

Effect of refeeding raw and cooked starches on hepatic enzyme activities of rats

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1. Responses of hepatic glucose-6-phosphate dehydrogenase (*EC* 1.1.1.49; G6PD), malic enzyme (*EC* 1.1.1.40; ME), acetyl-CoA carboxylase (*EC* 6.4.1.2; ACAC), and fatty acid synthetase (FAS) were studied in male Wistar rats after a period of starvation and refeeding of diets containing 400 g glucose, or raw or cooked starches as the source of carbohydrate/kg. Starches fed included maize, potato, wheat, rice, and tapioca.

2. When compared to the responses of rats given the glucose-containing diet, rats given raw maize- or rice-starch-containing diets had a significantly lower ME response, and rats given raw potato starch had significantly lower responses of G6PD, ME, ACAC, and FAS. The enzyme responses of rats given cooked starches were similar to those of glucose-fed rats, except that rats given cooked wheat starch had significantly lower G6PD and ME responses than did glucose-fed rats.

3. When the enzyme responses to refeeding of the same starch source in either raw or cooked form were compared, it was found that (a) the FAS response was significantly higher to cooked than to raw maize starch, (b) the G6PD and ACAC responses were significantly higher to cooked than to raw tapioca starch, (c) the G6PD, ME, ACAC, and FAS responses were significantly higher to cooked than to raw potato starch.

4. The results suggest that reported differences in the lipogenic enzyme responses between simple sugars and starch may in some instances be magnified because of the use of uncooked starches in experimental diets.

The greater induction of lipogenic liver enzymes by diets containing simple sugars, e.g. sucrose or glucose, than by diets containing starches has been well documented (Aitken, Robinson & Yudkin, 1967; Chang, Lee, Schuster & Trout, 1971; Cohen, Briller & Shafrir, 1972; Naismith & Rana, 1974; Reiser, Michaelis, Putney & Hallfrisch, 1975). However, the magnitude of the difference in liver enzyme responses to dietary carbohydrates has in many animal studies been based on a comparison of feeding simple sugars to that of feeding raw rather than cooked starches. Whether the starch used was raw or cooked is generally not specified in publications; however, this can often be ascertained by noting the commercial source of the starch. With the exception of starches from some root vegetables, e.g. potato (Booher, Behan & McMeans, 1951; Jelinek, Katayama & Harper, 1952; Reussner, Andros & Thiessen, 1963), and starch from palm (Booher *et al.* 1951) and high-amylose maize (Borchers, 1962; Sandstedt, Strahan, Ueda & Abbot, 1962), no appreciable differences in various kinds of responses to feeding of raw or cooked starches to rats have been reported. These comparisons, however, were based primarily on body-weight gains (Booher *et al.* 1951; Jelinek *et al.* 1952; Reussner *et al.* 1963), *in vitro* (Jelinek *et al.* 1952; Sandstedt *et al.* 1962) and *in vivo* (Booher *et al.* 1951; Borchers, 1962) digestibility studies, or liver lipid levels (Womack & Marshall, 1955; Reussner *et al.* 1963), and not on enzyme responses to feeding of the raw or cooked starches.

The present study was therefore conducted to compare liver lipogenic enzyme responses in rats that had been starved and re-fed diets containing 400 g carbohydrate as glucose, or raw or cooked starches from maize, potato, wheat, rice or tapioca/kg. The starvation and refeeding regimen was used in this study, since it had been previously shown to induce a greater lipogenic enzyme response than did *ad lib.* feeding (Tepperman & Tepperman, 1958).

METHODS

Male Wistar rats (Hilltop Laboratory Animals, Scottsdale, Pennsylvania, USA; mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the US Department of Agriculture, and does not imply its approval to the exclusion of other products that may also be suitable) weighing 175–200 g were used. To minimize the possibility of stress during shipment (Szepesi, 1973), rats were packed and delivered the same morning. In order to insure uniformity of experimental conditions in the laboratory, rats were caged individually in stainless steel cages in the same room. Cages were arranged in eleven vertical columns of five units each. Each column represented a different treatment. Rats had access at all times to distilled water and were equilibrated with a commercial laboratory chow preparation (D & G Rat and Mouse Diet: baked biscuits, specific pathogen free; The Price-Wilhoite Company, Frederick, Maryland, USA) for at least 3 d before dietary treatments. Rats were then subjected to 2 d of starvation followed by feeding of the carbohydrate diets (glucose or raw or cooked maize, potato, wheat, rice or tapioca starch) for 2 d. All carbohydrates with the exception of tapioca starch were obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio, USA. Tapioca starch was obtained from National Starch and Chemical Corporation, Plainfield, New Jersey, USA. Food cups were attached to the cages by means of a tunnel in order to minimize the spillage of food and to prevent excretal contamination of the food. Diets contained (g/kg) 400 carbohydrate, 140 water, 350 casein, 50 maize oil, 40 Bernhart-Tomarelli salt mix (Bernhart & Tomarelli, 1966), 10 cellulose and 10 vitamin mix (Vitamin fortification mix (no. 40060); Teklad Test Diets, Madison, Wisconsin, USA). The same batch of starch was used to prepare both the raw and cooked starch-containing diets. Starches were cooked by the process of drum-drying (Powell, 1967). Starches were drum-dried by National Starch and Chemical Corporation, Plainfield, New Jersey, USA. The amount of water added to each diet was adjusted to account for the water of hydration of the carbohydrates used.

Rats were killed in the morning by decapitation, and the livers were quickly removed, chilled over ice-cold glass, and weighed. A 1.0 g sample of liver was used for the assay of glucose-6-phosphate dehydrogenase (*EC* 1.1.1.49; G6PD) (Freedland, 1967), malic enzyme (*EC* 1.1.1.40; ME) (Freedland, 1967), acetyl-CoA carboxylase (*EC* 6.4.1.2; ACAC) (Gregolin, Ryder, Kleinschmidt, Warner & Lane, 1966), and fatty acid synthetase (FAS) (Michaelis, Nace & Szepesi, 1975). Enzyme activity was expressed as units/100 g of final body-weight. One unit of enzyme activity was defined as that amount of enzyme producing 1 μ mol measured product/min, under the conditions of the assay (see Szepesi (1973) and Freedland (1967)). Relative liver size (RLS) was calculated as (liver weight \times 100) \div final body-weight. Food intake was determined from the difference in the weight of the food cup before and after 2 d of feeding. Any food which remained in the tunnel or which had been spilled was carefully collected and added back to the food cup before weighing. Food intake was calculated as g food eaten/2 d per 100 g of initial body-weight = (total food intake \times 100) \div body-weight before fed. Statistical differences between treatments were tested by Student's *t* test. Differences with *P* < 0.05 were considered to be statistically significant.

RESULTS

The results are summarized in Table 1.

Enzyme activities of the groups refed the different starch-containing diets were compared with those of the group refed the 400 g glucose/kg diet. Rats refed diets containing raw maize or rice starch had significantly lower levels of ME activity than did those refed the glucose-containing diet, but did not differ significantly in the activities of the other enzymes

Table 1. Effect of raw and cooked starches on hepatic glucose-6-phosphate dehydrogenase (EC 1.1.1.49; G6PD), malic enzyme (EC 1.1.1.40; ME), acetyl-CoA carboxylase (EC 6.4.1.2; ACAC), fatty acid synthetase (FAS), relative liver size (RLS) and food intake when rats were starved for 2 d and refed for 2 d diets* containing 400 g carbohydrate/kg

Dietary carbohydrate source	Enzyme activity (units/100 g body-wt)												Food intake (g/2 d per 100 g body-wt)			
	G6PD			ME			ACAC			FAS			RLS†		Food intake	
	Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE	Mean	SE
Glucose	59.0	3.8		35.7	3.5		7.62	0.33		12.1	1.7		5.93	0.12	29.0	0.72
Raw starch																
Maize	48.2	2.9		20.2	1.9 ^a		7.36	0.51		8.64	1.0		5.47	0.13 ^a	28.5	0.86
Potato	14.4	1.8 ^a		7.58	0.7 ^a		3.22	0.23 ^a		1.85	0.2 ^a		3.92	0.08 ^a	24.7	2.00
Wheat	56.2	13.0		27.0	5.7		8.54	1.70		11.1	1.7		5.58	0.17	25.4	1.10 ^b
Rice	42.8	7.1		23.0	3.2 ^a		7.42	0.57		8.80	1.5		5.62	0.14	26.7	0.39 ^a
Tapioca	51.1	5.7		25.7	3.7		7.56	0.60		9.57	1.4		5.71	0.11	28.3	0.69
Cooked starch†																
Maize	58.1	5.1		28.4	4.4		9.15	1.10		13.9	2.0 ^b		5.70	0.13	27.7	0.57
Potato	53.0	7.5 ^b		28.7	3.7 ^b		7.77	0.45 ^b		11.6	1.5 ^b		5.63	0.16 ^b	27.9	1.20
Wheat	44.1	5.2 ^a		22.0	1.8 ^a		6.86	0.81		8.09	0.6		5.81	0.30	27.6	0.70
Rice	57.4	5.0		28.0	2.7		7.20	0.64		9.70	2.1		5.62	0.16	27.9	1.10
Tapioca	75.8	8.5 ^b		34.0	3.8		9.81	0.75 ^{a,b}		14.3	2.1		5.75	0.06	28.2	1.00

a. Significantly different ($P < 0.05$) from the response of rats refed the glucose-containing diet.

b. Significantly different ($P < 0.05$) from the response of rats refed the corresponding raw starch-containing diet.

* For details of diets, see p. 86.

† (Liver wt × 100) ÷ final body-wt.

‡ Drum-dried.

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measured. No significant differences in enzyme activities were found between the groups refed raw wheat or tapioca starch and the group refed glucose. However, the group refed raw potato starch had significantly lower levels of all the enzymes measured (G6PD, ME, ACAC, and FAS) than did the group refed glucose. RLS was significantly smaller in rats refed raw maize or potato starch than in rats refed glucose. Food intake was not correlated with the levels of activity of any of the enzymes measured.

Refeeding corresponding diets containing cooked maize, potato or rice starch abolished the statistical differences which were seen when the responses to the raw starch-containing diets were compared with the response to the glucose-containing diet. An opposite effect, however, was found with wheat starch in that the response of liver G6PD and ME to refeeding of cooked wheat starch was significantly less than the response to refeeding of glucose, whereas there were no significant differences between the responses to refeeding of raw wheat starch and glucose.

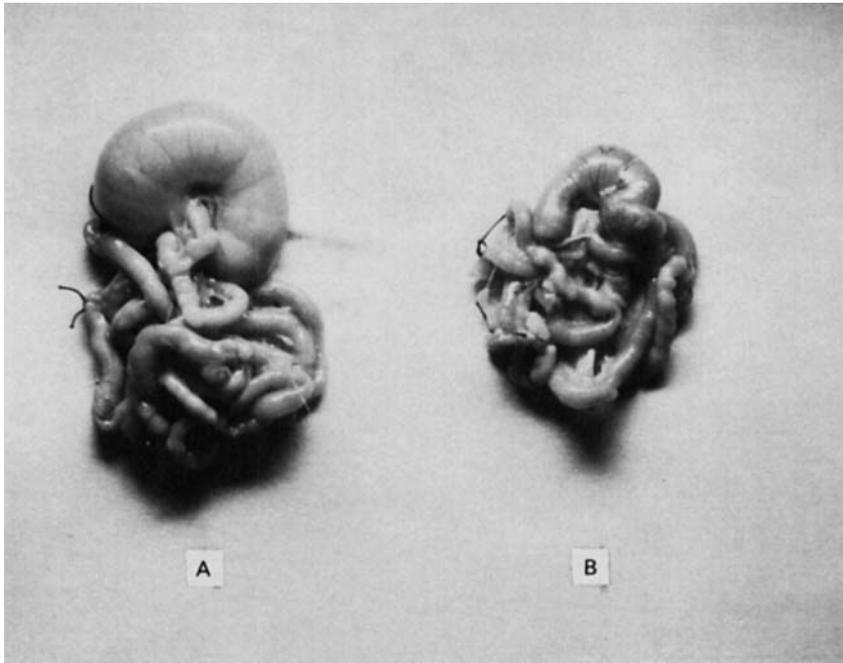
Differences in liver enzyme activities and RLS were also found when the responses of rats refed diets containing raw starch were compared to the responses of rats refed the corresponding cooked-starch-containing diets (Table 1). When the enzyme responses to refeeding of the same kind of starch in raw and in cooked form were compared, we found that the FAS response was significantly higher to cooked than to raw maize starch; that the G6PD and ACAC responses were significantly higher to cooked than to raw tapioca starch; and that G6PD, ME, ACAC, and FAS responses were significantly higher to cooked than to raw potato starch. RLS was also significantly higher after refeeding of cooked potato starch than after refeeding of raw potato starch. There were no significant differences in food intake between rats refed the raw and cooked-starch-containing diets.

The caecum and small intestine of the rats refed the raw-potato-starch-containing diet appeared distended, enlarged and gaseous when they were compared with those of rats refed the diets containing cooked potato starch or other raw starch or glucose (Plate 1).

DISCUSSION

The present study demonstrates that there are statistically significant differences in the activities of liver G6PD, ME, ACAC, and FAS when the responses of rats refed diets containing raw maize, potato or tapioca starch are compared with the responses of rats refed diets containing the corresponding cooked starches (Table 1). Earlier studies reported no significant differences in total liver lipid content between rats given either raw or cooked maize or tapioca starch (Womack & Marshall, 1955; Reussner *et al.* 1963). In the study reported here, starvation-refeeding was used to exaggerate the lipogenic response to feeding of the starch diets. It is possible that the differences reported in lipogenic enzyme responses may not be seen after longer periods of feeding or under *ad lib.* feeding conditions, since Varnell & Chang (1972) reported that rats fed *ad lib.* for 1 month had similar G6PD activity whether given raw or cooked potato starch. The reason for the slightly higher enzyme response to raw wheat starch than to cooked wheat starch in the study reported here is not known. It is possible that raw wheat starch may contain a contaminant which increases the activities of certain liver enzymes.

It has been reported that feeding rats diets containing starch results in lower liver lipogenic enzyme responses than does feeding an equivalent glucose-containing diet (Chang *et al.* 1971; Naismith & Rana, 1974). In the study reported here, lower liver enzyme responses to refeeding of the starch-containing diets than to refeeding of the glucose-containing diet were observed only when raw starches (maize, potato or rice) or cooked wheat starch were refed (Table 1). Since refeeding of cooked starch diets, with the exception of the wheat-starch-containing diet, resulted in hepatic enzyme responses that were not significantly lower than those of rats refed the glucose-containing diet, it is possible that the reported



differences in lipogenic response between feeding of simple sugars (e.g. sucrose) and starch may in some instances be magnified because of the use of raw starches in the experimental diets. In addition, the results obtained in this study as well as those reported by Vijayagopalan & Kurup (1972) suggest that the type of raw starch used may affect the lipogenic response.

Distention and enlargement of the caecum and small intestine of rats as the result of feeding raw potato starch was previously reported (Jelinek *et al.* 1952; Tomarelli, Hartz & Bernhart, 1960; Reussner *et al.* 1963). In the experiment reported in this communication, changes in the caecum and small intestine of the rats re-fed the raw-potato-starch-containing diet were apparent after only 2 d of refeeding (Plate 1). At other times we observed similar changes in the intestine and caecum when rats were given whole raw potatoes for only 12 h (over night). It is possible that the altered digestive tract of rats given raw potato starch could have significant effects on subsequent intestinal responses to experimental diets. It is a common practice to provide rats with raw potatoes during shipment; however, our findings would strongly suggest that an alternate source of water and food energy should be used for the shipment of rats; for example, fresh apples (Michaelis *et al.* 1975) and a gel made from agar (50 g/kg) – ground chow (1:1, w/w) (Szepesi, 1973) have been substituted for potatoes.

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EXPLANATION OF PLATE

Caecum and intestines from a rat re-fed the (A) raw and (B) cooked potato-starch-containing diets for 2 d after 2 d starvation. For details of diets and experimental procedure, see p. 86.