica rice (Taiwan No. 67), amylase content 18.6%), were removed by a rice-husking machine in the laboratory to yield polished rice. Polished rice was ground with approximately equal amounts of aqueous 0.025 mol/L sodium hydroxide by weight at 5°C in a blender. The filtrate was centrifuged at 3875 × g for 3 min at 4°C. The precipitate obtained was continuously washed with water on a glass filter until the supernatant did not cause a protein reaction; then it was dried in a oven at 50°C. The rice starch, with added water, was cooked in an electrical cooker [water:rice (wt/wt) = 1:1], and then dried under hot air at a temperature of 60°C for 16 h. The amylose content of cornstarch, purchased from Roquette Freres Lestrem USP (Lestren, France), was 25%. The contents of all five diets were then ground and sifted using a 0.42-mm diameter mesh analytical sifter.

The diet content was a modified AIN-76 diet (Bieri et al. 1977 and 1980; Table 1) in which carbohydrates account for 63 g/100 g diet protein for 20 g/100 g, and fat for 10 g/100 g. Casein and soybean oil were used to make the total energy and the proportions of the three major nutritional elements of each diet treatment the same. The contents of the diets were homogenized, placed in plastic bags, tightly sealed and refrigerated at 4°C. The test diets were fed to groups of rats (n = 7/group) for 4 wk. During the 4-wk feeding period, feces were collected once every 2 d and stored at −20°C until analysis. Food was withheld for 12 h at the end of wk 4. The rats were anesthetized with 1 g/L sodium pentobarbital and dissected. Blood was collected from the abdominal aorta, incubated at room temperature for 45 min and centrifuged at 4000 × g for 15 min. The serum was then stored in a freezer at −70°C. The livers were excised, rinsed in 9 g/L NaCl solution and stored tightly sealed at −70°C.

Fecal moisture contents were analyzed according to the AOAC (1980) method. Resistant starch was measured according to the method of Berry (1986). Proximate compositions of cereals and diets are shown in Table 1. The serum triglyceride concentration was determined as described by Megowan et al. (1983), and the procedure of Richmond (1973) was used to determine serum total cholesterol concentration. Phosphotungstic acid and magnesium were added to the serum, causing VLDL and LDL to precipitate, and the sample was then centrifuged at 4000 × g for 15 min. The upper liquid contained HDL; using the method of Richmond (1973), the HDL cholesterol concentration was measured.

Heparin and sodium citrate were added to the serum, causing LDL to precipitate; the serum was then centrifuged for 15 min at 4000 × g. The upper liquid contained VLDL; using the method of Richmond (1973), by subtracting the cholesterol content of the upper liquid from the total cholesterol level, the LDL cholesterol concentration could be calculated.

The frozen liver was defrosted according to the method of Foch (1957). Liver (1.5 g) was cut and extracted with 20 mL chloroform/methanol (2:1, v/v). After the addition of 4 mL of a 0.5 g/L CaCl

### TABLE 1

##### Composition of the experimental diets

<table>
<thead>
<tr>
<th>Group</th>
<th>C</th>
<th>R15</th>
<th>R30</th>
<th>R45</th>
<th>R63</th>
</tr>
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<tr>
<td>Ingredient</td>
<td>C</td>
<td>R15</td>
<td>R30</td>
<td>R45</td>
<td>R63</td>
</tr>
<tr>
<td>Rice starch</td>
<td>0</td>
<td>150</td>
<td>300</td>
<td>450</td>
<td>630</td>
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<tr>
<td>Cornstarch</td>
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<td>480</td>
<td>330</td>
<td>180</td>
<td>0</td>
</tr>
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<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
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<tr>
<td>Soybean oil</td>
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<td>35</td>
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<td>35</td>
</tr>
<tr>
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<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>α-Methionine</td>
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<td>10</td>
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<tr>
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<td>3</td>
<td>3</td>
<td>3</td>
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</tr>
<tr>
<td>Enzyme-resistant starch</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

1 Diet abbreviations: C, 63% cornstarch + 1% cholesterol (AIN-76 diet + 1% cholesterol); R15, 15% rice starch + 48% cornstarch + 1% cholesterol; R30, 30% rice starch + 33% cornstarch + 1% cholesterol; R45, 45% rice starch + 18% cornstarch + 1% cholesterol; R63, 63% rice starch + 1% cholesterol. All diets contained the same kind of Japonica rice (Taijun No. 67), which was provided by the Taiwan Agricultural Research Institute. Cornstarch was purchased from Roquette Freres Lestrem USP (Lestren, France).

2 All values, except enzyme-resistant starch, are diet formulations.

3 Rice starch was isolated from TNU-67 polished rice. Cornstarch was the same as that used in the AIN-76 diet.

4 Soybean oil was procured from Tung Yi (Taipei, Taiwan). Casein, α-cellulose, AIN-76 mineral mixture, and AIN-76 vitamin mixture were procured from ICN Biochemicals (Costa Mesa, CA). α-Methionine, choline bitartrate, and cholesterol were procured from Sigma Chemical (St. Louis, MO).

5 AIN (Bieri et al. 1977 and 1980).

6 Enzyme-resistant starch was measured by the method of Berry (1986). Values are means ± SEM (n = 3).
solution to the extract, it was collected in a sample flask and stored. The liver lipid extract was measured accurately into a glass tube with a screw top, according to the method of Soloni (1971), compared with a triolein standard solution for calculations and used to determine the hepatic triglyceride concentration. The liver lipid extract was determined according to the method of Carlson and Goldfarb (1977); liver total cholesterol concentrations were determined according to the method of Carlson and Goldfarb (1977). Liver total cholesterol concentrations were determined according to the method of Carlson and Goldfarb (1977) and the enzymatic method of Richmond (1973) was used to determine liver total neutral steroids concentration.

Fecal lipids were extracted by addition of ethanol, then concentrated and dried using a vacuum process. Petroleum ether was added to remove the extract. The residue was evaporated completely; methanol was added and the sample collected in a flask. The method of Mashige et al. (1981) was used to determine fecal bile acids.

Fecal starch was selected using an iodine solution, i.e., iodine and sodium iodide dissolved in 50 mL purified water, with additional purified water added to make 100 mL. Dried fecal starch was then loaded onto aluminum studs and coated with gold for 3 min at 8 mA under a pressure of 13.3 Pa. The samples were scanned and examined using a Hitachi model S-2400 SEM (Tokyo, Japan).

Short-chain fatty acids (SCFA) were measured by gas-liquid chromatography using serum samples after ethanolic extraction (Demigné and Rémy 1982).

**Statistical analysis.** Differences among treatment group means were assessed by one-way ANOVA (SAS Institute, Cary, NC). Group means were considered to be significantly different at P < 0.05.

### RESULTS

The daily food intake during the experimental period did not differ among groups [19.8 g/(rat ⋅ d), pooled SEM 0.05 g]. Weight gain also was unaffected by diet (140.9 g, pooled SEM 6 g).

Serum total (r = −0.94) and LDL cholesterol (r = −0.67) concentrations decreased with increasing dietary rice starch (P < 0.05). Propionate was not detected in serum of rats fed the R and R15 diets, but rats fed the R30, R45 and R63 diets had propionate concentrations that increased with increasing dietary rice starch (r = 0.95; P < 0.05) (Table 2).

Rats fed the R63 diet had lower liver weights than the C, R15 and R30 groups (P < 0.05). The hepatic total cholesterol concentrations of the R30, R45 and R63 groups were significantly lower than those of the C and R15 groups. The hepatic triglyceride concentration of rats fed the R63 diet was significantly lower than that of rats fed the control diet (P < 0.05) (Table 2).

In rats fed the R45 or R63 diet, fecal moisture contents were greater than those of other groups. Rats fed the R30 and R45 diets had significantly lower fecal total neutral steroid excretions than those of the C and R15 groups (Table 2).

The two kinds of starch had different structures as seen by SEM (Figs. 1, 2). The rice starch seemed to be an aggregation (n = 20–60) of smaller granules (3–8 μm in diameter), whereas the cornstarch was composed of larger (5–15 μm in diameter), single granules. The compound rice starch (0.99 kg/L) was larger in size and denser in structure than the cornstarch (0.63 kg/L).

**Figures 3 and 4 show results of SEM of cornstarch or rice starch from fecal material after rats were fed the cornstarch (C) or rice starch (R63) diets for 4 wk.**

### DISCUSSION

We examined the effects of the consumption of rice starch on a number of variables in rats. One of the potentially important effects of rice starch is the lowering of serum cholesterol concentrations with the attendant reduction in the risk of coronary disease. Rice starch also reduced the level of liver cholesterol and triglycerides, i.e., the higher the dietary rice starch concentration, the lower the liver triglycerides.

Resistant starch, so termed because of its resistance to amylase degradation, lowers plasma lipids in rats, but not all resistant starches have the same effect in humans (Noakes et al. 1996) or pigs (Topping et al. 1997). Cereals starches, which differ greatly in structure and chemical properties, can reduce
the danger of arterial blood diseases (Anderson 1969, Anderson and Gustafson 1988, Cheng and Yu 1997). This research examined the effects of the intake of different ratios of rice starch to cornstarch on serum and liver lipids in rats by feeding the AIN-76 diet with cholesterol supplemented at a high level. Resistant starch in cornstarch has been shown to reduce serum cholesterol in rats (De Deckere et al. 1992, Morand et al. 1992). A shift to a high rice starch Chinese diet (Muir et al. 1998) in humans resulted in unexpected changes.

Examination of starch granules of different sizes from cassava and corn suggests that the smaller the granule, the greater the extent of in vitro digestion by bacterial α-amylases and fungal amylglucosidase (Franco and Ciacco 1992).

We used SEM to observe the structure of rice and cornstarch in diets, and undigested starch in feces. The diameter of rice starch granules seemed to be an aggregation (n = 20–60) of smaller granules, whereas the cornstarch was composed of large (5–15 μm), single granules. The compound rice starch was larger in size and denser (0.99 kg/L) in structure than cornstarch (0.63 kg/L); it was digested and absorbed more slowly. We also observed undigested rice (R64) and cornstarch (C) in feces. The 45 and 63% rice starch diets favored higher propionic acid fermentations.

Propionate may inhibit the synthesis of fatty acids in the liver (probably through competition with lactate), thereby lowering the rates of triacylglycerol secretion (Chen et al. 1984). Propionate also may be involved in the control of hepatic cholesterol synthesis. It has been proposed that rice starch lowers plasma cholesterol concentrations by inhibiting hepatic cholesterogenesis via propionate formed through large-bowel fermentation (Chen et al. 1984).
Resistant starch has been defined by Berry (1986) and Englyst et al. (1987) as starch that escapes small intestinal digestion, but the actual definition (and that used in Table 1) is a chemical one. It is possible that starch, not measured by the method of Berry (1986), may also reach the small intestine. It appears inappropriate to conclude that the additional serum propionic acid (40–60 μmol/L) in rats fed R30, R45, and R60 was derived from the modest increase in resistant starch in the diet (see Table 1). The 1.2–6% increase in rats fed RS in these three diets vs. the other two (assuming a baseline RS value of 13.0%) amounts to 0.1–0.6 g fermentable carbohydrate (19.8 g/d × % increase in RS). However, in this study, it might be appropriate to define RS as an enzyme-resistant starch. Such dietary fibers may be defined in two ways, i.e., by an analytical approach and by a physiologic approach.

Rice starch cannot be completely digested by enzymes in the small intestine. Cheng and Yu (1997) reported that little of the rice starch was exerted into the feces, indicating that most of the 63% rice starch diet was fermented by bacteria in the intestines of rats. Resistant starch is fermented by the gut microflora into SCFA in the large intestines (Demigne and Rémésy 1982). Chen et al. (1984) reported that the propionate generated by bacterial fermentation of fibers reduced cholesterol accumulation in both serum and liver of cholesterol-fed rats. The hypocholesterolemic effect of propionate may be related to altered hepatic cholesterol synthesis. Nishina and Freedland (1990) reported that the effect of propionate on lipid metabolism is apparently limited to inhibition of de novo fatty acid synthesis. Because rice starch can generate SCFA by fermentation, some researchers (Suzuki and Kajuu 1983) determined that SCFA can inhibit the synthesis of hepatic triglycerides and reduce serum lipids. Nishina and Freedland (1990) showed that propionate can inhibit the activity of pyruvate dehydrogenase in liver and thus reduce the synthesis of fatty acids. Hara et al. (1999) pointed out that a decrease in hepatic cholesterol synthesis rate contributes mainly to the lowering of plasma cholesterol in rats.

In summary, the results presented here indicate that resistant rice starch was fermented to produce propionic acid, which reduced serum total cholesterol, serum LDL cholesterol, hepatic cholesterol, and hepatic triglyceride in rats.

LITERATURE CITED


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