

Comparison of tissue pyruvate dehydrogenase activities on re-feeding rats fed *ad libitum* or meal-fed rats with a chow-diet meal

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Meal-fed rats and rats fed *ad libitum* had similar rates of hepatic glycogenesis at 60 min after the initiation of re-feeding a chow meal after 22 h starvation, but hepatic PDH_a (active form of pyruvate dehydrogenase) activities were 4-fold higher in the meal-fed group. In heart, PDH_a activities were 3-fold higher before re-feeding and 2-fold higher after re-feeding in the meal-fed group compared with the group fed *ad lib*. The blood metabolite profile suggested diminished fat oxidation in starved meal-fed rats and accelerated flux through PDH in meal-fed re-fed rats compared with the group fed *ad lib*.

INTRODUCTION

It has been demonstrated recently that the antecedent pattern of food intake can markedly influence the hepatic response to re-feeding with a chow diet (Pallardo & Williamson, 1989). In rats fed *ad libitum* and then starved for 18–48 h before re-feeding, the primary response is net hepatic glycogen synthesis, which occurs via the indirect (gluconeogenic) pathway utilizing C₃ derivatives of glucose as precursors (reviewed by McGarry *et al.*, 1987; Landau & Wahren, 1988; Sugden *et al.*, 1989): rates of hepatic lipogenesis remain low for at least 3 h after the resumption of food intake (Sugden *et al.*, 1983; Newgard *et al.*, 1984; Holness *et al.*, 1986, 1988a; Pallardo & Williamson, 1989). In contrast, it has been demonstrated that, in rats where access to food is limited to a short daily period (meal-feeding) with a 21 h interval between meals, rates of hepatic lipid synthesis increase by over 6-fold within 90 min of re-feeding (Pallardo & Williamson, 1989), and the proportion of hepatic glycogen synthesized directly from glucose is greatly increased (Huang & Veech, 1988).

The hepatic pyruvate dehydrogenase complex (PDH) occupies a key role in determining whether C₃ derivatives of glucose are used for glycogen synthesis via the indirect pathway or for lipogenesis (Holness *et al.*, 1986, 1988a; reviewed by Sugden & Holness, 1989). Thus the greater stimulation of hepatic glycogenesis than of lipogenesis during the initial (0–3 h) phase of re-feeding after prolonged starvation is facilitated by the continued suppression of hepatic PDH activity (Holness *et al.*, 1986, 1988a; reviewed by Sugden & Holness, 1989). Furthermore, the most rapid increases in lipogenesis are preceded by PDH re-activation and a marked increase in the use of citrate as lipogenic precursor (Holness *et al.*, 1988a).

There has been no direct comparison of the initial responses of hepatic PDH activities to re-feeding in meal-fed rats maintained on a chow diet and in rats fed *ad libitum* and subsequently starved for an equivalent period, and it is unknown to what extent carbohydrate can function as a lipogenic precursor in meal-fed rats. In

the present study, we have measured the activity of the active form of hepatic PDH (PDH_a) before and during the absorptive phase of feeding in meal-fed rats in order to assess the importance of changes in hepatic PDH_a activity in relation to the glycogenic and lipogenic responses. We have also measured cardiac PDH_a activities, decreases in which are more rapid and pronounced in response to starvation than those in liver (Holness & Sugden, 1989), and increases in which are even more refractory than those of hepatic PDH_a to re-feeding after prolonged starvation (Kerby & Randle, 1982; Holness & Sugden, 1989).

EXPERIMENTAL

Rats

Female albino Wistar rats (180–220 g) were fed on a chow diet consisting of 52% carbohydrate, 16% protein, 2% fat (all by wt.) and a non-digestible residue, and were kept at an ambient temperature of 22 ± 2 °C with a 12 h-light/12 h-dark cycle (light from 08:30 h). One group of rats was fed *ad libitum* (termed 'control'), and the other group (termed 'meal-fed') was allowed access to food for only 2 h daily (between 09:00 and 11:00 h) with a 22 h interval between meals. The meal-fed rats were on the restricted feeding schedule for 10 days, during which their food intake progressively increased from 57% to 86% of that of control rats fed *ad lib.*, with a mean chow intake over the entire period of 72 ± 6% that of control rats. On the day before the experiments, the mean chow intakes in the control and meal-fed rats were 15.7 ± 0.5 g and 14.3 ± 0.5 g respectively. The restricted feeding regime led to loss of weight of approx. 7% over the 10 days, the major weight loss occurring on day 1. The weight of the control rats increased by 3% over the 10-day period.

Re-feeding

Experiments were commenced at 09:00 h, and, except where stated, both groups of rats were starved for the previous 22 h. The rats were provided with 5 g of the chow diet (see Pallardo & Williamson, 1989). When

Abbreviations used: PDH, the pyruvate dehydrogenase complex; PDH_a, the active form of PDH; NEFA, non-esterified fatty acids.

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permitted access to chow *ad lib.*, the mean chow intake in the re-fed control rats over a 120 min period was 5.4 ± 0.4 g, whereas in the re-fed meal-fed rats the mean chow intake was 14.2 ± 1.4 g. The rats were sampled at 60 or 120 min after the provision of chow. In some experiments, mitochondrial long-chain fatty acid oxidation was inhibited by the intragastric administration of 2-tetradecylglycidate [120 min; 2.5 mg/100 g body wt.; see Holness & Sugden (1989) for further details].

Metabolite and enzyme assays

Rats were sampled while under sodium pentobarbital anaesthesia (5 min; 6 mg/100 g body wt.). Tissues were rapidly freeze-clamped and stored in liquid N₂. Blood was sampled from the aorta. Plasma non-esterified fatty acids (NEFA) were measured with a Wako C-test kit. Concentrations of glucose, lactate and the ketone bodies were measured in KOH-neutralized HClO₄ extracts of whole blood by standard enzymic methods (respectively: Slein, 1963; Hohorst, 1963; Williamson *et al.*, 1962). Frozen liver was powdered under liquid N₂ and liver glycogen was determined, without prior precipitation with ethanol, as glucose after hydrolysis with amyloglucosidase (Keppler & Decker, 1974).

PDH_a and citrate synthase activities were measured in freeze-clamped tissue extracts as described by Caterson *et al.* (1982). PDH_a activities are expressed relative to citrate synthase to correct for possible variation in the efficiency of mitochondrial extraction (see Caterson *et al.*, 1982; French *et al.*, 1988a; Holness *et al.*, 1988a,b). One unit of enzyme activity is defined as that which converts 1 μ mol of pyruvate into acetyl-CoA per min at 30 °C.

Expression of results

Statistical significance of difference was assessed by Student's unpaired *t* test. Results are given as means \pm S.E.M. for the numbers of rats specified in parentheses.

RESULTS AND DISCUSSION

Hepatic PDH_a activities in relation to lipogenesis in starvation

Meal-fed rats have been demonstrated to exhibit 3-fold higher rates of hepatic fatty acid synthesis in the starved state than do control rats fed *ad lib.* before starvation, but no information was provided about the identity of the lipogenic precursors utilized (Pallardo & Williamson, 1989). Hepatic PDH_a activities of rats meal-fed and fed *ad lib.* after starvation are similar, and significantly (68–79%) lower than those observed in rats with continuous access to chow (Table 1). Thus the differences in rates of hepatic lipogenesis in the starved state cannot be attributed to an altered capacity for the use of carbohydrate via PDH as lipogenic precursor.

Hepatic glycogen deposition of re-feeding after starvation

In confirmation of the findings by Pallardo & Williamson (1989), hepatic glycogen concentrations immediately before re-feeding were low and similar in both meal-fed rats and rats fed *ad lib.* (Table 1). Approximately linear rates of hepatic glycogen deposition over a 180 min

period after re-feeding with a test (5 g) chow meal have been reported by others (Pallardo & Williamson, 1989), but in the present experiments, although hepatic glycogen deposition at 60 min after re-feeding was similar in the two groups, significant increases in hepatic glycogen content were not observed in meal-fed rats during the subsequent 60 min (Table 1). However, significant increases were observed if the meal-fed rats were allowed free access to chow, with a mean carbohydrate intake of approx. 7.4 g as opposed to 2.6 g over the 2 h period (Table 1). In post-absorptive rats, with a glucose turnover rate of 0.21 g/h per 200 g body wt. (Issad *et al.*, 1987), the carbohydrate content of the 5 g chow meal could sustain rates of glucose utilization for approx. 12 h. However, in post-absorptive rats, the activities of both hepatic and cardiac PDH_a are low, approx. 55 and 20% of the fed values respectively (Holness & Sugden, 1989).

Hepatic PDH_a activities in relation to glycogenesis and lipogenesis after re-feeding

Although hepatic PDH_a activities increased in response to re-feeding in control rats fed *ad lib.* before starvation, the response was refractory (Table 1). Thus, although hepatic PDH_a activities had significantly increased after 60 min of chow re-feeding, the values observed were only 67% of those of rats with uninterrupted access to food (termed 'fed' in Table 1), although by 120 min the activity had been restored. It is notable that, during the initial 60–120 min after re-feeding, net glycogenesis occurs despite an increased hepatic capacity for conversion of pyruvate into acetyl-CoA. The hepatic response to chow re-feeding after 22 h starvation is therefore quite distinct from that which is observed after re-feeding with carbohydrate alone after more prolonged (48 h) starvation, where retarded PDH re-activation appears to be essential for sparing carbon for hepatic glycogenesis (Holness *et al.*, 1986).

Re-feeding of meal-fed rats elicited an rapid and marked (14-fold) increase in hepatic PDH_a activity (Table 1). The activity of the complex at 60 min after re-feeding was thus 3-fold higher than the control fed value. The high activity of hepatic PDH_a was maintained if the meal-fed rats were permitted free access to chow, but declined between 60 and 120 min if the chow intake was restricted to 5 g (Table 1). However, PDH_a activities at 120 min after re-feeding still exceeded the pre-feeding value, and were not significantly different from those observed in control rats provided with continuous access to food ($P > 0.05$).

It is notable that rates of hepatic lipogenesis also fluctuate during the first 180 min after the provision of a 5 g chow meal to starved previously meal-fed rats, reaching a maximum at 90 min, and then declining to the control fed value at 180 min (Pallardo & Williamson, 1989). Rates of hepatic lipogenesis in re-fed meal-fed rats are approx. 3-fold higher than in rats provided with continuous access to chow, and 6-fold higher than in starved meal-fed rats (Pallardo & Williamson, 1989). These differences are reflected in the relative activities of hepatic PDH_a (Table 1). The striking parallelism between rates of lipogenesis and PDH-complex activities in livers of meal-fed rats in response to changes in carbohydrate supply suggests either co-ordinate regulation of PDH and lipogenesis or a primary role for PDH in determining the rate of lipogenesis.

Table 1. Comparison of the effects of re-feeding on hepatic glycogen deposition and hepatic and cardiac PDH_a activities in control and meal-fed rats

For full details see the Experimental section. Rats of both groups were provided with 5 g of chow diet after 22 h starvation. Hepatic and cardiac citrate synthase activities (units/g wet wt.) were as follows: liver, control, 7.9 ± 0.3 , meal-fed, 7.5 ± 0.2 ; heart, control, 74.0 ± 4.4 , meal-fed, 68.7 ± 3.5 . Values that are significantly different between control and meal-fed rats are indicated by: † $P < 0.05$; †† $P < 0.01$. Values in re-fed rats that are significantly different from the appropriate starved value are indicated by: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Experimental group	Glycogen (mg/g)	PDH _a (m-units/unit of citrate synthase)	
		Liver	Heart
Control			
Starved (22 h)	1.6 ± 0.6 (7)	5.7 ± 1.2 (5)	1.1 ± 0.2 (4)
Re-fed (60 min)	11.8 ± 4.7 (6)*	11.8 ± 2.1 (9)*	9.1 ± 1.4 (6)***
Re-fed (120 min)	33.6 ± 4.1 (5)***	19.0 ± 1.8 (4)***	8.3 ± 0.7 (8)***
Fed (<i>ad lib.</i>)	61.2 ± 4.5 (5)	17.7 ± 1.3 (6)	22.0 ± 3.5 (11)
Meal-fed			
Starved (22 h)	0.6 ± 0.4 (4)	3.7 ± 1.3 (5)	3.7 ± 0.5 (5)††
Re-fed (60 min)	12.8 ± 1.8 (7)***	50.2 ± 9.6 (7)***††	21.3 ± 4.3 (7)***†
Re-fed (120 min)	14.1 ± 5.0 (6)*†	14.5 ± 3.4 (6)*	15.5 ± 2.5 (6)***†
Fed (<i>ad lib.</i> , 60 min)	15.7 ± 2.1 (10)***	43.3 ± 2.9 (7)***	24.2 ± 4.5 (7)***
Fed (<i>ad lib.</i> , 120 min)	45.5 ± 4.1 (7)***	29.6 ± 2.0 (10)***	14.3 ± 2.0 (7)***

Carbohydrate as lipogenic precursor

The potential for the use of carbohydrate as oxidative or lipogenic substrate is clearly increased under conditions where the proportion of PDH in the active form is increased. It can be calculated that, at 37 °C, flux through PDH at 60 min after re-feeding of meal-fed rats could contribute as much as 500 nmol of C₂ equivalents/min per g to lipogenesis. This may be compared with an observed rate of lipid synthesis of approx. 600 nmol of C₂ equivalents/min per g (as calculated by Pallardo & Williamson, 1989). Therefore up to 80% of the acetyl-CoA required for lipogenesis could be provided via flux through PDH.

Cardiac PDH_a activity in starvation and after re-feeding

Meal-feeding has been demonstrated to increase the activities of key regulatory enzymes in the lipogenic pathway (Leveille, 1972). It seemed plausible that the altered regulatory characteristics of hepatic PDH might therefore be specifically related to its role in lipogenesis. To investigate this possibility, PDH_a activities were also measured in the heart, a tissue in which PDH has a predominantly energetic function. Cardiac PDH_a activity was significantly higher in starved meal-fed rats than in controls (Table 1), although still only 17% of the control fed value (Table 1). Furthermore, although cardiac PDH_a activity exhibited a refractory response to re-feeding in 22 h-starved controls, with only 38% re-activation after 120 min, cardiac PDH_a activities in re-fed meal-fed rats increased more rapidly, approximating to 97% of the activity observed in continuously fed rats within 60 min (Table 1). In contrast with the situation in the liver, the activity of cardiac PDH_a in meal-fed rats did not exceed the control fed value at either 60 or 120 min after re-feeding, and did not decline significantly during the period from 60 to 120 min after re-feeding the 5 g test meal.

Changes in substrate supply during starvation

The immediacy of the response of hepatic and cardiac PDH_a to re-feeding in meal-fed rats resembles that observed on re-feeding after short-term (6 h) starvation (see Holness & Sugden, 1989). However, in short-term starvation, inactivation of hepatic and cardiac PDH occurs in the absence of marked changes in lipid (NEFA and ketone body) supply, or in glycaemia and lactataemia (M. J. Holness & M. C. Sugden, unpublished work; see also McGarry *et al.*, 1973), whereas the blood metabolite profile in meal-fed rats more closely resembles that observed in more prolonged starvation (Table 2). Thus there is a marked increase in the circulating concentrations of both NEFA and ketone bodies, and glycaemia and lactataemia are decreased.

Ketone bodies are produced by the partial oxidation of NEFA in the liver, and the rate of hepatic ketogenesis is directly related to the exogenous NEFA supply. However, when presented with an identical fatty acid load, livers from starved rats convert a relatively higher proportion of the load into ketone bodies, whereas livers from fed rats convert relatively more into triacylglycerol (Mayes & Felts, 1967; reviewed by McGarry & Foster, 1980). The blood concentrations of ketone bodies relative to the circulating NEFA concentration thus reflect the carbohydrate status of the liver, with a higher ketone-body/NEFA concentration ratio in the starved than in the fed state. In the present study, this ratio increased on starvation for 22 h from 1.4 to 1.9 in control rats, but was only 1.0 in the starved meal-fed group (Table 2). Thus, despite similar or increased circulating NEFA concentrations in the starved state, ketonaemia was significantly lower in rats subjected to a meal-feeding regime rather than being fed *ad lib.* before starvation (Table 2). As the rate of utilization of ketone bodies is directly related to their circulating concentration (Reed *et al.*, 1984), this suggests either that in livers of starved meal-fed rats a greater proportion of available NEFA is esterified to

Table 2. Comparison of the effects of re-feeding on blood metabolite concentrations in control and meal-fed rats

For full details see the Experimental section. Rats of both groups were provided with 5 g of chow diet after 22 h starvation. The results are mean values \pm S.E.M. for 4–9 rats. Values that are significantly different between control and meal-fed rats are indicated by: † $P < 0.05$; †† $P < 0.01$. Values that are significantly different from the appropriate starved value are indicated by: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Experimental group	Metabolite concn. (mM)			
	Glucose	Lactate	NEFA	Ketone bodies
Control				
Starved (22 h)	5.24 \pm 0.19	1.59 \pm 0.19	0.49 \pm 0.05	0.93 \pm 0.05
Re-fed (60 min)	5.68 \pm 0.31	3.34 \pm 0.20***	0.08 \pm 0.03***	0.12 \pm 0.03***
Re-fed (120 min)	6.40 \pm 0.34*	3.17 \pm 0.24***	0.12 \pm 0.04***	0.14 \pm 0.02***
Fed (<i>ad lib.</i>)	6.28 \pm 0.45	1.92 \pm 0.17	0.15 \pm 0.03	0.21 \pm 0.04
Meal-fed				
Starved (22 h)	5.22 \pm 0.34	1.33 \pm 0.30	0.61 \pm 0.09	0.58 \pm 0.14†
Re-fed (60 min)	4.94 \pm 0.21	2.12 \pm 0.21††	0.18 \pm 0.04***	0.09 \pm 0.02**
Re-fed (120 min)	6.40 \pm 0.25*	2.15 \pm 0.31†	0.20 \pm 0.01***	0.28 \pm 0.07
Fed (<i>ad lib.</i> , 60 min)	4.75 \pm 0.10	2.33 \pm 0.38	0.14 \pm 0.03***	0.21 \pm 0.03*
Fed (<i>ad lib.</i> , 120 min)	5.79 \pm 0.22	2.84 \pm 0.40*	0.12 \pm 0.03***	0.26 \pm 0.04

triacylglycerol rather than oxidized to ketone bodies, or that the hepatic uptake of NEFA is decreased. The lower blood ketone-body concentrations after starvation in the meal-fed group may in part explain the maintenance of a higher cardiac PDH_a activity in the starved state (Table

1), as cardiac PDH is particularly responsive to changes in the exogenous supply of non-carbohydrate fuels (French *et al.*, 1988a,b).

To investigate further the role of changes in lipid fuel oxidation in the regulation of cardiac PDH_a activities in starved meal-fed rats, PDH_a activities were measured after inhibition of mitochondrial long-chain fatty acid oxidation at the level of carnitine palmitoyltransferase 1 by 2-tetradecylglycidate. Whereas in 22 h-starved control rats re-activation of cardiac PDH within a 120 min period was incomplete (achieving only 34% of the fed value), the administration of 2-tetradecylglycidate to 22 h-starved meal-fed rats led to a 5.7-fold increase in activity, and a value was achieved which was not significantly different from that observed in 2-tetradecylglycidate-treated control rats permitted continuous access to chow (Fig. 1). As is the case after re-feeding, the response is more reminiscent of that observed after short-term (6 h) starvation than that observed after prolonged (22–48 h) starvation (Fig. 1). The decline in the availability (and therefore oxidation) of NEFA and ketone bodies which is observed after re-feeding (Table 2) may thus assume greater regulatory importance for cardiac PDH re-activation in meal-fed than in control rats. The mechanism(s) responsible for the differential responses of the two groups requires further investigation. The meal-feeding regime may have long-term effects either to suppress the extent or duration of lipid oxidation (as is indeed suggested by decreased ketonaemia in the starved state) and/or have a direct effect to promote carbohydrate oxidation.

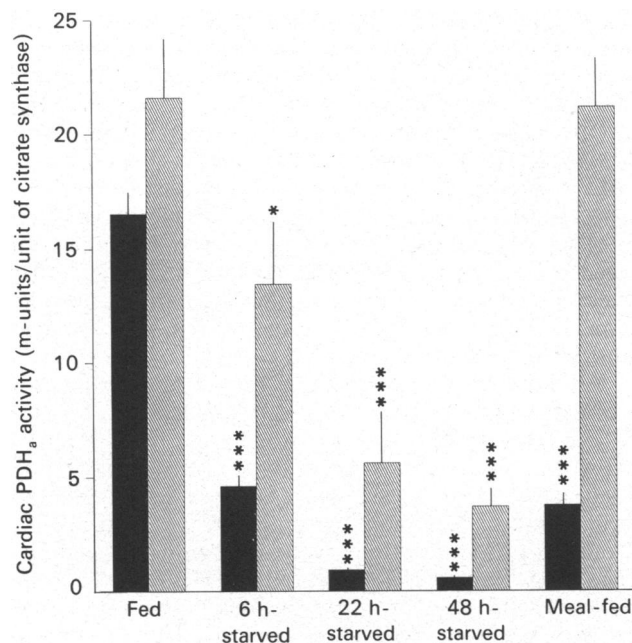


Fig. 1. Response of cardiac PDH_a to inhibition of mitochondrial fatty acid oxidation in control and meal-fed rats

For full details see the Experimental section. Control (fed, or 6 h-, 22 h- or 48 h-starved) rats or meal-fed (22 h-starved) rats were administered 2-tetradecylglycidate by intragastric intubation, and sampled after 120 min. PDH_a activities were measured in extracts of freeze-clamped hearts. Statistically significant effects of 2-tetradecylglycidate were observed in all groups ($P < 0.01$). Statistically significant differences from the appropriate (control or 2-tetradecylglycidate-treated) fed values are indicated by: * $P < 0.05$; *** $P < 0.001$. Key: ■, control; ▨, 2-tetradecylglycidate.

Changes in substrate supply in relation to glycogenesis and lipogenesis after re-feeding

Pallardo & Williamson (1989) have demonstrated that hepatic glucose and lactate concentrations after re-feeding are similar in both the control and the meal-fed groups of rats. However, hepatic lactate failed to increase in response to feeding the 5 g chow meal in meal-fed rats, whereas a 3–3.5-fold increase was observed in the control group. In the present experiments, blood lactate concentrations doubled after re-feeding a 5 g chow meal in the

control group, whereas only a moderate (60%) increase in lactataemia was observed after re-feeding in the meal-fed group (Table 2). These results are consistent with the concept that accelerated PDH re-activation is associated with an increase in flux from pyruvate to acetyl-CoA, and thus an increased rate of irreversible carbohydrate-carbon disposal. This will limit the extent to which carbohydrate carbon can be recycled to glucose 6-phosphate via hepatic gluconeogenesis, for subsequent use either for glucose production or for glycogen synthesis.

The failure to maintain a linear rate of hepatic glycogen deposition after re-feeding a 5 g chow meal to starved meal-fed rats might theoretically be a consequence of an irreversible drain on available glucose imposed by accelerated re-activation of PDH. However, there is no evidence from measurements of glycaemia for accelerated glucose clearance (Table 2), and decreased glucose availability cannot therefore account for the decline in the rate of hepatic glycogen deposition. Although it has been suggested that in meal-fed rats hepatic glycogen is predominantly (> 90%) synthesized directly from circulating glucose, rather than from C₃ derivatives of glucose (Huang & Veech, 1988), it remains possible that in meal-fed rats there is increased hepatic glycogen turnover, with accelerated flux from glycogen to lipid via PDH. This could be analogous to the situation which pertains during re-feeding after prolonged starvation in hyperthyroidism (Holness *et al.*, 1988b).

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