The Maximum Activities of Enzymes that are Involved in Substrate Cycles in Liver and Muscle of Obese Mice

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The maximum activities of some dehydrogenases and enzymes involved in substrate cycles are increased in both liver and muscle of obese mice. This suggests that endocrine control rather than maximum capacity of the activities of enzymes constituting such cycles is impaired in obese mice.

The presence of enzymes that catalyse opposing non-equilibrium reactions in the same cell first suggested the existence of substrate (sometimes known as futile) cycles [see Newsholme (1976) for an historical account of the development of the concept of substrate cycles]. Direct evidence for net rates of cycling in the liver between glucose and glucose 6-phosphate, fructose 6-phosphate and fructose 1,6-bisphosphate (D. G. Clark *et al.*, 1973) and pyruvate and phosphoenolpyruvate (Friedmann *et al.*, 1971) has been obtained. This is also the case for the fructose 6-phosphate/fructose bisphosphate cycle in mammalian muscle (M. G. Clark *et al.*, 1973). An analysis of the problems of control of the flux through non-equilibrium reactions in some metabolic pathways has led to the proposal that substrate cycles provide one means of improving sensitivity of such reactions to changes in the concentrations of metabolic regulators. However, in order to increase sensitivity, the ratio cycling rate/flux through the pathway must be high. Nonetheless, it is not expected that the rate of cycling will be high for prolonged periods of time, since the conversion of chemical energy to heat, which occurs in these cycles, could cause hyperthermia. A hypothesis has been put forward that cycling rates can be varied according to hormonal or nervous activity, so that when sensitivity is required (e.g. for glycolysis in muscle when exercise is anticipated), cycling rates are increased (see Newsholme & Crabtree, 1976, 1978; Newsholme, 1978).

An extension of this hypothesis is that the variability in substrate cycling could be used for weight control: if excess energy is consumed, the rate of substrate cycles will be increased (via hormonal control) to 'burn off' this excess (Newsholme & Crabtree, 1976, 1978; Newsholme, 1978). Consequently, obesity could result from an inability to increase the rate of substrate cycling. This could be explained either by a decreased capacity of such cycles, so that hormonal stimulation would produce a lower rate of energy expenditure than in the normal

animals, or by a hormone concentration imbalance that prevents the stimulation of the rates of such cycles. In order to investigate the former possibility, the maximum catalytic activities of enzymes involved in three cycles have been measured in obese mice and their lean controls: the glucose/glucose 6-phosphate and pyruvate/phosphoenolpyruvate cycles in liver and the fructose 6-phosphate/fructose 1,6-bisphosphate cycle in muscle and liver. In addition, the maximum activities of some dehydrogenases that might be involved in the production of reducing equivalents (NAD⁺- and NADP⁺-linked isocitrate dehydrogenases, NADP⁺-linked malate dehydrogenase, glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase) have been measured as 'control' activities. The results are reported and discussed in this paper.

Materials and Methods

Chemicals, enzymes and animals

Female obese mice, C57 BL/6J (*ob/ob*), and lean controls were kindly provided by the Nutritional Research Centre, Beecham Pharmaceuticals Research Division, Tadworth, Surrey, U.K. All chemicals and enzymes were obtained from sources given previously (Crabtree & Newsholme, 1972; Crabtree *et al.*, 1972; Zammit & Newsholme, 1976).

Preparation of homogenates and assay of enzyme activities

Except for glucose 6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase and liver NADP⁺-linked malate dehydrogenase, liver and quadriceps muscle were extracted and enzyme activities measured as described elsewhere: glucokinase (Walker & Holland, 1965); glucose 6-phosphatase (Kitcher *et al.*, 1978); phosphofructokinase (Opie & Newsholme, 1967; Start & Newsholme, 1970); fructose 1,6-bisphosphatase and pyruvate carboxylase (Crabtree *et al.*, 1972); pyruvate kinase (Feliu *et al.*, 1977;

Zammit *et al.*, 1978); phosphoenolpyruvate carboxykinase and muscle NADP⁺-linked malate dehydrogenase (Newsholme & Williams, 1978); NAD⁺- and NADP⁺-linked isocitrate dehydrogenase (Alp *et al.*, 1976). For glucose 6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase and NADP⁺-linked malate dehydrogenase, liver was extracted in the following medium: 20 mM-Tris/HCl, 250 mM-sucrose, 10 mM-dithiothreitol, 1 mM-MgCl₂ and 100 mM-KCl at pH 7.6. Glucose 6-phosphate dehydrogenase was assayed by the method of Matsuda & Yugari (1967) in assay medium that contained 50 mM-Tris/HCl, 20 mM-MgCl₂, 0.2 mM-NADP⁺ and 2 mM-glucose 6-phosphate at pH 7.4. 6-Phosphogluconate dehydrogenase was assayed by the method of Procsal & Holten (1972) in assay medium that contained 50 mM-Tris/HCl, 20 mM-MgCl₂, 0.2 mM-NADP⁺, 100 mM-KCl and 2 mM-6-phosphogluconate at pH 7.4. Liver NADP⁺-linked malate dehydrogenase was assayed by the method of Saito *et al.* (1971) in assay medium that contained 50 mM-Tris/HCl, 1 mM-MnCl₂, 0.2 mM-NADP⁺, 100 mM-KCl and 1 mM-L-malate (neutralized with KOH) at pH 7.4. All extractions and assays were carried out in such a way as to ensure that maximum enzyme activities were measured (see Crabtree *et al.*, 1979).

Protein was determined by the method of Bradford (1976). Enzyme activities are presented as nmol/min per mg of protein.

Results and Discussion

It has been reported previously that the activities of dehydrogenases that are involved in the production of reducing power for fatty acid synthesis are in-

creased in livers of obese mice (Fried & Antopol, 1966; Martin *et al.*, 1973). This result has been confirmed in the present work: the activities of hepatic NADP⁺-linked malate dehydrogenase, glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase are increased by 126, 28 and 110% respectively (Table 1). Although there was no change in the activity of NADP⁺-linked malate dehydrogenase in skeletal muscle of obese mice, in which the enzyme is unlikely to be involved in lipogenesis, there was an increase (46%) in activity of NADP⁺-linked isocitrate dehydrogenase in this tissue (Table 1). However, there was no change in the activity of this enzyme in the liver of obese mice. The reason for the increase in activity in muscle is unclear.

In the present work it has been shown that in obese mice the activities of fructose bisphosphatase were increased in both the liver (by 72%) and the muscle (by 30%), whereas the activity of phosphofructokinase in muscle was decreased (by 15%), although that of the liver was unchanged. Previous reports of changes in the activities of these enzymes in obese mice both agree and disagree with these findings: Seidman *et al.* (1967) report that hepatic fructose bisphosphatase activity is increased, whereas Willms *et al.* (1970) report that there is no change. Adolfsson *et al.* (1974) report that phosphofructokinase is decreased in muscle of obese mice, whereas Berne & Westman-Naeser (1978) report that the activity of this enzyme in the liver is increased. If the activity of the bisphosphatase is limiting for the operation of the substrate cycle [this is probable in muscle in which the maximum activities of fructose bisphosphatase are much lower than those of

Table 1. *Activities of some dehydrogenases and enzymes of substrate cycles in muscle and liver of lean and obese mice*
Enzyme activities are presented as means $\pm$ S.E.M. with the numbers of separate animals used in parentheses. The statistical significance (Student's *t* test) of the difference in activity between obese and lean mice is indicated by * ($P < 0.05$), ** ($P < 0.025$) and † ($P < 0.001$).

	Enzyme activities (nmol/min per mg of protein at 25°C)			
	Quadriceps muscle		Liver	
	Lean control	Obese	Lean control	Obese
Glucokinase	—	—	9.0 $\pm$ 1.47 (6)	17.7 $\pm$ 1.86** (6)
Glucose 6-phosphatase	—	—	117 $\pm$ 5.0 (6)	193 $\pm$ 11.0† (7)
Phosphofructokinase	227 $\pm$ 12.0 (12)	192 $\pm$ 16.5† (12)	10.5 $\pm$ 0.7 (12)	11.8 $\pm$ 0.9 (12)
Fructose 1,6-bisphosphatase	0.46 $\pm$ 0.047 (12)	0.60 $\pm$ 0.045† (12)	9.9 $\pm$ 0.45 (12)	17.0 $\pm$ 1.20* (12)
Pyruvate kinase	3283 $\pm$ 308 (5)	3078 $\pm$ 274 (5)	316 $\pm$ 25.8 (6)	497 $\pm$ 35.6* (6)
Pyruvate carboxylase	—	—	17.1 $\pm$ 1.6 (11)	37.0 $\pm$ 3.0* (12)
Phosphoenolpyruvate carboxykinase	5.6 $\pm$ 0.80 (6)	1.8 $\pm$ 0.50* (6)	2.2 $\pm$ 0.43 (6)	2.6 $\pm$ 0.25 (6)
NAD ⁺ -linked isocitrate dehydrogenase	15.0 $\pm$ 0.08 (6)	16.0 $\pm$ 0.11 (6)	4.9 $\pm$ 1.5 (6)	2.3 $\pm$ 0.43 (6)
NADP ⁺ -linked isocitrate dehydrogenase	11.4 $\pm$ 1.8 (6)	16.6 $\pm$ 2.0† (6)	122.0 $\pm$ 8.5 (6)	119.0 $\pm$ 3.9 (6)
NADP ⁺ -linked malate dehydrogenase	4.9 $\pm$ 0.60 (6)	4.3 $\pm$ 0.50 (6)	29.8 $\pm$ 3.3 (6)	67.5 $\pm$ 10.9† (6)
Glucose 6-phosphate dehydrogenase	—	—	4.6 $\pm$ 0.34 (6)	5.9 $\pm$ 0.58* (7)
Phosphogluconate dehydrogenase	—	—	9.6 $\pm$ 0.44 (6)	20.2 $\pm$ 1.20† (7)

phosphofructokinase (Crabtree *et al.*, 1972), although it may not be the case for liver (see Table 1)], the present findings suggest that the maximum capacities of the cycle are increased in both muscle and liver of obese mice.

Although the activity of phosphoenolpyruvate carboxykinase is not increased in liver of obese mice, those of pyruvate carboxylase and pyruvate kinase are increased (Table 1). The results for hepatic pyruvate kinase are in agreement with those of Seidman *et al.* (1967) and Willms *et al.* (1970), whereas those for muscle pyruvate kinase disagree with the results of Adolfsson *et al.* (1974). It is not clear which is the limiting reaction in the pyruvate/phosphoenolpyruvate cycle, but, if it is the activity of the carboxykinase, these changes suggest that the capacity of the pyruvate/phosphoenolpyruvate cycle is not increased in liver of obese mice.

The activities of both glucokinase and glucose 6-phosphatase are increased (by 97 and 65% respectively) in the liver of obese mice (Table 1). These findings are in agreement with those of Seidmann *et al.* (1967), Willms *et al.* (1970) and Martin *et al.* (1973). This suggests that the maximum capacity of the glucose/glucose 6-phosphate cycle is increased in this tissue.

These results suggest that, if the rate of substrate cycling is reduced in obese mice, it is not due to a decrease in the capacity of such cycles. However, in the absence of any information on the actual rate of cycling in the intact tissue or in the tissues of the intact animal, it is not possible to infer that the rates of these cycles have been changed *in vivo*. Nonetheless, it is tempting to speculate that the endocrinological disturbances that are characteristic of these mice (Mayer *et al.*, 1953; Genuth *et al.*, 1971) result in decreased activities of the rate-limiting enzymes of the cycles, via effects on the activities of interconverting enzymes; it is now established that hepatic phosphofructokinase, pyruvate kinase and fructose biphosphatase exist in interconvertible forms (Brand *et al.*, 1976; Feliú *et al.*, 1977; Riou *et al.*, 1977). Furthermore, it is possible that the varied changes in activities of some of these enzymes reported in the literature (see above) may be explained by failure of some workers to extract and assay the enzymes under conditions that measure total activity (i.e. conditions that either activate the less-active form of the enzyme or favour conversion of the less-active to the more-active form). In the present study, conditions of extraction and assay were chosen so that total activity of the enzyme was measured, and any changes in enzyme activity should reflect changes in concentration of enzyme. Perhaps the observed increases in activities of these substrate-cycling enzymes represent a stimulation of the rate of their synthesis in an attempt to overcome the reduced rate of

cycling due to hormonal effects on the interconversion enzymes.

York *et al.* (1978) have observed that the activities of the ($\text{Na}^+ + \text{K}^+$)-dependent ATPase in the kidney and liver of the obese mouse are reduced. This suggests that the capacities of the Na^+ -translocation cycle (see Newsholme & Crabtree, 1976) in these tissues are reduced, and this could be responsible for the obesity. The finding contrasts with the lack of any decrease in capacity of the metabolic cycles. Unfortunately, the rates of Na^+ leakage, the other component of the translocation cycle, have not been reported, which casts doubt on the physiological significance of the decrease in activities of the ($\text{Na}^+ + \text{K}^+$)-dependent ATPases.

One additional important finding in this work is the marked reduction in activity of phosphoenolpyruvate carboxykinase in the muscle of obese mice. Since this enzyme is considered to be involved in amino acid oxidation and/or conversion of amino acids to alanine in muscle (see Goldstein & Newsholme, 1976; Snell & Duff, 1977; Newsholme & Williams, 1978), it is possible that in obese mice, the amino acids glutamate, aspartate, isoleucine and valine are metabolized to a greater extent in the lipogenic tissues (i.e. liver and adipose tissue). It is, however, unclear whether this could play any role in the development of obesity.

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