Pyruvate dehydrogenase activities during the fed-to-starved transition and on re-feeding after acute or prolonged starvation

Mark J. HOLNESS and Mary C. SUGDEN*

Departments of Chemical Pathology and Biochemistry, London Hospital Medical College, Turner Street, London E1 2AD, U.K.

We investigated the temporal relationship between hepatic glycogen depletion and cardiac and hepatic PDH (pyruvate dehydrogenase complex) activities during the acute phase of starvation. There was a striking correlation between the decline in hepatic glycogen and PDH inactivation during the first 10 h of starvation. Re-feeding after 6 h starvation was associated with complete re-activation of PDH in liver and re-activation to approx. 75 % of the fed value in heart, whereas in rats previously starved for 24–48 h re-activation was delayed in liver and diminished in heart. The results are discussed with reference to the fate of dietary carbohydrate after re-feeding.

INTRODUCTION

The requirement of several tissues for carbohydrate as the sole or the major energy substrate necessitates the maintenance of the blood glucose concentration within a relatively narrow range. In starvation, where dietary carbohydrate is unavailable, such homeostasis is achieved by restricting the loss of carbohydrate carbon as CO₂ by phosphorylation and inactivation of the pyruvate dehydrogenase complex (PDH), by replacing (via glycogenolysis and/or gluconeogenesis) any glucose which is utilized, and by providing alternative non-carbohydrate substrates (e.g. fatty acids and ketone bodies) for use by oxidative tissues. The preferential use of non-carbohydrate substrates in starvation forms the basis of the glucose/fatty acid cycle (Randle *et al.*, 1964).

Many investigations of the regulation of glucose production and conservation in the intact animal after nutritional deprivation have been carried out after rather prolonged periods of starvation (24–48 h). Starvation of this duration is associated with a marked elevation in circulating fatty acid and ketone-body concentrations (see, e.g., Holness *et al.*, 1988) and a 30-fold decrease in the percentage of cardiac PDH in the active form (reviewed by Randle *et al.*, 1984; see also French *et al.*, 1988). Although the decline in hepatic PDH activity is less dramatic (a 3-fold change in activity; reviewed by Wieland, 1983), it is accompanied by greatly enhanced rates of gluconeogenesis, at least in part as a consequence of adaptive increases in the activities of key gluconeogenic enzymes, e.g. glucose-6-phosphatase and phosphoenolpyruvate carboxykinase. The response to short-term starvation has been less extensively studied. McGarry *et al.* (1973) demonstrated that a period as short as 6–12 h after the last feeding can induce profound effects on hepatic carbohydrate metabolism, including glycogen depletion and activation of gluconeogenesis, and, in lactating rats, starvation for 6 h is associated not only with hepatic glycogen depletion but also with a decline of over 85 % in mammary-gland lipogenesis (Jones *et al.*, 1984). However, measurements of arterio-venous differ-

ences did not suggest inactivation of mammary-gland PDH (Williamson *et al.*, 1983). These findings raised the possibility that there might not be co-ordinate control of glucose production and utilization during the transition from the fed to the fasted state, and prompted an investigation of the temporal relationship between hepatic glycogen depletion and PDH activities during the acute phase of starvation (0–10 h after food withdrawal).

The provision of carbohydrate after prolonged (48 h) starvation leads neither to immediate suppression of hepatic gluconeogenic flux (Sugden *et al.*, 1983; Newgard *et al.*, 1984) nor to immediate re-activation of cardiac or hepatic PDH (Kerbey & Randle, 1982; Holness *et al.*, 1986). Significant re-activation of hepatic PDH occurs only after 3–4 h (Holness *et al.*, 1988), and it has been proposed that such delayed re-activation may permit the preferential use of lactate and pyruvate for hepatic glycogenesis rather than lipogenesis (Holness *et al.*, 1988). However, Wieland *et al.* (1972) observed more rapid re-activation of hepatic PDH in response to re-feeding in rats starved for only 24 h. We therefore considered it important to determine whether the immediacy of the response of tissue PDH to re-feeding was dependent on the antecedent period of starvation.

MATERIALS AND METHODS

Materials

Sources of materials were as in Holness *et al.* (1988). 2-Tetradecylglycidate was generously given by McNeil Pharmaceuticals, Spring House, PA, U.S.A.

Rats

Female albino Wistar rats (180–220 g) were subjected to a 12 h-light/12-dark cycle (light from 08:30 h), and were fed *ad libitum* on a standard rodent diet containing 52 % digestible 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 the food was removed. Animals either were starved thereafter, or were provided with

Abbreviation used: PDH, pyruvate dehydrogenase complex; PDH_a, its active form.

* To whom correspondence and reprint requests should be addressed.

Table 1. Chow intakes after the provision of chow *ad libitum* to 6 h-, 24 h- or 48 h-starved rats

For experimental details see the text.

Time after chow administration (h)	Rats...	Chow intake (g/100 g body wt.)		
		6 h-starved	24 h-starved	48 h-starved
0.5		0.40±0.23 (5)	—	—
1.0		0.75±0.20 (5)	1.44±0.47 (5)	1.95±0.23 (10)
2.0		1.30±0.14 (7)	2.09±0.24 (6)	2.67±0.54 (8)
3.0		—	3.24±0.21 (6)	3.09±0.36 (9)
4.0		1.58±0.11 (6)	3.89±0.43 (5)	3.51±0.36 (7)

laboratory chow *ad libitum*, the periods of starvation preceding re-feeding being either 6, 24 or 48 h. Chow intakes after re-feeding were monitored, and are shown in Table 1. For the study of the effect of re-feeding in 6 h-starved rats, lights were dimmed in order to provide conditions conducive to food consumption. Even so, intakes were considerably less than those observed after more prolonged starvation (24 or 48 h, Table 1). No attempt was made to increase intake by intubation of a liquid diet (see, e.g., McGarry *et al.*, 1973), as significant effects were achieved at these lower intakes (see below). 2-Tetradecylglycidate [2.5 mg/100 g body wt., suspension in 0.5% (w/v) CM-cellulose] was administered intragastrically under light diethyl ether anaesthesia at 3 h after food withdrawal. Rats were sampled after a further 3 h.

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. Glycogen concentrations were measured in extracts of freeze-clamped liver (for details see Holness *et al.*, 1988).

The active form of the pyruvate dehydrogenase complex (PDH_a) and citrate synthase activities were measured in freeze-clamped tissue extracts as described by Caterson *et al.* (1982). PDH activities have been expressed relative to citrate synthase to correct for possible variation in the efficiency of mitochondrial extraction (see Caterson *et al.*, 1982; French *et al.*, 1988). Total PDH activities are unchanged by the nutritional status. One unit of enzyme activity is that which converts 1 µmol of substrate into product per min at 30 °C.

Expression of results

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

RESULTS AND DISCUSSION

Hepatic glycogen concentrations and hepatic and cardiac PDH activities during the initial 10 h of food deprivation

Several investigators have studied the pattern of changes in hepatic glycogen concentrations over the diurnal cycle (e.g. Fuller & Diller, 1970; Cohn & Joseph, 1971; Bouillon & Berdanier, 1981). Peak glycogen values are observed at the end of the dark phase of the cycle.

Concentrations decline during the light phase, the minimum concentration being reached at the end of the light phase. Thus, in the present experiments, rats were provided with standard laboratory chow *ad libitum* until the end of the dark phase (08:30 h). Food was then removed. Hepatic glycogen concentrations measured at 08:30 h (approx. 60–70 mg/g of tissue; Fig. 1a) were comparable with those observed in other studies (e.g. Bouillon & Berdanier, 1981; see also Jones *et al.*, 1984), and were maintained for the first 3 h after food withdrawal (Fig. 1a). Thereafter concentrations rapidly declined, and by 6 h were only approx. 40% of the fed value. The concentration continued to decrease over the next 4 h, and by 24 h of fasting was only 5% of the fed value (Fig. 1a).

McGarry *et al.* (1973) have also studied the time course of changes in hepatic glycogen concentrations over the initial 10 h of starvation. The protocol followed in that study differed from our own in that rats previously provided with free access to food were given a liquid diet by stomach intubation as a final feeding. The period over which hepatic glycogen concentrations were maintained was extended to 6 h, presumably as a result of continued carbohydrate absorption from the gastrointestinal tract. However, as in our experiments, the most rapid rates of depletion of hepatic glycogen occurred over a relatively short period (of 4–6 h duration), and was essentially complete after 24 h of fasting.

There was a striking correlation between the hepatic glycogen concentration and the activity of hepatic PDH over the first 10 h of starvation (Fig. 1a). This correlation was even more apparent when PDH activity was plotted as a function of the hepatic glycogen concentration (Fig. 1b). Little change in liver PDH activity was observed during the first 3 h, but thereafter the activity steadily decreased. This activity was approx. 55% of the fed value by 6 h and 31% by 8 h (Fig. 1a). The activity of hepatic PDH was not further decreased if the starvation period was extended (Fig. 1b). Previous studies (e.g. Caterson *et al.*, 1982; Fatania *et al.*, 1986; Marchington *et al.*, 1987; Holness *et al.*, 1988) have reported a similar extent of inhibition of hepatic PDH activity (to 29–48% of the fed value) during prolonged starvation.

A high activity of cardiac PDH complex was also maintained for 3 h, but in the heart the activity of the complex declined precipitously between 3 and 4 h after food withdrawal (Fig. 1a). Thus, at 4 h, although the hepatic glycogen concentration was still approx. 70% of that observed in the fed state, cardiac PDH activity had already declined by more than 68%, and, in contrast

Fig. 1. Hepatic glycogen concentrations and tissue PDH activities during the fed-to-starved transition

(a) Hepatic glycogen concentration ($\diamond$) and the activities of hepatic ($\blacktriangle$) and cardiac ($\bullet$) PDH for up to 48 h after food withdrawal. Each point represents the mean value $\pm$ S.E.M. for 4–8 animals. Statistically significant effects of starvation are indicated by: ‡ $P < 0.01$; * $P < 0.001$. (b) Relationship between the activity of hepatic PDH and the liver glycogen concentration in individual rats in the fed state ($\circ$), and at 2 ($\triangle$), 3 (∇), 4 ($\square$), 6 ($\diamond$), 7 ($\blacktriangledown$), 8 ($\blacktriangleleft$) and 10 ($\ominus$) h after food withdrawal. This was a linear relationship ($y = 2.02x + 2.72$; $r = 0.80$).

with the liver, activity was negligible ($< 5\%$ of the fed value) after 24–48 h starvation (see also Kerbey & Randle, 1982; Sale & Randle, 1982).

The effects of short-term starvation on hepatic metabolism have been attributed to a decreased insulin/glucagon ratio (McGarry *et al.*, 1975; Boyd *et al.*, 1981), and liver PDH is sensitive to both of these hormones (reviewed by Wieland, 1983), although, paradoxically, glucagon may increase hepatic PDH under some circumstances (Oviasu & Whitton, 1984; Fatania *et al.*, 1986). Observations of direct hormonal regulation of the PDH complex in heart are few (reviewed by Wieland, 1983). In this study we have not attempted to define the importance of hormonal changes in the response to acute starvation. However, as it has been noted that cardiac PDH is sensitive to elevated fatty acid concentration (Jin *et al.*, 1988) and that oleate can oppose the effects of insulin to increase hepatic PDH *in vivo* (Wieland *et al.*, 1972), we examined the effects of inhibition of mitochondrial fat oxidation on cardiac and hepatic PDH_a over the period associated with the most marked decline in activity (3–6 h after food withdrawal). The administration of 2-tetradecylglycidate (an inhibitor of carnitine palmitoyltransferase 1) abolished the effects of starvation to decrease cardiac PDH activity (values of cardiac PDH at 6 h of 13.9 ± 1.5 m-units/unit of citrate synthase; $P < 0.001$ versus control). In contrast, a decline in hepatic PDH activity was still observed (results not shown). Caterson *et al.* (1982) have previously observed

that 2-tetradecylglycidate fails to re-activate hepatic PDH in previously starved rats. The results indicate that the response of cardiac PDH to starvation is likely to be mediated via increased fatty acid oxidation, whereas in liver the response is dictated by factors other than, or in addition to, increased fat oxidation.

In the intact animal, the inactivation of hepatic PDH which accompanies glycogen mobilization may be important in directing available C_3 intermediates towards gluconeogenesis, rather than to oxidation or incorporation into lipid. In support of this suggestion, McGarry *et al.* (1973) have demonstrated that both glycogenolysis and gluconeogenesis contribute to glucose production by perfused livers from rats sampled during the first 10 h of starvation. The finding of rapid inactivation of hepatic and cardiac PDH during the early stages of starvation has a further important implication for glucose homeostasis. Since the inactivation of tissue PDH either precedes (heart) or accompanies (liver) hepatic glycogenolysis (Fig. 1a), oxidation of glucose made available by hepatic glycogen mobilization is minimized. Since the metabolism of glucose can proceed only to pyruvate and lactate and the capacity of the liver for gluconeogenesis is increased, glycaemia can subsequently be maintained via the operation of the Cori cycle.

It is noteworthy that rates of glucose turnover are significantly higher in rats starved for only 3–6 h compared with rates in 48 h-starved rats [17.6 ± 21.0 and 11.0 mg/min per kg respectively; see Issad *et al.* (1987)

Fig. 2. Hepatic (a) and cardiac (b) PDH activities after chow re-feeding

Rats fasted for 6 (▲), 24 (●) or 48 (◆) h were re-fed with chow *ad libitum* for the indicated time points as described in the Materials and methods section. Each point represents the mean value $\pm$ S.E.M. for 4–8 animals. Statistically significant effects of re-feeding are indicated by: † $P < 0.05$; ‡ $P < 0.01$; * $P < 0.001$.

and Smadja *et al.* (1988) for details]. This raises the possibility that the rate of operation of the Cori cycle during the fed-to-starved transition may actually exceed that observed during prolonged starvation.

Response of tissue PDH activity to re-feeding

Marchington *et al.* (1987) studied the time course of PDH re-activation in response to insulin in cultured hepatocytes from 48 h-starved rats. Re-activation took 4 h to complete and was associated with a decrease in PDH kinase activity. In parallel studies *in vivo*, we observed a similar period of hysteresis before re-activation in response to chow re-feeding in 48 h-starved rats (Holness & Sugden, 1987; Holness *et al.*, 1988). In contrast, Wieland *et al.* (1972) demonstrated restoration of the activity of hepatic PDH within 90 min of the intragastric administration of glucose to 24 h-starved rats. The responses of hepatic PDH to chow re-feeding in rats previously starved for 6, 24 or 48 h are compared in Fig. 2(a).

A major consequence of an increase in the duration of starvation was to introduce a lag phase before significant re-activation. Thus, in contrast with the delay of approx. 3 h before re-activation of hepatic PDH in rats previously starved for 48 h, re-activation of the enzyme complex was apparent within 1 h of re-feeding of 6 h-starved rats, with an intermediate period of hysteresis (1–2 h) in 24 h-starved rats. The restraint on the activity of hepatic PDH after re-feeding therefore appears to be related to the duration of starvation. In all cases re-activation of hepatic PDH to the fed value (approx. 15 m-units/unit of citrate synthase) was achieved.

The time course of cardiac PDH re-activation on chow re-feeding after 40 h starvation has been examined previously by Kerbey & Randle (1982), albeit in less detail than in the present study. Only partial (approx. 50%) re-activation was observed even after 15 h, although full re-activation was observed after re-feeding for 24–30 h. In the present experiments, limited (26%) re-activation was observed between 1 and 2 h after re-feeding of 48 h-starved rats, with limited further re-activation over the subsequent 2 h (Fig. 2b). Even at 8 h after re-feeding, cardiac PDH activity was only $29.8 \pm 1.2\%$ of the fed value. Limited (35%) re-activation of cardiac PDH was also observed within 1 h of re-feeding of 24 h-starved rats. The activity of the complex over the subsequent 3 h reached a mean value of 41.2% of that observed in the fed state. In marked contrast, in rats starved for only 6 h, re-activation of cardiac PDH was rapid, and within 1 h values did not differ significantly from those found in the fed state (Fig. 2b).

The activity of kinase and kinase activator protein ('KAP') in heart increases with the duration of starvation, being significantly less in rats starved for 20 h than in rats starved for 40–48 h (Kerbey & Randle, 1982). The induction of PDH kinase in hepatocytes from fed rats by fatty acids/glucagon also occurs progressively, with major increases between 4 and 24 h (Fatania *et al.*, 1986). It is attractive to suggest that the effects of prolonged starvation to restrict PDH re-activation in response to re-feeding are linked to these stable adaptive changes in PDH kinase activities. However, whereas the activity of liver PDH is restored to fed values within 4 h, cardiac PDH activities remain relatively low for at least 8 h. Thus it would appear either that the regulation of kinase activity during the re-feeding transition varies

between the two tissues, or that additional factors [e.g. multi-site phosphorylation (see, e.g., Sugden *et al.*, 1978; Sale & Randle, 1982), changes in phosphatase activity] may be of regulatory importance.

Hepatic glycogen repletion

Although livers of 6 h-starved rats contained a relatively high concentration of residual glycogen (approx. 40% of peak values; Fig. 1a) compared with 24 h/48 h-starved rats (approx. 5% of peak values), the provision of chow nevertheless led to significant hepatic glycogen deposition within 2 h, and rates of glycogen deposition during this period were similar to those observed in rats previously starved for 24 or 48 h (10–15 mg/h per g of liver; results not shown). Thus in 6 h-starved rats, where rapid re-activation of hepatic PDH activity is observed, pyruvate oxidation does not exclude glycogen deposition, as is the case after prolonged starvation (Holness *et al.*, 1986). Therefore, either the supply of C₃ intermediates for glycogen synthesis via gluconeogenesis (the indirect pathway of glycogen synthesis; reviewed by McGarry *et al.*, 1987) is not rate-limiting, or hexose monophosphate formation via glucokinase (the direct pathway) assumes greater significance, as is observed in meal-fed rats (Huang & Veech, 1988).

Concluding remarks

This study has demonstrated that inactivation of the PDH complex in at least two oxidative tissues is an early response to starvation, occurring concomitantly with hepatic glycogenolysis. However, in contrast with the situation after more prolonged starvation, complete re-activation of the complex is rapidly achieved by re-feeding.

Although inhibition of fat oxidation reversed the effects of acute starvation on cardiac PDH, this was not the case in liver. This implies that hepatic energy requirements during the early stages of starvation can be met by glycolysis or by the oxidation of substrates other than carbohydrates or fatty acids. The possibility is raised that inhibition of hepatic glycolysis may occur after hepatic PDH inactivation. Alternatively, amino acids (or their keto derivatives) may be used as oxidative substrates.

In the present study we have examined the response to re-feeding after a very brief period of starvation, which may possibly have more relevance to normal feeding patterns than that seen after long-term starvation. Nevertheless, the response to re-feeding after prolonged starvation may more closely reflect the metabolic pattern which exists in certain physiological (pregnancy, lactation) or pathological (cancer, trauma) states, where additional substrate requirements have to be met during periods of food deprivation.

We thank the British Heart Foundation and the Medical Research Council for financial support, David Kelm for help

with some of the experiments and Lena Purvis for expert secretarial assistance.

REFERENCES

- Bouillon, D. J. & Berdanier, C. D. (1981) *J. Nutr.* **111**, 1462–1474
- Boyd, M. E., Albright, E. B., Foster, D. W. & McGarry, J. D. (1981) *J. Clin. Invest.* **68**, 142–152
- Caterson, I. D., Fuller, S. J. & Randle, P. J. (1982) *Biochem. J.* **208**, 53–60
- Cohn, C. & Joseph, D. (1971) *Proc. Soc. Exp. Biol. Med.* **137**, 1303–1306
- Fatania, H. R., Vary, T. C. & Randle, P. J. (1986) *Biochem. J.* **234**, 233–236
- French, T. J., Holness, M. J., MacLennan, P. A. & Sugden, M. C. (1988) *Biochem. J.* **250**, 773–779
- Fuller, R. W. & Diller, E. R. (1970) *Metab. Clin. Exp.* **19**, 226–229
- Holness, M. J. & Sugden, M. C. (1987) *Biochem. J.* **247**, 627–634
- 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
- Huang, M.-T. & Veech, R. L. (1988) *J. Clin. Invest.* **81**, 872–878
- Issad, T., Pénicaud, L., Ferré, P., Kandé, J., Baudon, M.-A. & Girard, J. (1987) *Biochem. J.* **246**, 241–244
- Jin, D.-Y., Goode, A. W., MacLennan, P. A. & Sugden, M. C. (1988) *Biochem. Soc. Trans.* **16**, 553
- Jones, R. G., Ilic, V. & Williamson, D. H. (1984) *Biochem. J.* **223**, 345–351
- Kerbey, A. L. & Randle, P. J. (1982) *Biochem. J.* **206**, 103–111
- Marchington, D. R., Kerbey, A. L., Jones, A. E. & Randle, P. J. (1987) *Biochem. J.* **246**, 233–236
- McGarry, J. D., Meier, J. M. & Foster, D. W. (1973) *J. Biol. Chem.* **248**, 270–278
- McGarry, J. D., Wright, P. H. & Foster, D. W. (1975) *J. Clin. Invest.* **55**, 1202–1209
- McGarry, J. D., Kuwajima, M., Newgard, C. B., Foster, D. W. & Katz, J. (1987) *Ann. Rev. Nutr.* **7**, 51–73
- Newgard, C. B., Moore, S. V., Foster, D. W. & McGarry, J. D. (1984) *J. Biol. Chem.* **259**, 6958–6963
- Oviasu, O. A. & Whitton, P. D. (1984) *Biochem. J.* **224**, 183–186
- Randle, P. J., Newsholme, E. A. & Garland, P. B. (1964) *Biochem. J.* **93**, 652–665
- Randle, P. J., Fuller, S. J., Kerbey, A. L., Sale, G. J. & Vary, T. C. (1984) *Horm. Cell Regul.* **8**, 139–150
- Sale, G. J. & Randle, P. J. (1982) *Biochem. J.* **206**, 221–229
- Smadja, C., Morin, J., Ferré, P. & Girard, J. (1988) *Am. J. Physiol.* **254**, E407–E413
- Sugden, M. C., Watts, D. I., Palmer, T. N. & Myles, D. D. (1983) *Biochem. Int.* **7**, 329–337
- Sugden, P. H., Hutson, N. J., Kerbey, A. L. & Randle, P. J. (1978) *Biochem. J.* **169**, 433–435
- Wieland, O. H. (1983) *Rev. Physiol. Biochem. Pharmacol.* **96**, 123–170
- 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