Effects of high-carbohydrate diets on lipogenesis in rat interscapular brown adipose tissue

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Cytoplasmic preparations from brown and white adipose tissues were assayed for three lipogenic enzymes throughout a programme of starvation followed by refeeding on either a normal or a white-bread diet. In the brown adipose tissue of rats fed on a white-bread diet the three enzymes were elevated to levels significantly higher than those in white adipose tissue.

Brown adipose tissue has been shown to be a major site of lipogenesis both in rats that have been cold adapted (McCormack & Denton, 1977; Trayhurn, 1979) and in rats that have been subjected to intragastric feeding (Agius & Williamson, 1980). These results suggest that in conditions of cold-induced and dietary-induced thermogenesis a significant proportion of the accumulated lipid in brown adipose tissue may have been synthesized *in situ* rather than in liver or white adipose tissue.

Since thermogenesis in brown adipose tissue necessitates high rates of β -oxidation, high rates of lipogenesis would seem to be incompatible with this proposition. However, carnitine palmitoyltransferase in brown adipose tissue from cold-adapted rats has been shown to be extremely sensitive to inhibition by malonyl-CoA (Saggerson & Carpenter, 1982) and this effect has been suggested as a means by which rates of fatty acid oxidation may be regulated.

Previous studies have utilized the incorporation of ^3H from $^3\text{H}_2\text{O}$ for measurement of rates of lipogenesis and parallel changes in the initial activities of both acetyl-CoA carboxylase and pyruvate dehydrogenase have also been shown, in response to insulin (McCormack & Denton, 1977; Trayhurn, 1979; Agius & Williamson, 1980). An alternative means of assessing the lipogenic capacity of a tissue is by the assay of enzymes associated with the provision of cytoplasmic acetyl units and NADPH (Martin & Denton, 1970; Saggerson, 1974*a,b*). In particular, levels of the enzymes ATP citrate lyase, malate dehydrogenase and glucose 6-phosphate dehydrogenase have been shown to rise and fall coordinately in rat liver (Gibson *et al.*, 1972) in

response to dietary variation. In this study, these three enzymes, together with lactate dehydrogenase, were assayed in cytoplasmic preparations from the brown and white adipose tissues of rats that had been maintained on normal and white-bread diets. The lipogenic response of brown adipose tissue to feeding on a white-bread diet was greater than that of white adipose tissue taken from the same animals.

Experimental

Full grown male Sprague–Dawley rats were maintained on Labsure CRM Diet (normal) or white bread for up to 5 days. The carbohydrate, fat and protein contents of Labsure CRM Diet are 56.9g, 2.4g and 18.1g/100g respectively. The corresponding values for white bread are 49.7g, 1.7g and 7.8g/100g (Paul & Southgate, 1978). Interscapular brown adipose tissue and perigenital white adipose tissue was removed from individual rats into 0.25 M-sucrose/20 mM-Tris/HCl (pH 7.4)/2 mM-EDTA at 25°C and any traces of adhering muscle or fibrous tissue were removed. Brown adipose tissue was trimmed free of white fat as far as possible. The tissues were homogenized in 5 cm³/g of the above medium, with the minimum force, using a Potter–Elvehjem homogenizer fitted with a motor-driven Teflon pestle. A temperature of 25°C was chosen to avoid solidification of the fat and the subsequent necessity for the use of excessive force during the homogenization procedure. The homogenates were centrifuged at 12000g (r_{av} , 8 cm) rotor for 20 min at 4°C. The supernatants were carefully removed from the solidified lipid layer and stored on ice for assay. Protein contents were determined by using the

method of Lowry *et al.* (1951) with bovine serum albumin as the standard. Enzyme activities were assayed spectrophotometrically by using a Varian Techtron 635 u.v./visible recording spectrophotometer. Simultaneous blanks were performed by the omission of a substrate from the assay. In all cases rates were constant over the measured period and were proportional to the volume of preparation used. ATP citrate lyase (EC 4.1.3.8), malate dehydrogenase (EC 1.1.1.40) and glucose 6-phosphate dehydrogenase (EC 1.1.1.49) were assayed as described by Martin & Denton (1970). Lactate dehydrogenase (EC 1.1.1.27) was assayed as described by Saggerson & Greenbaum (1969) with the substitution of 0.4 M-triethanolamine/HCl, pH 7.6, as the assay buffer.

Results and discussion

Before refeeding, the rats were subjected to a 48 h starvation period in order to reduce the activities of ATP citrate lyase, malate dehydrogenase and glucose 6-phosphate dehydrogenase to the lowest possible levels. During the subsequent refeeding period marked differences were observed in the levels attained by these enzymes, in response to a white-bread diet, in white and brown adipose tissue. Mean levels of ATP citrate lyase and malate dehydrogenase increased in brown adipose tissue by 452% and 558% of their levels before starvation (Table 1) and glucose 6-phosphate dehydrogenase by 30%. When compared with the results in Table 2, in the case of ATP citrate lyase this represents a 4-fold greater level than the highest activity achieved in white adipose tissue, whereas the levels of malate dehydrogenase and glucose 6-phosphate dehydrogenase are 2-fold greater in brown adipose tissue.

The significance of these three enzymes in the supply of lipogenic precursors in the white adipose tissue and liver of non-ruminants is well documented (Kornacker & Lowenstein, 1965; Martin & Denton, 1970; Gibson *et al.*, 1972; Saggerson, 1974*a,b*). The results presented here compare favourably with those of Agius & Williamson (1980), who reported an 8-fold stimulation in lipogenesis in rat brown adipose tissue, in response to intragastric feeding with glucose. The stimulation of the enzyme levels in brown and white adipose tissue is not parallel, those in white adipose tissue reaching peak values on day 3 or 4 of refeeding on a white-bread diet (Table 2), whereas levels in brown adipose tissue were still increasing at an undiminished rate on day 5 of refeeding on a white-bread diet (Table 1).

Levels of lactate dehydrogenase activity were also monitored throughout the experimental period, in order to provide a comparison, since this enzyme would be expected to show little, if any variation in

Table 1. *Activities of lipogenic enzymes in interscapular brown adipose tissue*
Enzyme activities were assayed as stated in the Experimental section. The rates are means $\pm$ S.E.M. of at least three but usually four or five separate determinations and are expressed as nmol/mg of protein per min.

	ATP citrate lyase		Malate dehydrogenase		Glucose 6-phosphate dehydrogenase		Lactate dehydrogenase	
	Normal diet	White bread	Normal diet	White bread	Normal diet	White bread	Normal diet	White bread
Normal level	33.63 $\pm$ 10.45	24.93 $\pm$ 8.13	28.65 $\pm$ 6.69	41.33 $\pm$ 21.3	50.14 $\pm$ 3.91	40.92 $\pm$ 8.89	610 $\pm$ 260	1971 $\pm$ 1250
48 h starvation	16.3 $\pm$ 4.5	66.35 $\pm$ 12.20	65.33 $\pm$ 29.89	73.55 $\pm$ 11.42	50.43 $\pm$ 7.62	65.55 $\pm$ 10.66	988 $\pm$ 147	2512 $\pm$ 710
Refeeding								
1 day	23.04 $\pm$ 6.06	84.77 $\pm$ 23.45	82.90 $\pm$ 15.79	130.30 $\pm$ 27.88	73.93 $\pm$ 14.89	82.52 $\pm$ 18.82	1845 $\pm$ 293	1516 $\pm$ 477
2 days	20.70 $\pm$ 10.49	108.75 $\pm$ 12.53	54.80 $\pm$ 13.37	176.05 $\pm$ 46.91	64.33 $\pm$ 22.75	140.23 $\pm$ 22.85	1688 $\pm$ 426	2051 $\pm$ 688
3 days	45.86 $\pm$ 3.66	152.00 $\pm$ 22.18	82.50 $\pm$ 21.24	265.33 $\pm$ 25.27	67.66 $\pm$ 15.49	161.72 $\pm$ 29.25	1471 $\pm$ 279	1803 $\pm$ 256
4 days	39.33 $\pm$ 8.10							
5 days	42.23 $\pm$ 7.79							

Table 2. *Activities of lipogenic enzymes in perigenital white adipose tissue*
 Enzyme activities were assayed as stated in the Experimental section. The rates are means $\pm$ S.E.M. of at least three but usually four or five separate determinations and are expressed as nmol/mg of protein per min.

	ATP citrate lyase		Malate dehydrogenase		Glucose 6-phosphate dehydrogenase		Lactate dehydrogenase	
	Normal diet	White bread	Normal diet	White bread	Normal diet	White bread	Normal diet	White bread
Normal level	10.69 $\pm$ 4.69	15.3 $\pm$ 3.95	37.90 $\pm$ 7.44	33.23 $\pm$ 11.33	32.13 $\pm$ 10.70	50.42 $\pm$ 10.70	544 $\pm$ 233	1287 $\pm$ 317
48 h starvation	6.87 $\pm$ 1.36	30.94 $\pm$ 6.26	37.05 $\pm$ 12.22	41.82 $\pm$ 12.36	—	65.9 $\pm$ 6.15	1164 $\pm$ 628	1457 $\pm$ 322
Refeeding								
1 Day	3.14 $\pm$ 0.39	31.60 $\pm$ 15.08	8.81 $\pm$ 4.6	117.18 $\pm$ 47.47	30.66 $\pm$ 11.40	69.5 $\pm$ 18.05	1360 $\pm$ 500	1227 $\pm$ 287
2 Days	17.17 $\pm$ 5.92	37.60 $\pm$ 7.70	30.25 $\pm$ 13.8	88.93 $\pm$ 16.45	39.83 $\pm$ 1.87	71.07 $\pm$ 17.09	982 $\pm$ 251	1289 $\pm$ 354
3 Days	13.26 $\pm$ 6.36	25.25 $\pm$ 7.11	17.52 $\pm$ 8.73	38.85 $\pm$ 13.47	38.20 $\pm$ 9.68	75.2 $\pm$ 17.24	1172 $\pm$ 296	1323 $\pm$ 438
4 Days	4.73 $\pm$ 1.26		14.76 $\pm$ 2.10					
5 Days	8.03 $\pm$ 2.09		18.74 $\pm$ 4.39					

response to dietary changes. As expected, little change was observed in either brown or white adipose tissue.

When these results are taken in conjunction with the observations of McCormack & Denton (1977), that the levels of the active forms of pyruvate dehydrogenase and acetyl-CoA carboxylase change in response to both insulin levels and glucose administration, it is apparent that rat interscapular brown adipose tissue has a greater capacity for lipogenesis, under conditions of a high-carbohydrate diet, than white adipose tissue. The necessary conclusion that must be drawn from this observation is that during the state of dietary-induced thermogenesis, much of the fatty acid being oxidized in brown adipose tissue has been synthesized *in situ* from glucose. The question of how such high rates of lipogenesis are compatible with simultaneous high rates of lipolysis and β -oxidation has already been posed by Saggerson & Carpenter (1982). The reason why the lipogenic activity of brown adipose tissue is so much greater on a white-bread diet than on the normal diet is not clear, since both diets have a similar high-carbohydrate content. However, the normal diet contains higher levels of both protein and fat than white bread and therefore a difference in energy consumption on the two diets may be significant in causing this effect.

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