Pyruvate dehydrogenase activities and rates of lipogenesis during the fed-to-starved transition in liver and brown adipose tissue of the rat

Mark J. HOLNESS and Mary C. SUGDEN*

Department of Biochemistry, London Hospital Medical College, Turner Street, London E1 2AD, U.K.

The percentages of pyruvate dehydrogenase complex (PDH) in the active form (PDH_a) in two lipogenic tissues (liver and brown adipose tissue) in the fed state were 12.0% and 13.4% respectively. After acute (0.5 h) insulin treatment, PDH_a activities had increased by 77% in liver and by 234% in brown fat. Significant decreases in PDH_a activities were observed in both tissues by 5 h after the removal of food. The patterns of decline in PDH_a activities in the two lipogenic tissues were similar in that the major decreases in activities were observed within the first 7 h of starvation. The significant decreases in PDH_a activities observed after starvation for 6 h were accompanied by decreased rates of lipogenesis. Hepatic and brown-fat PDH_a activities after acute (30 min) exposure to exogenous insulin were less in 6 h-starved than in fed rats, but the absolute increases in PDH_a activities over the 30 min exposure period were similar in fed and 6 h-starved rats. Increases in PDH_a activities were paralleled by increases in lipid synthesis in both tissues. Re-activation of PDH in response to insulin treatment or chow re-feeding after 48 h starvation occurred more rapidly in brown adipose tissue than in liver. The results are discussed in relation to the importance of the activity of the PDH complex as a determinant of the total rate of lipogenesis during the fed-to-starved transition and after insulin challenge or re-feeding.

INTRODUCTION

In mammals, the principal sites of fatty acid synthesis are white and brown adipose tissue, liver and, during lactation, mammary gland. Rates of lipid synthesis in these lipogenic tissues vary with the nutritional status. Under certain conditions, such as the administration of high-carbohydrate or -lipid diets, meal-feeding or prolonged carbohydrate deficiency, changes in rates of lipid synthesis can be effected by long-term changes in the concentrations of enzymes in the pathway (reviewed by Block & Vance, 1977). In the shorter term, rates of fatty acid synthesis may be influenced by hormones such as insulin. These effects are exerted through changes in the catalytic properties of key regulatory enzymes, which include pyruvate kinase, the pyruvate dehydrogenase complex (PDH) and acetyl-CoA carboxylase (reviewed by Denton & Brownsey, 1983). For PDH, this involves interconversion between an active form (PDH_a) and a phosphorylated form (PDH_b), which is devoid of activity.

An increase in the circulating insulin concentration in the fed state stimulates lipid synthesis in all four lipogenic tissues (Volpe & Vagelos, 1976; McCormack & Denton, 1977; Agius & Williamson, 1980; Hardie, 1980; Munday & Williamson, 1981). In contrast, whereas acute insulin deficiency (induced by short-term treatment with streptozotocin or anti-insulin serum) leads to a 7-fold decrease in lipogenesis in lactating mammary gland (Robinson *et al.*, 1978) and a 3-fold decrease in brown-adipose-tissue lipogenesis (McCormack & Denton, 1977), its effects on hepatic lipogenesis are much more modest or are not observed (see, e.g., Stansbie *et al.*, 1976; Agius & Williamson, 1980). These findings raise the possibility that rates of lipogenesis in different tissues may not change in parallel in response to insulin deficiency, which, in non-pathological circumstances, occurs between meals.

Decreases in rates of fatty acid synthesis elicited by the administration of anti-insulin serum in the fed state are accompanied by decreases in PDH_a activity in brown adipose tissue (McCormack & Denton, 1977). Furthermore, even in the liver a

close relationship exists between increases in lipid synthesis and in PDH_a activities during periods over which starvation-induced decreases in insulin concentrations are reversed, namely postprandially in meal-fed rats (Holness & Sugden, 1989b; Pallardo & Williamson, 1989), and during the first few hours of re-feeding after prolonged starvation (Holness *et al.*, 1988). We have recently defined the pattern of changes in hepatic PDH_a activities during the fed-to-starved transition (Holness & Sugden, 1989a), which is associated with progressive and sustained insulin deficiency (McGarry *et al.*, 1973). In the present study, we have extended this work to investigate the relationship between changes in hepatic PDH_a activities and rates of hepatic lipogenesis during short-term starvation and after insulin challenge after short-term starvation. We have also compared the time course of PDH inactivation in liver with that in brown fat, a lipogenic tissue which, in view of its rapid and marked response to anti-insulin serum, might be expected to exhibit a more pronounced response than the liver to acute starvation. In addition, the relationship between PDH_a activity and lipogenic rate in brown adipose tissue has been defined.

MATERIALS AND METHODS

Sources of materials were as specified in Holness *et al.* (1988).

Female albino Wistar rats (170–220 g) were subjected to a 12 h-light/12 h-dark cycle (light from 08:30 h), and were fed *ad libitum* on a standard rodent diet (52% carbohydrate, 16% protein, 2% lipid and 30% non-digestible residue, all by wt.) before use. Water was provided *ad libitum*. Experiments were started at 08:30 h, at which time food was removed. In some experiments, insulin (2 units) was injected subcutaneously (Agius & Williamson, 1980) or dichloroacetate [DCA; 0.25 ml of a 5% (w/v) solution in 0.15 M-NaCl (pH 7.4)/100 g body wt.] was injected intraperitoneally (at 30 min intervals; for details see Holness *et al.*, 1986). In addition, a group of rats previously

Abbreviations used: PDH, pyruvate dehydrogenase complex; PDH_a, active PDH complex; DCA, dichloroacetate.

* To whom correspondence should be addressed.

starved for 48 h were provided with chow *ad libitum*. Rats were sampled at 0.5 h or 1 h after insulin injection, at 2 h after injection of dichloroacetate (DCA) or at the specified intervals after food removal or replacement. Samples of liver and brown fat were obtained from the same animal in each experiment.

For measurements of rates of lipid synthesis, $^3\text{H}_2\text{O}$ (5 mCi in 0.5 ml of 0.15 M-NaCl) was injected at 1 h before sampling (Agius & Williamson, 1980). A blood sample was taken at the time of sampling for determination of plasma $^3\text{H}_2\text{O}$ specific radioactivity. Tissue lipid was saponified and extracted as described by Stansbie *et al.* (1976). In both adipose tissue and liver, it has been estimated that 13–14 g-atoms of H is incorporated per mol of fatty acid synthesized. For the purpose of this paper, the total rate of lipid synthesis was calculated from the μg -atoms of ^3H in tissue lipid by dividing by 13.2 (Windmueller & Spaeth, 1966, 1967; see Hems *et al.*, 1975).

PDH_a and citrate synthase activities were measured in tissue extracts as described by Caterson *et al.* (1982). One unit of enzyme activity is that which converts 1 μmol of substrate into product/min at 30 °C. PDH_a activities are expressed on a wet-weight basis. The efficiency of mitochondrial extraction was assessed by measurement of citrate synthase activities (see Caterson *et al.*, 1982; Denyer *et al.*, 1989), and no significant variation was observed between the experimental groups (results not shown). Values for citrate synthase activities, expressed on a wet-weight basis, were similar to those obtained previously by Caterson *et al.* (1982) and Denyer *et al.* (1989). Total PDH (PDH_a+PDH_i) activities were measured after incubation of tissue extracts with PDH phosphatase (Whitehouse *et al.*, 1974). Total PDH activities in liver and brown fat are unchanged by alterations in the nutritional status (Caterson *et al.*, 1982; Denyer *et al.*, 1989; M. J. Holness & M. C. Sugden, unpublished work). In the liver, both PDH activity and lipogenic rate were estimated in the same sample. In brown fat, because of the smaller amount of tissue available, PDH activities and lipogenic rates were estimated in separate experiments.

Statistical significance of differences was assessed by Student's unpaired *t* test. Results are means $\pm$ S.E.M. for the numbers of rats specified.

RESULTS AND DISCUSSION

Hepatic and brown-fat PDH activities in the fed state and during the fed-to-starved transition

Initial PDH activities in liver and brown adipose tissue in the fed state (measured between 08:30 and 09:30 h) and total PDH activities in both tissues are shown in Tables 1 and 2 and Fig. 1. The percentages of PDH in the active (dephosphorylated) form were similar in brown fat and liver. Values of total PDH and citrate synthase activities in liver and brown fat were similar to those obtained previously (Caterson *et al.*, 1982; Denyer *et al.*, 1989) and were unaffected by the duration of starvation (results not shown).

The decline in hepatic PDH_a activity elicited by starvation is slow in onset, occurring only after the initiation of depletion of hepatic glycogen at 3–4 h after food removal (Holness & Sugden, 1989a). This, in turn, is paralleled by a steady decline in glycaemia and plasma insulin concentrations (McGarry *et al.*, 1973). In the present experiments, a significant decrease in hepatic PDH_a activity was observed at 4 h after food withdrawal (Fig. 1). Stansbie *et al.* (1976) failed to demonstrate any effect of acute (10–30 min) exposure to anti-insulin serum on hepatic PDH_a in fed rats. It therefore appears that, if insulin is involved in the regulation of hepatic PDH_a activity during the fed-to-starved transition, a sustained decline in its concentration may be necessary to evoke a response.

Fig. 1. Time courses of PDH inactivation and re-activation after starvation and re-feeding in liver and brown adipose tissue

PDH_a activities in liver (○) and brown fat (▲) are expressed as percentages of total PDH activities in the respective tissues. Mean total PDH activities are shown in the legends to Tables 1 and 2. Statistically significant effects of starvation were first observed at 4 h in liver ($P < 0.01$) and at 5 h in brown adipose tissue ($P < 0.05$). Statistically significant effects of re-feeding were first observed at 1 h in brown adipose tissue ($P < 0.01$) and at 2.7 h in liver ($P < 0.05$). Results are means $\pm$ S.E.M. for 6–12 rats.

Despite quite distinct metabolic functions of liver and brown fat and selective inhibition of PDH_a activity by acute treatment with anti-insulin serum in brown fat (McCormack & Denton, 1977; Stansbie *et al.*, 1976), the time courses of the decreases in PDH_a activities during the fed-to-starved transition in brown fat and liver were quite similar (Fig. 1). A decline in PDH_a activity in brown adipose tissue was evident at 4–5 h after the initiation of starvation, at which time approx. 5% of total PDH remained active (Fig. 1). After 6 h of starvation brown-fat PDH_a activity had decreased to 27% of the fed value (Fig. 1). Inactivation of brown-fat PDH was essentially complete after 48 h starvation (Fig. 1).

Hepatic and brown-fat PDH_a activities after insulin treatment in the fed state

Wieland *et al.* (1972) were the first to demonstrate that the administration of insulin to fed rats increases hepatic PDH_a activity, a result that has been confirmed subsequently (French *et al.*, 1988). In isolated hepatocytes from fed rats, the addition of insulin increases the percentage of PDH in the active form from 23% to 32% (Assimakopoulos-Jeannet *et al.*, 1982). In the present series of experiments, the acute administration of insulin to fed rats induced a 77% increase in the activity of hepatic PDH_a, such that at 30 min after insulin treatment approx. 21% of the complex was present in the active form (Table 1). The response to insulin treatment in brown fat over the same period was much more dramatic: PDH_a activity increased by 234%, representing an increase in the percentage of active complex to 45% of the total (Table 2). The magnitude of the response of brown adipose tissue to an increase in the circulating insulin

Table 1. PDH_a activities and rates of lipogenesis in livers of fed, 6 h-starved or 48 h-starved rats

For experimental details see the text. PDH_a activities were measured at 30 °C in extracts of freeze-clamped livers from fed, 6 h-starved or 48 h-starved rats. Fed, 6 h-starved or 48 h-starved rats were also treated with insulin or DCA as described in the Materials and methods section. Total PDH activity (mean of values obtained in fed and starved rats) was 1106 ± 72 m-units/g wet wt. (136.2 ± 13.6 m-units/unit of citrate synthase). PDH_a activity (on a unit/wet wt. basis) has also been expressed in terms of C₁₆-unit equivalents/h (column 4) to facilitate direct comparison with rates of lipid synthesis, measured *in vivo* by using ³H₂O. It has been assumed that 13.2 g-atoms of H are incorporated per mol of fatty acid synthesized (column 6) (see Windmueller & Spaeth, 1966, 1967). Values are means $\pm$ s.e.m. for 6–10 rats. Statistically significant effects of starvation are shown by: ††† $P < 0.001$. Statistically significant effects of insulin or DCA are given by: *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$.

Rats	Treatment	PDH _a activity			Lipogenesis	
		(% active)	(m-units/g wet wt.)	(μ mol of C ₁₆ units/h per g wet wt.)	(μ g-atoms of 'H'/h per g wet wt.)	(μ mol of C ₁₆ units/h per g wet wt.)
Fed	Control	12.0 ± 1.2	114.5 ± 12.9	0.86	21.5 ± 1.5	1.63
	Insulin	$21.2 \pm 3.6^*$	$204.8 \pm 40.0^*$	1.54	$51.0 \pm 4.8^{***}$	3.86
	DCA	$100.6 \pm 4.2^{***}$	$1120.6 \pm 57.8^{***}$	—	—	—
6 h-starved	Control	$3.7 \pm 0.3^{\dagger\dagger\dagger}$	$35.9 \pm 3.0^{\dagger\dagger\dagger}$	0.27	$9.5 \pm 0.6^{\dagger\dagger\dagger}$	0.72
	Insulin	$13.4 \pm 1.8^{***}$	$127.8 \pm 17.3^{***}$	0.96	$19.0 \pm 2.3^{\dagger\dagger\dagger\dagger}$	1.44
	DCA	$86.9 \pm 11.4^{***}$	$1169.5 \pm 70.3^{***}$	—	—	—
48 h-starved	Control	$1.4 \pm 0.5^{\dagger\dagger\dagger}$	$15.7 \pm 6.2^{\dagger\dagger\dagger}$	0.12	$7.1 \pm 0.4^{\dagger\dagger\dagger}$	0.54
	Insulin	$2.0 \pm 0.7^{\dagger\dagger\dagger}$	$25.9 \pm 8.5^{\dagger\dagger\dagger}$	0.19	$7.3 \pm 0.5^{\dagger\dagger\dagger}$	0.55
	DCA	$9.1 \pm 1.6^{***\dagger\dagger\dagger}$	$86.0 \pm 24.0^{***\dagger\dagger\dagger}$	—	—	—

Table 2. PDH_a activities and rates of lipogenesis in brown adipose tissue of fed, 6 h-starved or 48 h-starved rats

Full details are provided in the text and the legend in Table 1. Total PDH activity was 6822 ± 251 m-units/g wet wt. (80.0 ± 5.8 m-units/unit of citrate synthase). Statistically significant effects of starvation are shown by: ††† $P < 0.001$; †† $P < 0.01$; † $P < 0.05$. Statistically significant effects of insulin or DCA are shown by: *** $P < 0.001$; ** $P < 0.01$.

Rats	Treatment	PDH _a activity			Lipogenesis	
		(% active)	(m-units/g wet wt.)	(μ mol of C ₁₆ units/h per g wet wt.)	(μ g-atoms of 'H'/h per g wet wt.)	(μ mol of C ₁₆ units/h per g wet wt.)
Fed	Control	13.4 ± 3.4	670.5 ± 129.1	5.03	114.0 ± 11.6	8.63
	Insulin	$44.7 \pm 5.2^{***}$	$2642.6 \pm 515.4^{**}$	19.82	$348.4 \pm 29.6^{***}$	26.40
	DCA	$85.8 \pm 14.8^{***}$	$5691.9 \pm 1245.2^{**}$	—	—	—
6 h-starved	Control	$3.7 \pm 1.3^{\dagger}$	$250.6 \pm 74.6^{\dagger}$	1.90	$41.0 \pm 7.0^{\dagger\dagger\dagger}$	3.11
	Insulin	$24.2 \pm 2.8^{***\dagger\dagger}$	$1448.1 \pm 139.6^{***\dagger}$	10.86	$318.8 \pm 28.1^{***}$	24.15
	DCA	$98.0 \pm 7.4^{***}$	$6885.6 \pm 701.4^{***}$	—	—	—
48 h-starved	Control	$0.5 \pm 0.2^{\dagger}$	$397 \pm 12.2^{\dagger\dagger\dagger}$	0.30	$44.6 \pm 5.7^{\dagger\dagger\dagger}$	3.37
	Insulin	$7.6 \pm 1.4^{***\dagger\dagger\dagger}$	$689.0 \pm 183.4^{***\dagger\dagger}$	5.17	$234.2 \pm 17.1^{***\dagger\dagger}$	17.74
	DCA	$76.8 \pm 12.2^{***}$	$5309.3 \pm 868.8^{***}$	—	—	—

concentration is emphasized by the more than 12-fold difference between brown-fat PDH_a activities under conditions in which insulin concentrations were acutely elevated (by insulin injection) or acutely decreased [by short-term (6 h) starvation] (Table 2), compared with a 6-fold difference in the liver (Table 1).

Hepatic and brown-fat PDH_a activities after insulin treatment during the fed-to-starved transition

In the present study, an acute (30 min) elevation in the insulin concentration after 6 h starvation led to a clear (262%) increase in hepatic PDH_a activity, reversing the decline in activity elicited by starvation (Table 1). Although the percentage of the PDH complex in the active form at 30 min after insulin injection was less in 6 h-starved rats than in fed rats, net PDH_a dephosphorylation (i.e. the increase in PDH_a activity) over the 30 min period of exposure to increased insulin was similar in fed or 6 h-starved rats (Table 1). Furthermore, if the period of exposure to insulin was increased to 1 h, hepatic PDH_a activity

exceeded the fed value, and approached the value found in insulin-treated fed rats, with $27.7 \pm 3.0\%$ of the complex in the active form. There is therefore no evidence for an impaired responsiveness of hepatic PDH to insulin administration after short-term starvation. In contrast, no acute effect of insulin administration to increase hepatic PDH_a activity was observed after 48 h starvation (Table 1; see also Holness & Sugden, 1988). There are therefore striking parallels between the latency in the response of hepatic PDH_a activity to insulin administration (Table 1) and that to re-feeding after prolonged (48 h) starvation (Fig. 1; see also Holness *et al.*, 1988). The values for the percentages of the complex in the active form after insulin treatment in livers of rats starved for 6 h or 48 h in the present experiments are comparable with those obtained by Kruszynska & McCormack (1989) during a 2.5 h hyperinsulinaemic glucose clamp.

Treatment of 6 h-starved rats with insulin for 30 min elicited a 6.5-fold increase in PDH_a activity in brown fat (Table 2). In this

case, PDH_a activity after insulin treatment exceeded that found in the control fed state. However, it was apparent that PDH in brown adipose tissue was resistant to complete re-activation by insulin treatment within a 30 min period of exposure if the duration of starvation was extended to 48 h: although a 15-fold increase in PDH_a activity was observed, the absolute increment in activity was almost 3-fold lower than that observed in fed or 6 h-starved rats (Table 2).

By analogy with the incomplete re-activation observed after acute treatment with insulin, only partial PDH_a re-activation in brown fat was observed over the first 1 h after the initiation of chow re-feeding (Fig. 1). Prolonged starvation therefore renders the PDH complex resistant to rapid re-activation in at least two lipogenic tissues (i.e. liver and brown fat). However, full re-activation of brown-fat PDH was observed if the re-feeding period was extended to 2–3 h (Fig. 1), compared with a more sluggish response in liver (Holness *et al.*, 1988; Holness & Sugden, 1989b).

Prolonged starvation induces stable increases in the activity of PDH kinase, which (in cultured hepatocytes) are only slowly reversed by exposure to an elevated insulin concentration (Marchington *et al.*, 1987). We demonstrate in the present experiments that a delayed response of hepatic PDH_a to insulin administration is also observed in the intact animal (Table 1). Hepatic PDH also becomes resistant to re-activation by DCA, a pyruvate analogue which inhibits PDH kinase (Table 1; see also Holness *et al.*, 1986). Commensurate with the more rapid complete re-activation of PDH_a in brown fat than in liver, there was no evidence for any marked effect of prolonged starvation to impair the re-activation of brown-fat PDH by DCA (Table 2).

Hepatic and brown-fat lipogenesis in relation to PDH activities in the fed state

It is currently thought that the precursors used for lipid synthesis in the fed state differ in liver and brown adipose tissue (reviewed by McCormack, 1982; Katz & McGarry, 1984). Glucose itself is considered to be a major precursor for lipogenesis in brown fat (see McCormack, 1982), whereas lactate or glycogen is thought more likely to serve as the lipogenic precursor in the liver (Hems *et al.*, 1972, 1975; reviewed by Katz & McGarry, 1984). In the fed state, the rate of hepatic lipogenesis bore a reasonable correlation with the measured activity of PDH_a expressed on a wet-weight basis, bearing in mind that PDH_a activity was measured at 30 °C rather than 37 °C (Table 1). This finding is consistent with the idea that pyruvate (derived from lactate, glycogen or possibly dietary protein) is a major precursor for hepatic lipogenesis, although it does not exclude the possibility of additional regulatory sites for lipogenesis distal to PDH.

Ferré *et al.* (1986) have measured rates of glucose uptake and phosphorylation *in vivo* in brown adipose tissue in 2 h-starved rats under basal conditions and also after an acute elevation in the circulating insulin concentration (insulin clamp). In the basal state, the rate of glucose utilization (uptake and phosphorylation) in brown adipose tissue was relatively low (approx. 33 nmol/min per g of tissue). The maximum possible rate of production of pyruvate from circulating glucose is therefore 66 nmol/min per g of tissue. PDH_a activity in brown fat in the fed state (measured at 30 °C) is sufficient for conversion of at least approx. 670 nmol of pyruvate to acetyl-CoA/min per g of tissue (Table 2). The capacity for pyruvate decarboxylation to acetyl-CoA thus greatly exceeds the rate of glucose uptake and phosphorylation (and presumably glycolytic flux from exogenous glucose to pyruvate). In contrast, potential carbon flux via PDH to acetyl-CoA, when expressed in terms of C₁₆ (palmitate) equivalents, compares well with the total rate of brown-fat lipogenesis (Table 2).

It would appear that under basal (fed or immediately post-

absorptive) conditions the rate of glucose uptake and phosphorylation rather than PDH_a activity limits the use of glucose itself as a lipogenic precursor in brown fat. Indeed, the rate of glucose uptake and phosphorylation observed in the experiments of Ferré *et al.* (1986) could supply only approx. 5% of the carbon required for lipid synthesis. However, since the rate of lipogenesis approximates to the potential for production of precursor (acetyl-CoA) from pyruvate via PDH (Table 2), it is attractive to suggest that use of glucose itself as a lipogenic precursor may be supplemented by the use of circulating lactate/pyruvate. Lipogenesis in both liver and brown fat in the fed state may thus be viewed as a mechanism to scavenge surplus C₃ derivatives of glucose of exogenous origin for biosynthetic purposes.

Hepatic and brown-fat lipogenesis in relation to PDH activities during the fed-to-starved transition

The decline in hepatic and brown-fat PDH_a activities observed after 6 h of starvation was in both cases accompanied by significantly decreased rates of lipid synthesis (Table 1). A 55% decrease in the rate of lipid synthesis was observed in the liver. This approximates to the decline in hepatic lipogenesis which has been observed in response to 6 h starvation in lactating rates (Gibbons *et al.*, 1983; reviewed by Williamson *et al.*, 1983) and closely correlates with the 69% decrease in hepatic PDH_a activity (Table 1). Starvation for a more prolonged period (48 h) further decreased rates of hepatic lipogenesis, to approx. 33% of the fed value (Table 1). In brown fat, the 72% decline in PDH_a observed after acute (6 h) starvation was accompanied by a 64% decrease in brown-fat lipogenesis (Table 2).

It is remarkable that, of the 67% decline in hepatic lipid synthesis observed after 48 h starvation, 83% of the total response is achieved within the first 6 h of the onset of starvation (Table 1). Indeed, in brown fat, rates of lipogenesis after 6 h starvation were not significantly different from those observed after 48 h starvation (Table 2), when lipogenic rates may be sustained by increased use of non-carbohydrate precursors, which may include ketone bodies (Agius & Williamson, 1981) or acetate (Steiner & Cahill, 1966; Sugden *et al.*, 1982).

Hepatic and brown-fat lipogenesis in relation to PDH activities after insulin treatment in the fed state

The rate of brown-fat lipogenesis increases by approx. 18 µmol of fatty acid/h per g after acute insulin treatment (Table 2). This increase approximates to the increase in the capacity for conversion of pyruvate into acetyl-CoA via PDH (approx. 15 µmol of C₁₆ equivalents/h per g wet wt.; Table 2). The increases in rates of hepatic lipid synthesis observed in response to insulin administration in the fed state also closely paralleled increases in hepatic PDH_a activities (Table 1). Consequently, increases in lipogenesis in both liver and brown fat after insulin treatment in the fed state can be accommodated by the increased potential for conversion of pyruvate into acetyl-CoA via PDH.

Insulin treatment of 2 h-starved rats elicits a 60-fold increase in the rate of brown-adipose-tissue glucose uptake and phosphorylation (Ferré *et al.*, 1986) to a value of 2000–3000 nmol/min per g of tissue. Theoretically, this would permit a maximum rate of pyruvate production from glucose of 6000 nmol/min per g. This is comparable with or exceeds the capacity for conversion of pyruvate into acetyl-CoA via PDH (see Table 2). Thus glucose uptake and phosphorylation do not necessarily limit the use of glucose itself as a lipogenic precursor after an elevation in the circulating insulin concentration from the basal (fed) value. The possibility is raised that insulin-stimulated brown adipose tissue may produce significant amounts

of lactate, and may thereby play an important role in the Cori cycle.

Hepatic and brown-fat lipogenesis in relation to PDH activities after insulin treatment during the fed-to-starved transition

The administration of insulin to 6 h-starved rats elicits a 100 % increase in hepatic lipogenesis (Table 1). Increased flux from glucose to lipid is presumably facilitated by the parallel increases in PDH_a activity. Both PDH_a activity and lipogenic rates remain relatively low after acute challenge with insulin after 48 h starvation (Table 1; see also Sugden *et al.*, 1981, 1982; Holness *et al.*, 1988).

Marked increases in brown-fat lipogenesis are observed in response to insulin administration after acute (6 h) starvation (Table 2) and also after prolonged (48 h) starvation (Table 2; see also Sugden *et al.*, 1982). The disproportionate increase in lipogenic rate relative to PDH_a observed in insulin-treated 48 h-starved rats presumably reflects the fact that PDH_a activities were measured after only 30 min exposure to insulin, whereas rates of lipogenesis were measured over a period of 1 h after insulin injection.

Insulin action in relation to substrate supply

In the current study *in vivo* we have not attempted to differentiate between the direct effects of insulin or effects which may be secondary to actions of insulin on other tissues or to changes in circulating concentrations of counter-regulatory hormones. In particular, suppression of lipolysis, with resultant decreases in fatty acid availability, might be expected either to reinforce or to be directly responsible for some of observed effects on either PDH or lipogenesis. In the present experiments, fatty acid concentrations were consistently lowered by the administration of insulin, even in the fed state (values of 0.19 ± 0.04 versus 0.04 ± 0.01 mM in the fed state; 0.18 ± 0.04 versus 0.07 ± 0.02 mM after 6 h starvation; 0.60 ± 0.04 versus 0.31 ± 0.03 mM after 48 h starvation). However, it has been excluded previously that the decline in hepatic PDH_a activity observed after acute (6 h) starvation is secondary solely to an increased fatty acid supply, since inhibition of mitochondrial long-chain fatty acid oxidation does not reverse the effect (Holness & Sugden, 1989a).

Concluding remarks

There are several novel findings from this study. First, the major decline in lipid synthesis which is elicited by starvation is observed within 6 h in two lipogenic tissues (liver and brown fat). Decreased lipid synthesis occurs concomitantly with a decline in PDH_a activity in both tissues. Secondly, restoration of lipid synthesis by the administration of insulin can be achieved rapidly after 6 h starvation in both liver and brown fat: increased lipid synthesis is in both cases associated with PDH re-activation. The acute regulation of lipid synthesis during the initial period of the fed-to-starved transition may thus be achieved via alterations in the use of pyruvate via PDH. Thirdly, prolonged (48 h) starvation renders the PDH complex resistant to rapid and complete re-activation by insulin administration or re-feeding in both liver and brown fat. The more pronounced effect is observed in liver, which also shows a more attenuated initial lipogenic response. The results demonstrate the sensitivity of PDH and lipogenesis

to acute starvation in liver and brown fat, and indicate the potential importance of the activity of the PDH complex in determining the rate of lipid synthesis during the fed-to-starved and starved-to-fed nutritional transitions in two tissues known to be quantitatively important sites of lipid synthesis.

This work was supported by the U.K. Medical Research Council.

REFERENCES

- Agius, L. & Williamson, D. H. (1980) *Biochem. J.* **190**, 477–480
- Agius, L. & Williamson, D. H. (1981) *Biochim. Biophys. Acta* **666**, 127–132
- Assimakopoulos-Jeannet, F., McCormack, J. G., Prentki, M., Jeanrenaud, B. & Denton, R. M. (1982) *Biochim. Biophys. Acta* **717**, 86–90
- Block, K. & Vance, D. (1977) *Annu. Rev. Biochem.* **46**, 263–298
- Caterson, I. D., Fuller, S. J. & Randle, P. J. (1982) *Biochem. J.* **208**, 53–60
- Denton, R. M. & Brownsey, R. W. (1983) *Philos. Trans. R. Soc. London B* **302**, 33–45
- Denyer, G. S., Lam, D., Cooney, G. J. & Caterson, I. D. (1989) *FEBS Lett.* **250**, 464–468
- Ferré, P., Burnol, A.-F., Leturque, A., Terretaz, J., Pénicaud, L., Jeanrenaud, B. & Girard, J. (1986) *Biochem. J.* **233**, 249–252
- French, T. J., Holness, M. J., Goode, A. W. & Sugden, M. C. (1988) *Clin. Sci.* **74**, 107–112
- Gibbons, G. F., Pullinger, C. R., Munday, M. R. & Williamson, D. H. (1983) *Biochem. J.* **212**, 843–848
- Hardie, D. G. (1980) *Mol. Aspects Cell. Regul.* **1**, 33–62
- Hems, D. A., Whitton, P. D. & Taylor, E. A. (1972) *Biochem. J.* **129**, 529–538
- Hems, D. A., Rath, E. A. & Verrinder, T. R. (1975) *Biochem. J.* **150**, 167–173
- Holness, M. J. & Sugden, M. C. (1988) *Biochem. Soc. Trans.* **16**, 773–774
- Holness, M. J. & Sugden, M. C. (1989a) *Biochem. J.* **258**, 529–533
- Holness, M. J. & Sugden, M. C. (1989b) *Biochem. J.* **262**, 321–325
- Holness, M. J., French, T. J. & Sugden, M. C. (1986) *Biochem. J.* **235**, 441–445
- Holness, M. J., MacLennan, P. A., Palmer, T. N. & Sugden, M. C. (1988) *Biochem. J.* **252**, 325–330
- Katz, J. & McGarry, J. D. (1984) *J. Clin. Invest.* **74**, 1901–1909
- Kruszynska, Y. T. & McCormack, J. G. (1989) *Biochem. J.* **258**, 699–707
- Marchington, D. R., Kerbey, A. L., Jones, A. E. & Randle, P. J. (1987) *Biochem. J.* **246**, 233–236
- McCormack, J. G. (1982) *Prog. Lipid Res.* **21**, 195–223
- McCormack, J. G. & Denton, R. M. (1977) *Biochem. J.* **166**, 627–630
- McGarry, J. D., Meier, J. M. & Foster, D. W. (1973) *J. Biol. Chem.* **248**, 270–278
- Munday, M. R. & Williamson, D. H. (1981) *Biochem. J.* **196**, 831–837
- Pallardo, F. V. & Williamson, D. H. (1989) *Biochem. J.* **257**, 607–610
- Robinson, A. M., Girard, J. R. & Williamson, D. H. (1978) *Biochem. J.* **176**, 343–346
- Stansbie, D., Brownsey, R. W., Crettaz, M. & Denton, R. M. (1976) *Biochem. J.* **160**, 413–416
- Steiner, G. & Cahill, G. F., Jr. (1966) *Can. J. Biochem.* **44**, 1587–1596
- Sugden, M. C., Watts, D. I. & Marshall, C. E. (1981) *Biosci. Rep.* **1**, 469–476
- Sugden, M. C., Watts, D. I., Marshall, C. E. & McCormack, J. G. (1982) *Biosci. Rep.* **2**, 289–297
- Volpe, J. J. & Vagelos, P. R. (1976) *Physiol. Rev.* **56**, 339–417
- Whitehouse, S., Cooper, R. H. & Randle, P. J. (1974) *Biochem. J.* **141**, 761–774
- Wieland, O. H., Patzelt, C. & Löffler, G. (1972) *Eur. J. Biochem.* **26**, 426–433
- Williamson, D. H., Munday, M. R., Jones, R. G., Roberts, A. F. C. & Ramsey, A. J. (1983) *Adv. Enzyme Regul.* **21**, 135–145
- Windmueller, H. G. & Spaeth, A. E. (1966) *J. Biol. Chem.* **241**, 2891–2899
- Windmueller, H. G. & Spaeth, A. E. (1967) *Arch. Biochem. Biophys.* **122**, 362–369