Effect of high-amylose starch on carbohydrate digestive capability and lipogenesis in epididymal adipose tissue and liver of rats

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This study was conducted to clarify whether feeding high-amylose cornstarch would delay digestion and absorption of the starch and lead to a decrease in lipogenesis in epididymal adipose tissue and liver. Two groups of five or six male Wistar rats were fed defined diets ad libitum for 14 days. The control group received a diet containing 53.7% standard cornstarch rich in amylopectin (control diet) and the experimental group received a diet containing 53.7% high-amylose cornstarch (70% amylose). Food intake during the experimental period did not differ between the two groups. Feeding the high-amylose diet resulted in significantly lower activities of sucrase, isomaltase, and maltase in the upper jejunum than in the animals fed the control diet. However, in the lower part of small intestine, disaccharidase activities were significantly elevated in the rats fed the high-amylose diet compared with those fed control diet. The activities of lipogenic enzymes, i.e., fatty acid synthetase, malic enzyme, and glucose-6-phosphate dehydrogenase were significantly lower in the liver as well as in the adipose tissue of the animals fed the high-amylose diet compared with the control group. The weights of both epididymal and mesentery adipose tissues were reduced by 30% in rats fed the high amylose diet, and the serum concentration of triglycerides was also reduced in rats fed the high-amylose diet. These results suggest that digestion and absorption of high-amylose starch may be slower than low-amylose starch and feeding a diet rich in amylose might produce lower glycemic response, consequently leading to declined lipogenesis in adipose tissue and liver.
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Keywords: starch; amylose; disaccharidase; lipogenic enzymes

Introduction

Physical form of complex carbohydrate was shown to be of particular importance in determining the postprandial glucose and insulin responses to rice.[1,2] Comparing glucose and insulin responses to meals of different variety of rice containing different levels of amylose, a straight-chain starch, Goddard et al.[3] demonstrated that the ingestion of rice containing 23 to 25% of carbohydrate in the form of amylose produced flatter glycemic and insulin responses than the consumption of rice without amylose starch.[3] Behall et al.[4] showed that the meal consisting of starch crackers made of high amylose (70%) cornstarch resulted in flatter glucose and insulin responses after amylose meal than after the meal made of regular cornstarch consisting of 30% amylose. They further demonstrated that long-term feeding (5 weeks) of the diet with the high amylose content in human subjects led to lower fasting plasma levels of triglycerides during the period when amylose was consumed.[5] These observations suggested that long-term intake of dietary amylose may be valuable in decreasing insulin response, which might lead to metabolic changes in lipogenesis.

Comprehensive studies have shown that feeding a high carbohydrate diet enhances lipogenesis in the liver[6–8] and in the adipose tissue.[9] The increased lipogenic enzyme activities with a high carbohydrate diet are mediated by hyperinsulinemia.[6] It is also known that the type of carbohydrate influenced the lipogenic enzyme activities; sucrose-fed rats had greater lipogenic enzyme activities in the liver than the starch-fed rats,[10] and that plasma insulin response to sucrose

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feeding is greater than to starch feeding.[11] Furthermore, inhibiting carbohydrate absorption by a glucosidase inhibitor (acarbose) causes a delay in energy accumulation during refeeding of rats, both in glycogen stores and more markedly in adipose tissue triglyceride stores.[9] Feeding acarbose-containing diet causes decrease in disaccharidase activities as well as their immunoreactive amounts in the small intestine,[12] suggesting a substrate-dependent response of intestinal digestive capability.

However, it was not clear whether small intestinal absorptive cells can respond to the intake of starch with reduced digestibility, and whether feeding the diet consisting of starch subjected to slower digestion can modulate lipogenic enzyme activities in the liver and the adipose tissue. Therefore, we considered it pertinent to examine whether the delay of carbohydrate digestion and absorption brought by feeding a high amylose diet might produce a decline of lipogenesis in epididymal adipose tissue as well as liver in the rat.

Methods and materials

Animals and diets

Wistar male rats, 6 weeks of age, were purchased from Japan SLC Inc. (Hamamatsu, Japan). They were housed in individual wire cages in a temperature- and humidity-controlled room (23° C, 53%) under a light cycle with 12 hours of darkness from 6:00 p.m. to 6:00 a.m. The animals had free access to a standard laboratory diet (MF, Oriental Yeast Co., Tokyo, Japan) for 10 days until the start of experimental diets. Five or six rats were used in the two experimental subgroups. The control group received a diet containing 53.7% regular cornstarch (α-type), which consists of 20 to 36% amylose[13] (control diet). The experimental group received a diet containing 53.7% high-amylose cornstarch (α-type), which consisted of 70% amylose (high-amylose diet). The details of the diet compositions are shown in *Table 1*. The animals were allowed free access to the diets and water for 14 days. At the end of feeding period, the animals were killed by decapitation between 10:00 a.m. and noon. Blood samples were collected and the entire small intestine, liver, epididymal fat pad (epididymal adipose tissue), and mesentery fat pad were immediately collected. The experimental procedures used in the present study met the guidelines for animal usage of the committee of the University of Shizuoka.

Table 1 Diet compositions

Ingredient	Control	High-amylose
	g/100g	
Cornstarch*	53.7	—
High amylose cornstarch*	—	53.7
Casein	16.0	16.0
Lard	15.0	15.0
Corn oil	5.0	5.0
Cellulose†	6.3	6.3
AIN[76] mineral mixture	2.8	2.8
AIN[76] vitamin mixture	0.8	0.8
DL-methionine	0.24	0.24
Choline bitartrate	0.16	0.16

*α-cornstarch was purchased from Oriental Yeast Co., Tokyo, Japan and α-high amylose cornstarch (HS7) from Honen Corporation, Tokyo, Japan.
†Cellulose powder "D" (Toyo Roshi Co., Tokyo, Japan).

Intestinal disaccharidase assay

The duodenum was discarded, and the jejunoileum was divided into four segments of equal length, referred to as upper jejunum, lower jejunum, upper ileum, and lower ileum, respectively. After each segment was flushed with ice-cold saline, mucosa was scraped from each segment with a glass microscope slide. Intestinal mucosa was homogenized in 10 volumes (vol/wt) of ice-cold 10 mmol/L potassium phosphate buffer (pH 7.0). Sucrase, isomaltase, and maltase activities were assayed according to Dahlqvist[14] using 28 mmol/L sucrose, palatinose, and maltose as substrate, respectively. Lactase activity was assayed according to Koldovský et al.[15] Protein was measured by the method of Lowry et al.[16] using bovine serum albumin as a standard.

Lipogenic enzyme assay

The fresh tissues of liver and epididymal fat pad were subjected to the lipogenic enzyme assays. All tissue preparations were performed at 0 to 4° C. Two g liver or 1 g epididymal fat pad was homogenized into two volumes (vol/wt) of 0.1 M potassium phosphate buffer (pH 7.4) containing 0.25 M sucrose, 0.07 M $KHCO_3$, 1 mM EDTA, and 1 mM dithiothreitol. The homogenate was centrifuged at 8,000*g* for 20 min, and the resulting postmitochondrial supernatant was centrifuged at 105,000*g* for 60 min. The clear supernatant was removed with care being taken not to disturb the pellet or the floating fat layer. Aliquot of the soluble supernatant (cytosol) was used to determine fatty acid synthetase (FAS) activity, and the remaining supernatant was kept at −20° C until use. Determination of malic enzyme (ME) and glucose-6-phosphate dehydrogenase (G-6-PDH) activities were carried out within 2 days. FAS, ME, and G-6-PDH activities were assayed spectrophotometrically as previously described.[17] The activities were expressed as nmoles of NADPH produced or decreased per minute per milligram of cytosol protein.

Other assays

Serum triglycerides were assayed using an assay kit containing lipoprotein lipase, glycerol-3-phosphate oxidase, and glycerokinase (Triglyceride E-test, Wako Pure Chemical Industries, Osaka, Japan). Serum total cholesterol was assayed using an assay kit containing cholesterol esterase and cholesterol oxidase (Cholesterol E-test, Wako Pure Chemical Industries).

Statistics

All results were subjected to one-way analysis of variance. *P* values of less than 0.05 were considered to indicate statistical significance.

Results

Effect of feeding high-amylose starch on the adipose tissue weight and serum levels of triglycerides

The average daily food intake of the rats fed the high amylose diet (12.3 ± 0.4 g, mean ± SEM) was similar to that of the control group (13.4 ± 0.4 g). No diarrhea was observed in either group. The body weights at the end of feeding period of the experimental diets and the weight of liver did not differ between the two groups *(Table 2)*. However, the weights of both epididymal and mesentery adipose tissues were significantly smaller ($P < 0.05$) in the animals fed the high-amylose diet than in the control animals *(Table 2)*. The serum triglyceride level in the rats fed the high-amylose diet was significantly ($P < 0.05$) lower than that of the control

Table 2 Effect of feeding high-amylose starch on the body weight and the weights of liver and epididymal and mesentery adipose tissues of the rat

Group =	Control	High-amylose
No. of rat =	5	6
Body weight (g)		
initial	181 ± 1	182 ± 2
final	222 ± 4	214 ± 3
Liver (g)	7.90 ± 0.29	7.32 ± 0.17
Epididymal adipose tissue (g)	5.14 ± 0.33	3.67 ± 0.19*
Mesentery adipose tissue (g)	3.50 ± 0.11	2.45 ± 0.13**

Mean ± SEM are shown.
*,**Significant differences from the control group at $P < 0.05$ and $P < 0.01$, respectively (ANOVA).

Table 3 Effect of feeding high-amylose starch on serum levels of triglycerides and total cholesterol in the rat

Group =	Control	High-amylose
No. of rat =	5	6
Serum triglycerides (mg/100 mL)	197 ± 11	144 ± 20*
Serum cholesterol (mg/100 mL)	40.5 ± 1.7	42.3 ± 1.7

Mean ± SEM are shown.
*Significant difference from the control group at $P < 0.05$ (ANOVA).

group, whereas the serum cholesterol level was similar between the two groups *(Table 3)*.

Effect of feeding high-amylose starch on small intestinal disaccharidase activities

To determine if small intestinal tissue responds to feeding the slowly digested starch, jejunoileum of the rats fed the high amylose diet and the control diet was divided into four segments of equal length, and the disaccharidase activities in the intestinal segments were determined. In all segments, mucosal weight and mucosal protein contents were unaffected by feeding the high-amylose diet. As shown in *Figure 1*, the activities of sucrase, isomaltase, and maltase of the control group were the highest in either upper jejunum or lower jejunum, whereas the levels of disaccharidase activities in the upper and lower ileum were similar (isomaltase) or much lower (sucrase and maltase) than the corresponding activities found in the lower jejunum. Compared with the control group, the high-amylose diet group showed significantly reduced ($P < 0.01$) sucrase, isomaltase, and maltase activities in the upper jejunum but exhibited significantly elevated ($P < 0.01$) sucrase, isomaltase, and maltase activities in the upper ileum, indicating an altered distribution of these disaccharidase activities along the jejunoileum, with the peaks shifting toward the lower part of small intestine *(Figure 1)*. The total activities of sucrase, isomaltase, and maltase calculated as the summed activities in the total jejunoileal segments of the rats fed the high-amylose diet were similar to those of rats fed the control diet (data not shown). Lactase activity in the upper jejunum was unaltered by the high-amylose diet, but feeding the high-amylose diet produced a significantly ($P < 0.05$) greater lactase activity in the lower jejunum and in the upper ileum, where maximal level of lactase activity was observed in both groups fed the high-amylose diet and control diet *(Figure 1)*.

Effect of feeding high-amylose starch on lipogenic enzyme activities in liver and adipose tissue

To determine whether the delay of carbohydrate digestion by the high-amylose diet would influence lipid metabolism, the lipogenic enzyme activities in liver and epididymal adipose tissue were determined *(Figure 2)*. The animals fed the high-amylose diet exhibited significantly reduced ($P < 0.05$) activities of FAS, ME, and G-6-PDH in the liver; the levels were decreased to 61%, 68%, and 52%, respectively, of the corresponding enzyme activity of the control group (Figure 2, upper panel). Likewise, in the epididymal adipose tissue the rats fed the high amylose diet showed reduced activities of FAS, ME, and G-6-PDH; the levels decreased to 63%, 45%, and 69%, respectively, of the corresponding enzyme activity of the control group (*Figure 2*, lower panel).

Discussion

In intestinal lumen, amylose is converted into polymers up to 9 glucose units. Amylopectin is broken down into branched segments of 5 to 9 glucose units. In turn, α-1, 4 glucosidic bonds of these polymers of both amylose and amylopectin

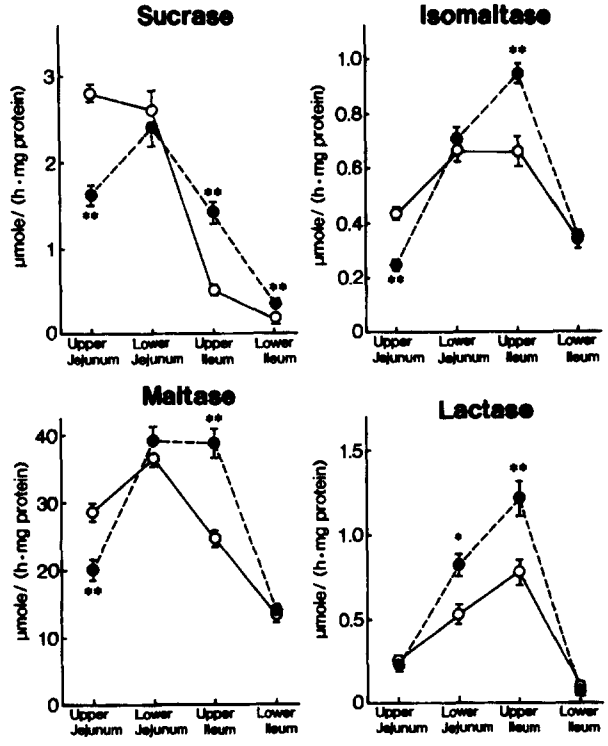

Figure 1 Effect of feeding a high-amylose diet on sucrase, isomaltase, maltase, and lactase activities in the small intestinal segments of the rat. Open circle indicates the control group and closed circle indicates the high-amylose group. Each circle represents the mean ± SEM for five (control) or six (high-amylose) rats. *,** denote significant differences from the control group at $P < 0.05$ and $P < 0.01$, respectively (ANOVA).

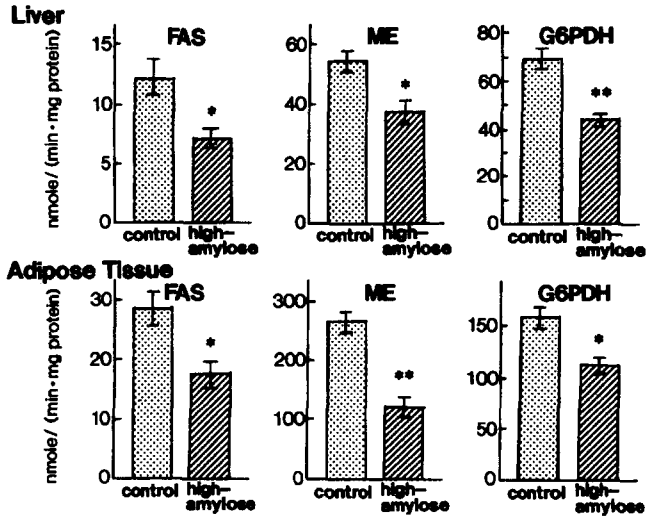

Figure 2 Effect of feeding a high-amylose diet on lipogenic enzyme activities in liver and epididymal adipose tissue of the rat. The upper panel shows the activities of FAS, ME, and G-6-PDH in the liver. The lower panel shows the activities of FAS, ME, and G-6-PDH in the epididymal adipose tissue. Each bar represents the mean ± SEM for five (control) or six (high-amylose) rats. *,** denote significant differences from the control group at $P < 0.05$ and $P < 0.01$, respectively (ANOVA).

are hydrolyzed by the action of α-glucosidases including sucrase, isomaltase, and maltase located in the intestinal brush border membranes.[18] In addition, isomaltase attacks α-1, 6 linkages of the branched segments. It was speculated that the helical structure of amylose, which is presumably stabilized by hydrogen bonding, is responsible for the decreased susceptibility of amylose to hydrolysis by the action of α-amylase.[19]

In the present study, we found that feeding the high amylose diet produced a marked reduction in the activities of all intestinal microvillar α-glucosidases examined, i.e., sucrase, isomaltase, and maltase, in the upper jejunum *(Figure 1)*. Because these small intestinal α-glucosidase activities are known to be influenced by the carbohydrate intake,[20] it is most likely that the decreased disaccharidase activities in the upper jejunum were caused by the decrease in the amount of substrates available for the disaccharidases in this segment, possibly due to a retarded hydrolysis of high-amylose starch. By contrast, the disaccharidase activities in the lower part of the small intestine were significantly elevated in the animals fed the high-amylose diet *(Figure 1)*. This result is consistent with the notion that the starch rich in amylose is digested and absorbed more slowly than the regular starch rich in amylopectin. Thus, we consider that the increase in disaccharidase activities in the ileum of animals fed the high-amylose starch might be an adaptive response of the small intestinal segment that is responding to the increased number of substrates escaped from the jejunum and present in the lower part of the small intestine. It should be noted that the total activities in the whole jejunoileum of all α-glucosidases examined in the animals fed the high-amylose starch were similar to those of animals fed the diet rich in amylopectin (control diet), suggesting that the carbohydrate digestive capability in the whole small intestine was preserved after feeding the high-amylose diet for 14 days. Both food intake and weight gain were unchanged by the high-amylose diet, suggesting that the total amount of starch hydrolyzed and absorbed in the small intestine might be similar between the two dietary groups.

It is well known that high-carbohydrate feeding is associated with elevated insulin-stimulated glucose uptake into adipocytes and enhanced lipogenic metabolism in adipose tissue[21,22] as well as in the liver.[6,23] Intake of a high-carbohydrate diet induces the lipogenic capacity of liver and adipose tissue by increasing lipogenic enzyme synthesis.[24]

However, we have shown in this study that in spite of a "high-carbohydrate" nature, the diet rich in amylose effectively reduced the lipogenic enzyme activities in both adipose tissue and liver, as compared with the diet containing the same amount of regular starch *(Figure 2)*. We consider that the delayed digestion and absorption of starch rich in amylose might be responsible for the reduced lipogenic enzyme activities. Further, we have demonstrated in this study that the decrease of lipogenic enzyme activities found in the animals fed the high-amylose diet might lead to the changes in blood and adipocyte lipid contents. First, the animals fed the high-amylose diet exhibited a reduced serum concentration of triglycerides *(Table 3)*. This result was in accordance with the report that showed that the volunteers who consumed the high-amylose cornstarch for 5 weeks exhibited significantly reduced blood triglyceride levels.[5] Secondly, feeding the high-amylose diet led to a decrease in both epididymal and mesentery adipose tissue weights by 30% *(Table 2)*.

The results of the present study strongly suggest that the use of amylose-rich starch might be effective in delaying digestion and absorption of carbohydrates and in reducing lipogenesis in adipose tissue and liver.

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