Hepatic glycogen synthesis on carbohydrate re-feeding after starvation

A regulatory role for pyruvate dehydrogenase in liver and extrahepatic tissues

Mark J. HOLNESS,* Timothy J. FRENCH† and Mary C. SUGDEN*‡

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

†Surgical Unit, The London Hospital, Whitechapel, London E1 1BB, U.K.

Glucose administration to 48 h-starved rats increased hepatic glucose, lactate, pyruvate and glycogen concentrations and re-activated PDH (pyruvate dehydrogenase complex) in kidney, but not in heart or liver. Dichloroacetate together with glucose re-activated PDH in all three tissues, decreased hepatic lactate and pyruvate concentrations and impaired glycogen resynthesis. Thus on re-feeding, delayed PDH re-activation is important for provision of precursors for hepatic glyconeogenesis.

INTRODUCTION

In the liver, mitochondrial acetyl-CoA can be used either for fat synthesis or for ketone-body formation, but, whereas under lipogenic conditions the acetyl-CoA is formed mainly from carbohydrate, under ketogenic conditions it is derived mainly from fat. In the fed (lipogenic) state, acetyl-CoA production from fat is restricted through inhibition of mitochondrial fatty acid uptake by malonyl-CoA, a lipogenic intermediate (for review, see [1]). Since the malonyl-CoA concentration is proportional to the rate of lipogenesis, this inhibition minimizes mitochondrial oxidation of newly synthesized fat, and ensures that hepatic fatty acid oxidation occurs only when the supply of carbohydrate is markedly decreased (for review, see [2]). Conversely, in the starved (ketogenic) state, acetyl-CoA formation from carbohydrate is restricted because of increased availability of fatty acids. This restriction is achieved, at least in part, through mitochondrial fat oxidation, which elevates mitochondrial ratios of [acetyl-CoA]/[CoA] and possibly [NADH]/[NAD⁺], thereby causing end-product inhibition of the pyruvate dehydrogenase complex (PDH; EC 1.2.4.1+2.3.1.12+1.8.1.4) and stimulating PDH kinase (EC 2.7.1.99), which phosphorylates and inactivates the complex (for review, see [3]). At the same time available oxaloacetate is utilized for gluconeogenesis, thereby minimizing use of acetyl-CoA for citrate synthesis (and lipogenesis from carbohydrate), and increasing ketogenesis.

Work by several groups has indicated that the administration of carbohydrate to 24 h-starved rats does not result in an immediate reversal of the direction of hepatic carbon flux to that observed in the 'fed' state. Lipogenesis remains depressed [4, 5], and the predominant direction of carbon flux is gluconeogenic rather than glycolytic. Furthermore, most of the hepatic glycogen deposited after carbohydrate administration to starved rats is synthesized from C₃ metabolites of glucose via the gluconeogenic pathway, rather than directly from the

glucose provided ([6–8]; for reviews see [9, 10]). It is uncertain whether the metabolism of glucose to its C₃ breakdown products occurs within the liver [11], in the gut [12–14], or in extra-splanchnic tissues. During the course of a study of the metabolic effects of glucose re-feeding in the rat, we observed that cardiac PDH was refractory to re-activation. In the present study we report these findings, and we have specifically addressed the question of whether the maintenance of relatively inactive PDH complex in liver and/or extrahepatic tissues is important for the provision of carbon for hepatic glyconeogenesis and the restriction of hepatic lipogenesis.

MATERIALS AND METHODS

Materials

Sources of materials were as in [15].

Treatment of rats

Female albino Wistar rats (150–200 g) were fed *ad libitum* on a standard rodent diet [E. Dixon and Son (Ware) Ltd., Ware, Herts., U.K.], or starved for 48 h (grid-bottomed cages). Experiments were started between 08:30 and 09:30 h, when glucose (2 mmol/100 g body wt.,; 2 M solution) or water (1 ml/100 g body wt.) was administered intragastrically under light anaesthesia as described previously [5]. In some experiments DCA [0.25 ml of a 5% (w/v) solution in 0.15 M-NaCl, pH 7.4] or 0.15 M-NaCl was injected intraperitoneally at the time of intubation and at 0.5 h intervals thereafter until rats were sampled at 2.0 h.

Measurements of lipogenesis

The rate of lipogenesis *in vivo* was estimated by the incorporation of ³H₂O into saponifiable fatty acids [16]. The rats were injected intraperitoneally with ³H₂O (5 mCi; 0.5 ml in 0.9% NaCl) either at the time of intubation or at 1 h after the intragastric load, and dissected after a further 60 min as described in [17].

Abbreviations used: PDH, pyruvate dehydrogenase complex; DCA, dichloroacetate; NEFA, non-esterified fatty acids.

‡ To whom reprint requests should be addressed.

Enzyme and metabolite assays

PDH complex, active form (PDH_a) and citrate synthase (EC 4.1.3.7) activities were measured in freeze-clamped tissue extracts as described in [18]. Further details are given in [19]. Serum was included to inhibit proteolysis. A unit of enzyme activity converts 1 μ mol of substrate into product/min at 30 °C. The results have been expressed relative to citrate synthase (see [18]) in order to correct for possible differences in extraction of mitochondria.

Plasma obtained at the time of sampling was assayed for NEFA [15] and concentrations of the ketone bodies (D-3-hydroxybutyrate and acetoacetate), glucose, lactate, pyruvate and glycogen were determined in KOH-neutralized HClO₄ extracts of blood and freeze-clamped liver [20–23].

Statistics

Results are presented as means $\pm$ S.E.M. for the numbers of rats shown in parentheses. Statistical differences were tested by Student's unpaired *t*-test.

RESULTS AND DISCUSSION

Metabolite concentrations after glucose re-feeding

The administration of glucose to 48 h-starved rats produced the expected decreases in NEFA and hepatic ketone-body concentrations, whereas hepatic glucose concentrations were elevated (Fig. 1). The effects of glucose were apparent within 0.5 h of its administration, when NEFA concentrations were similar to, and glucose concentrations exceeded, the fed value. Qualitatively similar changes in ketone-body and glucose concentrations were observed in the blood (results not shown).

Glycogen and fatty acid synthesis

Lactate and other C₃ compounds have been suggested to be important precursors for both glycogen and fatty acid synthesis ([9, 10]; see also [24]). Whereas glucose administration increased hepatic concentrations of lactate plus pyruvate and resulted in a steady rate of glycogen deposition, it failed to increase hepatic lipogenesis, suggesting a constraint on utilization of lactate and pyruvate for lipogenesis (Table 1). Similar observations have been made previously [4, 5]. Because the hepatic concentrations of lactate and pyruvate in glucose-re-fed rats are similar to those found in fed rats, it is considered unlikely that substrate availability is rate-limiting for lipogenesis.

Hepatic PDH activity after re-feeding

In 24 h-starved rats, re-activation of hepatic PDH after administration of glucose [25] has been correlated with decreased hepatic fat oxidation. In the present experiments with 48 h-starved rats, intragastric glucose re-feeding alone failed to re-activate hepatic PDH within 2.0 h (Table 2). The results indicate that, on carbohydrate re-feeding after prolonged starvation, a relatively inactive PDH could restrict utilization of C₃ intermediates for acetyl-CoA production, thereby ensuring maximal carbon flux towards glycogen synthesis.

PDH activity in extrahepatic tissues

Several groups have provided evidence (for reviews, see [9, 10]) that, after its administration to starved rats,

Fig. 1. Changes in blood NEFA (a) and hepatic glucose (b) and total ketone-body (c) concentrations after intragastric administration of glucose to 48 h-starved rats

For experimental details see the text. Each point is the mean value $\pm$ S.E.M. for six to nine rats. Statistically significant effects of glucose administration are indicated by: ****P* < 0.001. Corresponding values for fed rats are shown for comparison.

glucose is metabolized to a C₃ compound, probably lactate, before its conversion into hepatic glycogen through reactions of the gluconeogenic pathway. The source of the lactate utilized was not defined.

In cardiac muscle, re-activation of PDH by phosphorylation may be retarded by multi-site phosphorylation [26, 27] and/or biosynthesis of kinase/activator [28]. The results shown in Table 2 demonstrate that re-activation of cardiac PDH does not occur *in vivo* in the first 2 h after glucose administration to 48 h-starved rats. This finding raises the possibility that the prolonged inactivation of PDH in some extrahepatic tissues after carbohydrate re-feeding could lead to recycling of glucose carbon through the continued operation of the Cori cycle

Table 1. Effects of intragastric administration of glucose on hepatic lactate+pyruvate concentrations and rates of fatty acid and glycogen synthesis in 48 h-starved rats

For experimental details see the text. Statistically significant effects of glucose administration are indicated by: ** $P < 0.01$; *** $P < 0.001$.

Nutritional status	Treatment	Time of sampling (h after intubation)	Lactate+pyruvate ($\mu\text{mol/g wet wt.}$)	Lipogenesis ($\mu\text{g-atoms of 'H'}/\text{h per g wet wt.}$)	Glycogen (% wet wt.)
Fed	Water	1.0	2.38 ± 0.32 (6)	21.6 ± 2.8 (6)	5.42 ± 0.10 (5)
48 h-starved	Water	1.0	0.89 ± 0.09 (6)	7.5 ± 0.5 (12)	0.16 ± 0.05 (5)
	Glucose	0.5	2.16 ± 0.25 (6)***	—	0.17 ± 0.03 (5)
	Glucose	1.0	1.85 ± 0.19 (6)***	8.2 ± 0.6 (11)	0.69 ± 0.12 (5)**
	Glucose	1.5	1.59 ± 0.17 (6)**	—	0.83 ± 0.18 (5)**
	Glucose	2.0	1.83 ± 0.34 (6)**	8.4 ± 1.0 (9)	1.14 ± 0.09 (5)***

[29]. It should be noted that, despite a demonstration of multi-site phosphorylation of PDH in the kidney [30], re-activation of renal PDH was essentially complete within 2.0 h of intragastric administration of glucose (Table 2).

Re-activation of PDH and hepatic glycogen synthesis after re-feeding

The pyruvate analogue DCA activates tissue PDH through inhibition of PDH kinase, and decreases lactate and pyruvate concentrations *in vivo* in starved rats [31, 32]. To test the possible importance of maintenance of low tissue PDH activities for hepatic glycogen synthesis after re-feeding, glucose was administered in combination with DCA. In water-fed rats, DCA administration resulted in re-activation of cardiac and renal PDH, but re-activation of hepatic PDH did not occur (Table 2). In glucose-fed rats DCA-induced PDH re-activation was also observed in the liver (Table 2).

Hepatic concentrations of lactate plus pyruvate were decreased by DCA in both water-fed and glucose-fed rats (Table 3). Neither glycogen deposition nor lactate+pyruvate accumulation occurred in DCA-treated rats in response to glucose (Table 3). The results clearly indicate that activation of PDH in liver and extrahepatic tissues resulted in decreased hepatic glycogen synthesis, presumably because of a diminished precursor supply.

Gluconeogenesis and ketogenesis in DCA-treated rats

DCA decreased hepatic glucose concentrations only in starved rats not intubated with glucose, i.e. where maintenance of blood glucose was primarily dependent on gluconeogenesis (Table 3; see also [33]). Blood glucose concentrations were also decreased by DCA in water-fed, but not in glucose-fed, rats (results not shown). Activation of hepatic PDH by DCA did not occur in water-fed rats (Table 2), and therefore it can be concluded that the hypoglycaemic effect of DCA is predominantly the result of decreased release of lactate and pyruvate from extrahepatic tissues rather than a change in the hepatic disposal of these metabolites.

Increased blood ketone-body concentrations in 24 h-starved rats have been attributed to impaired ketone-body uptake [32]. In the present experiments with 48 h-starved rats, increases in hepatic ketone-body concentrations in response to DCA were more marked in the water-fed rats (Table 3). Studies both *in vivo* and *in vitro* [34–37] have demonstrated that, in starvation and experimental

diabetes, the availability of oxaloacetate for citrate synthesis represents a major regulatory factor in ketogenesis, particularly when rates of β -oxidation (acetyl-CoA production) are high, and increased availability of lactate and pyruvate is associated with decreased ketogenesis, through changes in oxaloacetate concentrations. The results shown in Table 3 therefore suggest that DCA may stimulate ketogenesis *in vivo* in 48 h-starved rats through decreased availability of lactate and pyruvate (and presumably oxaloacetate).

Regulation of hepatic PDH

DCA activation of hepatic PDH was observed only in the glucose-re-fed rats (Table 2), where plasma NEFA and hepatic ketone-body concentrations were low. It appears that in the liver high rates of fat oxidation (implied by the high [3-hydroxybutyrate]/[acetoacetate] ratio) may oppose the effects of DCA on PDH kinase. It is of interest that glucose feeding decreased hepatic ketone-body concentration and the [3-hydroxybutyrate]/[acetoacetate] ratio in both untreated and DCA-treated rats, but hepatic PDH was re-activated only in the latter group, suggesting that in liver decreased acetyl-CoA concentrations together with a more oxidized mitochondrial redox state are not of necessity associated with re-activation of PDH. The mechanism for the prolonged inactivation of hepatic PDH in starved-re-fed rats has yet to be determined.

General discussion

The results indicate that, in the first 2 h after the administration of glucose to 48 h-starved rats, PDH in at least two tissues (heart and liver) remains inactive. Where PDH is re-activated by the administration of DCA, net hepatic glycogen resynthesis in response to glucose is impaired. The results are important in that they imply that Cori (and probably alanine) recycling continues after carbohydrate re-feeding as a consequence of the prolonged inactivation of PDH in one or more oxidative tissues, and furthermore that the release of C_3 derivatives of glucose from these tissues is quantitatively important for the provision of precursors for net hepatic glycogen synthesis.

The relative contributions of the various extrahepatic tissues to lactate and pyruvate (and possibly alanine) production after carbohydrate re-feeding are unknown. Since the activity of PDH is dependent on the [ATP]/[ADP] ratio [38], it would appear likely that only

Table 2. Effects of administration of glucose and/or DCA on tissue active pyruvate dehydrogenase complex activities in 48 h-starved rats

For experimental details see the text. Statistically significant effects of glucose administration are indicated by: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Statistically significant effects of DCA are indicated by: ††† $P < 0.001$.

Nutritional status	Treatment	Time of sampling (h after intubation)	Active pyruvate dehydrogenase complex in:			
			Liver		Kidney	
			(munits/unit of citrate synthase)	(% of fed value)	(munits/unit of citrate synthase)	(% of fed value)
Fed 48 h-starved	Water	1.0	87.2 ± 9.3 (7)	100	43.5 ± 1.6 (13)	100
	Water	1.0	18.1 ± 1.9 (11)	21 ± 2	6.1 ± 0.8 (12)	14 ± 2
	Glucose	0.5	17.8 ± 4.3 (5)	20 ± 5	6.7 ± 1.0 (6)	15 ± 2
	Glucose	1.0	17.6 ± 2.2 (6)	20 ± 3	8.12 ± 1.4 (6)	19 ± 3
	Glucose	1.5	18.5 ± 0.8 (4)	21 ± 1	10.4 ± 1.6 (4)	24 ± 4
	Glucose	2.0	12.8 ± 3.1 (4)	21 ± 4	9.1 ± 1.4 (6)	21 ± 3
	Water + DCA	2.0	19.1 ± 1.1 (6)	22 ± 1	26.3 ± 2.0 (5)†††	60 ± 5
	Glucose + DCA	2.0	91.3 ± 1.6 (4)***†††	105 ± 2	35.0 ± 1.6 (5)*†††	80 ± 4
					78.6 ± 4.2 (6)	100
					37.7 ± 1.6 (9)	48 ± 2
					71.7 ± 11.0 (6)**	91 ± 14
					77.9 ± 10.3 (6)**	99 ± 13
					79.9 ± 12.1 (5)**	101 ± 15
					82.9 ± 9.6 (4)***	105 ± 12
					66.7 ± 4.2 (8)†††	85 ± 5
					72.2 ± 5.0 (7)†††	92 ± 6

Table 3. Effects of DCA alone or in combination with glucose on hepatic glycogen and metabolite concentrations and blood NEFA concentrations in 48 h-starved rats

For experimental details see the text. Statistically significant effects of glucose administration are indicated by: ** $P < 0.01$; *** $P < 0.001$. Statistically significant effects of DCA are indicated by: † $P < 0.05$; †† $P < 0.01$; ††† $P < 0.001$.

Intragastric load	Treatment	n	Liver metabolite					Plasma NEFA (mM)
			Glycogen (% wet wt.)	Lactate + pyruvate (μmol/g wet wt.)	Glucose (μmol/g wet wt.)	3-Hydroxybutyrate + acetoacetate (μmol/g wet wt.)	3-Hydroxybutyrate/ acetoacetate	
Water	NaCl	5	0.13 ± 0.01	1.03 ± 0.11	3.91 ± 0.24	1.35 ± 0.04	3.27 ± 0.24	0.67 ± 0.07
	DCA	8	0.20 ± 0.05	0.51 ± 0.04§§	3.20 ± 0.13†	2.74 ± 0.11†††	3.10 ± 0.25	0.76 ± 0.07
Glucose	NaCl	4	1.14 ± 0.10***	1.59 ± 0.14*	7.18 ± 0.11***	0.52 ± 0.05***	0.68 ± 0.14***	0.15 ± 0.01***
	DCA	5	0.28 ± 0.13††	0.46 ± 0.07†††	7.19 ± 0.03***	0.87 ± 0.13***†	1.07 ± 0.24***	0.22 ± 0.03***

those tissues capable of generating an adequate ATP supply by anaerobic glycolysis would maintain PDH in a relatively inactive form and release glycolytic products. The regulation of PDH in skeletal muscle resembles that in heart in that PDH kinase activity is increased and the complex is subject to multisite phosphorylation in starvation [39, 40]. Others have proposed (for review, see [41]) that activation of skeletal-muscle PDH is a major factor in the hypoglycaemic and hypolactaemic effects of DCA, and preliminary work in our laboratory has indicated that skeletal-muscle PDH in 48 h-starved rats is re-activated by DCA, but not by glucose re-feeding. It is therefore tempting to speculate that skeletal muscle, because of its large mass, will make a major contribution towards the provision of precursors for hepatic glycconeogenesis. Further measurements of release of lactate, pyruvate and alanine from skeletal muscle after carbohydrate re-feeding will confirm or refute this. It is relevant that, although the gut has been proposed as a possible source of lactate after carbohydrate re-feeding, extra-hepatic splanchnic production of alanine and lactate is unaffected by DCA [42].

This work was supported by a grant from The National Kidney Research Fund. We thank Dr. T. N. Palmer for helpful discussion, and Mrs. I. Austin for expert secretarial assistance.

REFERENCES

- McGarry, J. D. & Foster, D. W. (1980) *Annu. Rev. Biochem.* **49**, 395–420
- Sugden, M. C. & Williamson, D. H. (1982) in *Metabolic Compartmentation* (Sies, H., ed.), pp. 287–315, Academic Press, New York and London
- Randle, P. J., Fuller, S. J., Kerbey, A. L., Sale, G. T. & Vary, T. C. (1984) *Horm. Cell Regul.* **8**, 139–151
- Agius, L. & Williamson, D. H. (1981) *Biochim. Biophys. Acta* **666**, 127–132
- Sugden, M. C., Watts, D. I. & Marshall, C. E. (1981) *Biosci. Rep.* **1**, 469–476
- Sugden, M. C., Watts, D. I., Palmer, T. N. & Myles, D. D. (1983) *Biochem. Int.* **7**, 329–337
- Newgard, C. B., Hirsch, L. J., Foster, D. W. & McGarry, J. D. (1983) *J. Biol. Chem.* **258**, 8046–8052
- Williamson, D. H., Ilic, V. & Jones, R. G. (1985) *Biochem. J.* **228**, 727–733
- Foster, D. W. (1984) *Diabetes* **33**, 1188–1199
- Katz, J. & McGarry, J. D. (1984) *J. Clin. Invest.* **74**, 1901–1909
- Claus, T. H., Nyfeler, F., Muenkel, H. A., Burns, M. G., Patel, T. & Pilgis, S. J. (1984) *Biochem. Biophys. Res. Commun.* **125**, 655–661
- Windmueller, H. G. & Spaeth, A. E. (1978) *J. Biol. Chem.* **253**, 69–76
- Shapiro, A. & Shapiro, B. (1979) *Biochim. Biophys. Acta* **586**, 123–127
- Nicholls, T. T., Leese, H. J. & Bronk, J. R. (1983) *Biochem. J.* **212**, 183–187
- Schofield, P. S., French, T. J., Goode, A. W. & Sugden, M. C. (1985) *FEBS Lett.* **184**, 214–220
- Stansbie, D., Brownsey, R. W., Crettaz, M. & Denton, R. M. (1976) *160*, 413–416
- Agius, L. & Williamson, D. H. (1980) *Biochem. J.* **190**, 477–480
- Caterson, I. D., Fuller, S. J. & Randle, P. J. (1982) *Biochem. J.* **208**, 53–60
- Kerbey, A. L., Randle, P. J., Cooper, R. H., Whitehouse, S., Pask, H. T. & Denton, R. M. (1976) *Biochem. J.* **154**, 327–348
- Williamson, D. H., Mellanby, J. & Krebs, H. A. (1962) *Biochem. J.* **82**, 90–96
- Hohorst, H. J., Kreutz, F. H. & Bücher, T. (1959) *Biochem. Z.* **332**, 18–46
- Slein, M. W. (1963) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), pp. 117–123, Academic Press, New York and London
- Keppeler, D. & Decker, K. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), 2nd edn., pp. 1127–1131, Academic Press, New York and London
- Salmon, D. M. W., Bowen, N. L. & Hems, D. A. (1974) *Biochem. J.* **142**, 611–618
- Wieland, O. H., Patzelt, C. & Löffler, G. (1972) *Eur. J. Biochem.* **26**, 426–433
- Sugden, P. H., Hutson, N. J., Kerbey, A. L. & Randle, P. J. (1978) *Biochem. J.* **169**, 433–435
- Sale, G. J. & Randle, P. J. (1982) *Biochem. J.* **206**, 221–229
- Kerbey, A. L. & Randle, P. J. (1982) *Biochem. J.* **206**, 103–111
- Cori, C. F. (1931) *Physiol. Rev.* **11**, 143–275
- Sugden, P. H. & Simister, N. E. (1980) *FEBS Lett.* **111**, 299–302
- Whitehouse, S., Cooper, R. H. & Randle, P. J. (1973) *Biochem. J.* **141**, 761–774
- Blackshear, P. J., Holloway, P. A. H. & Alberti, K. G. M. M. (1974) *Biochem. J.* **142**, 279–286
- Agius, L., Watts, D. I. & Sugden, M. C. (1983) *Horm. Metab. Res.* **15**, 482–485
- Nosadini, R., Datta, M., Hodson, A. & Alberti, K. G. M. M. (1980) *Biochem. J.* **190**, 323–332
- Mooney, R. A. & Lane, M. D. (1982) *Eur. J. Biochem.* **121**, 281–287
- Watts, D. I. & Sugden, M. C. (1982) *Biochem. Int.* **4**, 255–261
- Siess, E. A., Kientsch-Engel, R. I. & Wieland, O. H. (1984) *Biochem. J.* **218**, 171–176
- Kerbey, A. L., Radcliffe, P. M. & Randle, P. J. (1977) *Biochem. J.* **164**, 509–519
- Ashour, B. & Hansford, R. G. (1983) *Biochem. J.* **214**, 725–736
- Fuller, S. J. & Randle, P. J. (1984) *Biochem. J.* **219**, 635–646
- Crabb, D. W., Yount, E. A. & Harris, R. A. (1981) *Metab. Clin. Exp.* **30**, 1024–1039
- Diamond, M. P., Rollings, R. C., Erlendson, L., Williams, P. E., Lacy, W. W., Rabin, D. & Cherrington, A. D. (1980) *Diabetes* **29**, 702–709

Received 20 September 1985/11 November 1985; accepted 10 December 1985