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Resistant starch suppresses postprandial hypertriglyceridemia in rats

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ABSTRACT

Postprandial increase in blood triglyceride levels is an independent risk factor for coronary artery disease, and dietary resistant starch (RS) is increasingly being considered for its contribution to disease prevention. Specifically, RS has beneficial effects on of the glycemic index, diabetes, cholesterol levels, and weight management. However, the effects of once-daily intake of RS on postprandial hypertriglyceridemia remain poorly characterized. In this study, the effects of a single administration of cornstarch-derived RS on postprandial increases in blood triglyceride levels were investigated in rats using oral fat tolerance/loading tests. Following the administration of lipid meals, increases in serum triglycerides levels were significantly reduced in rats fed corn oil containing 500 mg/mL RS. Moreover, fecal lipid volumes and wet weights following lipid meals were significantly greater in rats fed corn oil containing 500 mg/mL RS than in the corn oil only group, confirming the inhibition of dietary fat absorption. Finally, a significant positive correlation was observed between fecal lipid contents and wet weights in rats administered RS. These results suggest that RS intake with dietary fats induces defecation and confirm results of recent reports on the health-promoting potential of once-daily RS intake.

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1. Introduction

Dietary starches are the major nutritional source of carbohydrates for humans and are important sources of energy in many human societies (Rantnayake & Jackson, 2008). Starches are classified as rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS), according to the rates and degrees of digestion (Englyst, Kingman, & Cummings, 1992), and starchy foods may contain varied proportions of these fractions. Previous studies have suggested that RDS contributes to chronic disease in humans, and because of this problem, starches that are more resistant to digestive enzymes, such as SDS and RS, have been the focus of a growing research emphasis (Birt et al., 2013). Accordingly, RS has gained recognition as a source of dietary fibers (Fuentes-Zaragona, Riquelme-Navarrete, Sánchez-Zapata, & Pérez-Álvarez, 2010), and as a functional food, RS has desirable physicochemical properties including white appearance, bland flavor, swelling, gel formation, increased viscosity, and water-binding capacity (Sajilata, Singhal, & Kulkarni, 2006).

RS is naturally present in various foods and can also be added to domestic and industrial food preparations. Distinct classes of RS include (1) physically inaccessible starches (RS_1), which are found in whole or partly milled grains or seed; (2) raw starch granules and high-amylose starches (RS_2), which are found in bananas and potatoes as well as high-amylose corn, respectively; (3) retrograded starches (RS_3), which are either processed from unmodified starch or are the products of food processing; and (4) starches that are chemically modified to increase resistance to enzymatic digestion (RS_4), which include starch ethers, starch esters, and cross-linked starches. The general physiological behaviors of these types of RSs are similar to those of soluble fermentable fibers (Rantnayake & Jackson, 2008).

RS intakes vary between countries, and whereas Africans consume 20–30 g/day and Chinese consume 15 g/day, dietary RS intakes among Europeans and Americans are 3 and 3–8 g/day, respectively. Dietary RS sources predominantly include cereals (approximately 50%) such as bread, corn flakes, pastas, and vegetables (Chen et al., 2010; Maziarz et al., 2013; Murphy, Douglass, & Birkett, 2008).

Blood triglyceride levels are elevated in the postprandial state, and previous studies have indicated that the size of this increase is an independent risk factor for coronary artery disease (Gotto, 1998; Vogel, Corretti, & Plotnick, 1997). Dietary triglycerides are primarily hydrolyzed by pancreatic lipase in the proximal part of the small intestine, giving rise to *sn*-2 monoacylglycerols and free fatty acids. The products of fat digestion are solubilized by bile acids, which are synthesized in the liver from cholesterol and are combined with glycine and taurine to form mixed micelles that absorb dietary fats (Bernbäck, Bläckberg, & Hernell, 1990). Free fatty acids that are absorbed from the intestinal lumen are transported to lymphatic vessels and are then taken up into the liver and released as very low-density lipoprotein (VLDL; Iqbal & Hussain, 2009). Following the emulsification of bile with fat in the small intestine, most bile acids are actively reabsorbed through the terminal ileum into the enterohepatic circulation after digestion.

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Alternatively, bile acids can be trapped by dietary fibers in the large intestine and are then excreted with the feces.

RS has been shown to bind bile acids, and characterized disease-preventive effects of RS include the modulation of glycemic index and diabetes, cholesterol-lowering capability, and weight management (Sajilata et al., 2006). However, the effects of once-daily intake of RS on postprandial hypertriglyceridemia have not been previously shown. The oral fat tolerance test (OFTT) or oral fat loading test is a widely clinical tool for assessing the risk of coronary artery disease (Egashira et al., 2013; Kimura, Natsusaka, Endo, & Fujimoto, 2004; Mattes, 2002; Volek et al., 2001). Hence, in the present study, we performed OFTTs and investigated the effects of a single administration of RS on increases in postprandial blood triglyceride levels in rats fed high-fat meals. In addition, we examined *in vitro* bile acid-binding capacities and lipase inhibitory effects of RS and determined effects on fecal parameters, such as bile acid and lipid levels, *in vivo*.

2. Materials and methods

2.1. Sample materials

Cornstarch-derived RS (Amylofiber ®SH) was obtained from J-OIL MILLS (Tokyo, Japan). This product was produced using the methods described by Nagata, Kobayashi, Goto, Nakaura, and Inouchi (2013) and contains approximately 70% (w/w) RS_2.

2.2. Bile acid-binding capacity in vitro

Bile acid-binding capacities of RS were measured using the method of Eastwood, Anderson, Mitchell, Robertson, and Pocock (1976) with modifications, and the ratios of RS to Questran® containing approximately 45% cholestyramine (Sanofi; Tokyo, Japan) were determined. Briefly, RS (5, 15, or 30 mg) or cholestyramine (5 mg) was added to bile acid mixtures containing cholic acid, deoxycholic acid, chenodeoxycholic acid, and taurocholic acid at 1 mM in 1 mL of 0.1 M phosphate buffer (pH 6.8) and was incubated at 37 °C for 1 h in a shaking water bath. Supernatants were then centrifuged at 1750 ×*g* for 10 min, and bile acid contents were determined using an enzymatic assay kit (total bile acid test kit; Wako, Osaka, Japan).

2.3. In vitro lipase activity assays

The inhibition of pancreatic lipase activity by RS was measured using a Lipase Kit S (DS Pharma Biomedical, Osaka, Japan) with modifications in the manufacturer's instructions. Briefly, 0.1, 1, 10, and 100 mg/mL RS in ethanol was added to a coloring solution containing lipase from porcine pancreas (Wako) and an esterase inhibitor in 0.1 M citric acid buffer. Mixtures were incubated at 30 °C for 5 min and were then reacted with the substrate solution at 30 °C for 30 min in the dark. After the reactions were stopped, lipase activities were determined using a spectrophotometer at 412 nm.

2.4. Oral fat tolerance tests

Animals were handled in accordance with the guidelines established by the Institution Animal Care and Use Committee of the Tokyo University of Marine Science and Technology. Four-week-old male Sprague–Dawley rats were purchased from SLC Japan (Shizuoka, Japan) and were assigned to four experiment groups ($n = 4$) of equal weights (average, 153 g) after feeding on a commercial CE-2 pellet diet (Clea Japan, Tokyo, Japan) for 7 days. Animals were individually housed at 23 ± 2 °C with a 12-h light/12-h dark cycle, and water and food were provided *ad libitum*.

OFTTs were performed in rats as previously described (Egashira et al., 2013; Kimura et al., 2004). Briefly, lipid meals were prepared with corn oil (Wako) containing 0 (control group), 100, 250, or 500 mg/mL RS, and meals were orally administered at 5 mL/kg body weight after overnight fasting. Blood samples were collected from the tail veins before and 1–6 h after the administration. Serum triglyceride levels were determined using the TG E-test (Wako).

2.5. Measurements of fecal components

2.5.1. Animals

Four-week-old male Sprague–Dawley rats were purchased from SLC Japan and were assigned to four experimental groups of equal weights (157 g; $n = 7$) after feeding on a commercial CE-2 pellet diet for 7 days. Subsequently, animals were fed corn oil alone (control group) or 250, 500, or 1000 mg/mL RS at 5 mL/kg body weight after overnight fasting. Fecal samples were collected 16 h before and 5–16 h after the administration.

2.5.2. Fecal total lipids and moisture contents

Fecal pellets from individual rats were pooled and lyophilized. The contents of fecal total lipids, such as triglyceride and cholesterol, were determined in sufficient volumes of powdered feces (150 mg) using the gravimetric method described by Folch, Lees, and Sloane Stanley (1957). Stool moisture contents were determined by the difference between fresh fecal weights and dry fecal weights.

2.5.3. Fecal bile acid contents

Lyophilized and powdered feces (150 mg) were suspended in 5 volumes of 99.5% ethanol (Wako), were incubated at 70 °C for 1 h, and were then centrifuged at 3500 ×*g* for 15 min. Bile acid contents were then determined using a total bile acid test kit as described above.

2.6. Statistical analysis

Values were expressed as the means ± standard errors of the mean (SE). Differences between the treatment groups were identified using a two-tailed Student's *t*-test or Dunnett's multiple comparison tests and were considered significant when $^{*}p < 0.05$ or $^{**}p < 0.01$.

3. Results and discussion

3.1. RS bound bile acids but did not inhibit pancreatic lipase activity in vitro

Cholestyramine was used as a positive control, and binding values of 5, 15, and, 30 mg/mL RS were 7.2 ± 0.4%, 30.1 ± 0.9%, and 40.7 ± 2.6%, respectively, relative to that of cholestyramine (100%; Fig. 1). However, pancreatic lipase activities were not inhibited by RS doses of 0.1–100 mg/mL (Fig. 2).

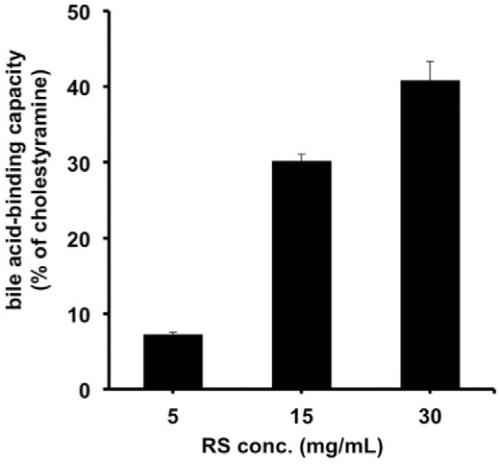

Fig. 1. *In vitro* bile acid-binding capacity of RS at 5, 15, and 30 mg/mL relative to 100% bile acid binding by cholestyramine. Values are presented as means ± SE ($n = 6$).

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