Effects of nutritional status and acute variation in substrate supply on cardiac and skeletal-muscle fructose 2,6-bisphosphate concentrations

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We examined the long-term effects of nutritional status and the acute effects of changes in exogenous carbohydrate- and lipid-substrate supply and utilization on fructose 2,6-bisphosphate (Fru-2,6- P_2) concentrations in heart, gastrocnemius and soleus. Starvation decreased Fru-2,6- P_2 concentrations in all three muscles. The acute administration of insulin and glucose increased skeletal-muscle Fru-2,6- P_2 in the fed, but not in the starved, state, but cardiac Fru-2,6- P_2 was unchanged. Cardiac and skeletal-muscle Fru-2,6- P_2 concentrations were unaffected by acute increases in fatty acid supply produced by the administration of corn oil plus heparin, or by acute decreases in fatty acid supply produced by inhibition of lipolysis. Differences in cardiac and skeletal-muscle Fru-2,6- P_2 concentrations observed in response to starvation were not reversed by administration of glucose or glucose plus insulin, or by inhibition of lipolysis, even though changes in citrate (heart), acylcarnitine (heart) and glycogen (skeletal muscle) were observed. Concentrations remained low for at least 8 h after chow re-feeding, but the fed value was restored by 24 h.

INTRODUCTION

The cardiac response to starvation is characterized by inactivation of the pyruvate dehydrogenase complex (PDH) (reviewed by Randle, 1986), a doubling of citrate content, changes in metabolite concentrations consistent with inhibition of phosphofructokinase (PFK), and increased glycogen (Randle *et al.*, 1963, 1964; Newsholme & Randle, 1964; Randle, 1966; Neely *et al.*, 1969, 1970; Zorzano *et al.*, 1985). It is generally accepted, on the basis of metabolite analysis and flux measurements, that the utilization of lipid fuels inhibits glycolysis at the level of PFK via the accumulation of citrate, with enhanced glycogen synthesis resulting from an increase in glucose 6-phosphate secondary to the block at PFK (reviewed by Randle & Tubbs, 1979). However, paradoxically, it has been reported that the increased rates of glycolysis observed in glucose-perfused hearts of fed rats in response to insulin are also accompanied by increased citrate concentrations (Opie *et al.*, 1971; Lawson & Uyeda, 1987).

The suggestion has been made that, in tissues (such as heart and red skeletal muscle) that are capable of oxidizing both carbohydrate and lipid fuels, a fall in fructose 2,6-bisphosphate (Fru-2,6- P_2) concentrations could signal the presence of non-carbohydrate substrates and limit glycolytic flux (Hue & Rider, 1987). Furthermore, Lawson & Uyeda (1987) have argued that increases in glycolytic rate in the Langendorff-perfused heart in response to insulin show a good correlation with increases in Fru-2,6- P_2 , but not with changes in citrate. The question therefore arises as to whether changes in citrate or in Fru-2,6- P_2 concentrations are primarily responsible for variation in glucose utilization in response to changes

in nutritional status in the heart *in vivo*. Similarly in skeletal muscles, where increases in citrate concentrations cannot explain the marked decreases in glucose utilization observed in response to starvation (Zorzano *et al.*, 1985; Issad *et al.*, 1987), it is unknown whether changes in Fru-2,6- P_2 may assume regulatory significance for glycolysis.

In the present series of experiments, we have varied both the nutritional status and the availability of exogenous carbohydrate and lipid substrates. We have measured cardiac concentrations of Fru-2,6- P_2 , citrate and glycogen, while at the same time assessing the potential for carbohydrate and long-chain fatty acid oxidation by measurement of PDH activities and acylcarnitine concentrations. In addition we have measured Fru-2,6- P_2 in soleus (a slow-twitch red muscle) and in gastrocnemius (which consists of a mixture of fast-twitch red and fast-twitch white fibres).

MATERIALS AND METHODS

Sources of materials were as described in Schofield *et al.* (1987).

Rats

Albino Wistar rats (180–220 g) were subjected to a 12 h-light/12 h-dark cycle (light from 08:30 h) and were either fed *ad libitum* or starved for 48 h (grid-bottomed cages) before use.

Experiments were started between 08:30 and 09:30 h. Glucose (2 mmol/100 g body wt.) or water (1 ml/100 g body wt.) was administered intragastrically at 2 h before sampling (see Sugden *et al.*, 1981). Insulin (2 units) was injected subcutaneously at the time of glucose administra-

Abbreviations used: PDH, pyruvate dehydrogenase complex; PFK, 6-phosphofructo-1-kinase; Fru-2,6- P_2 , fructose 2,6-bisphosphate; MPCA, 5-methylpyrazole-3-carboxylic acid; NEFA, non-esterified fatty acids.

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tion. 5-Methylpyrazole-3-carboxylic acid (MPCA; 0.66 mg/100 g body wt.) was administered by intraperitoneal injection at 2 h before sampling in order to inhibit lipolysis (Axelrod *et al.*, 1979). In experiments in which fatty acids were artificially increased, rats were given 5 ml of corn oil by intragastric intubation. After 3 h the animals were given 200 units of sodium heparin. The rats were killed after a further 40 min. Control rats were sampled concurrently [see Rennie *et al.* (1976) for details]. A further group of 48 h-starved rats were provided with standard laboratory chow. Mean chow intakes after 2, 4, 6, 8 and 24 h were 2.80 ± 0.32 , 3.87 ± 0.34 , 5.69 ± 0.34 , 6.18 ± 0.60 and 13.65 ± 1.20 g respectively.

Metabolite and enzyme assays

Rats were killed while under sodium pentobarbital anaesthesia (6 mg/100 g body wt., 5 min). Blood was sampled from the abdominal aorta. Muscles were freeze-clamped and stored in liquid N₂. Concentrations of glucose, lactate, pyruvate and the ketone bodies were measured in KOH-neutralized HClO₄ extracts of whole blood [see Sugden *et al.* (1982) for details of methods], non-esterified fatty acids (NEFA) in plasma, and glycogen (Keppler & Decker, 1974), free and acylated carnitine (Schofield *et al.*, 1985), citrate (Dagley, 1974) and Fru-2,6-P₂ were measured in extracts of freeze-clamped muscles. Fru-2,6-P₂ was assayed by activation of PFK as described by Richards & Uyeda (1980) after extraction and purification as described by Hue *et al.* (1982).

The active form of the PDH complex (PDH_a) and citrate synthase activities were measured in freeze-clamped heart extracts as described by Caterson *et al.* (1982). PDH activities were expressed relative to citrate synthase to correct for differences in mitochondrial extraction. Total PDH activity is unaffected by nutritional status. One unit of enzyme activity converts 1 μ mol of substrate into product per min at 30 °C.

Expression of results

Results are given as means $\pm$ S.E.M. Statistical significance of differences was assessed by Student's unpaired *t* test.

RESULTS AND DISCUSSION

Modulation of the supply of exogenous substrates

The differences in substrate supply between the fed and starved states are demonstrated by the data shown in Table 1. Starvation was characterized by decreased concentrations of glucose and the carbohydrate-derived fuels lactate and pyruvate, whereas NEFA and ketone-body concentrations were significantly increased.

The administration of glucose to fed rats was not associated with a marked alteration of the blood metabolite profile at the time of sampling (2 h after the intragastric load), except that NEFA concentrations were decreased, an effect mimicked by inhibition of lipolysis by the administration of MPCA. The administration of insulin in combination with glucose to fed rats led to hypoglycaemia together with increased concentrations of lactate and pyruvate, indicative of increased glycolysis in insulin-dependent tissues.

The changes in exogenous fuel supply observed in response to starvation were reversed by the intragastric administration of glucose. In rats given glucose plus insulin, the metabolic profile was independent of the prior nutritional status, except for slightly more pronounced hypoglycaemia in the starved group. Metabolite concentrations in starved rats treated with MPCA plus glucose were similar to those observed in fed rats treated with MPCA. Consequently any differences in terms of tissue fuel utilization between the previously fed or previously starved rats after these experimental manipulations can be attributed to factors other than the supply of blood-borne substrates.

Table 1. Effects of modulation of fuel supply by the administration of glucose, insulin or MPCA on blood metabolite concentrations in fed or 48 h-starved rats

Results are given as means $\pm$ S.E.M. for six to ten rats. Glucose was administered by intragastric intubation to previously fed or 48 h-starved rats at 2 h before sampling; MPCA or insulin was injected at 2 h before sampling. Further details are given in the text. NEFA concentrations were measured in plasma; other metabolites were measured in KOH-neutralized HClO₄ extracts of whole blood. Statistically significant effects of starvation are shown by: *P < 0.05; **P < 0.01; ***P < 0.001. Statistically significant effects of other treatments are shown by: †*P* < 0.05; ††*P* < 0.01; †††*P* < 0.001.

Treatment	Nutritional status	Glucose (mM)	Lactate (mM)	Pyruvate (mM)	Acetoacetate + 3-hydroxybutyrate (mM)	NEFA (mequiv./l)
Control	Fed	7.59 ± 0.21	2.27 ± 0.18	0.12 ± 0.02	0.19 ± 0.22	0.18 ± 0.01
	Starved	$5.67 \pm 0.08^{***}$	$0.93 \pm 0.08^{***}$	0.08 ± 0.02	$1.51 \pm 0.09^{***}$	$0.44 \pm 0.03^{***}$
Glucose feeding	Fed	7.62 ± 0.15	$3.12 \pm 0.16^{\dagger\dagger}$	0.15 ± 0.03	0.22 ± 0.02	$0.09 \pm 0.01^{\dagger\dagger\dagger}$
	Starved	$7.48 \pm 0.01^{\dagger\dagger\dagger}$	$2.22 \pm 0.12^{**\dagger\dagger\dagger}$	$0.18 \pm 0.03^{\dagger}$	$0.41 \pm 0.10^{\dagger\dagger\dagger}$	$0.20 \pm 0.05^{\dagger\dagger}$
Glucose + insulin	Fed	$2.84 \pm 0.25^{\dagger\dagger\dagger}$	$2.96 \pm 0.14^{\dagger}$	$0.24 \pm 0.04^{\dagger}$	0.22 ± 0.01	$0.06 \pm 0.02^{\dagger\dagger\dagger}$
	Starved	$1.72 \pm 0.07^{**\dagger\dagger\dagger}$	$3.16 \pm 0.32^{\dagger\dagger\dagger}$	$0.42 \pm 0.07^{*\dagger\dagger\dagger}$	$0.19 \pm 0.04^{\dagger\dagger\dagger}$	$0.10 \pm 0.02^{\dagger\dagger\dagger}$
MPCA	Fed	8.01 ± 0.27	1.97 ± 0.31	0.12 ± 0.02	0.25 ± 0.04	$0.10 \pm 0.02^{\dagger}$
	Starved	$3.01 \pm 0.18^{***\dagger\dagger\dagger}$	$1.13 \pm 0.09^*$	0.14 ± 0.02	$0.49 \pm 0.04^{**\dagger\dagger\dagger}$	$0.04 \pm 0.01^{*\dagger\dagger\dagger}$
MPCA + glucose	Starved	$7.88 \pm 0.22^{\dagger\dagger\dagger}$	$1.91 \pm 0.24^{\dagger\dagger}$	$0.29 \pm 0.04^{\dagger\dagger\dagger}$	$0.26 \pm 0.04^{\dagger\dagger\dagger}$	$0.01 \pm 0.01^{\dagger\dagger\dagger}$

Table 2. Effects of modulation of fuel supply on cardiac carnitine concentrations in fed or 48 h-starved rats

Results are given as means $\pm$ S.E.M. for six to ten rats. Treatment of rats was as in the legend to Table 1, with sampling after 2 h. Free (F), short-chain (S) and long-chain (L) acylcarnitine concentrations were measured in extracts of freeze-clamped hearts. Further details are given in the text. Statistically significant effects of starvation are shown by: * $P < 0.05$; ** $P < 0.01$. Statistically significant effects of other treatments are shown by: † $P < 0.05$; †† $P < 0.01$; ††† $P < 0.001$.

Treatment	Nutritional status	Carnitine concn. (nmol/g wet wt.)				
		Free (F)	Acid-soluble (S)	Acid-insoluble (L)	F/(S+L)	S/F
Control	Fed	731.4 $\pm$ 25.7	248.5 $\pm$ 22.4	31.4 $\pm$ 5.3	2.80 $\pm$ 0.40	0.34 $\pm$ 0.04
	Starved	570.4 $\pm$ 41.6**	419.3 $\pm$ 45.8**	51.0 $\pm$ 4.6*	1.33 $\pm$ 0.23**	0.78 $\pm$ 0.14*
Glucose feeding	Fed	809.2 $\pm$ 20.9†	216.3 $\pm$ 30.1	22.3 $\pm$ 3.9	3.36 $\pm$ 0.36	0.27 $\pm$ 0.03
	Starved	825.4 $\pm$ 45.6††	321.2 $\pm$ 65.2	30.4 $\pm$ 1.0††	3.01 $\pm$ 0.70†	0.35 $\pm$ 0.10†
Glucose + insulin	Fed	891.1 $\pm$ 19.4†††	137.2 $\pm$ 23.7††	18.9 $\pm$ 3.0	5.73 $\pm$ 0.47†††	0.15 $\pm$ 0.03††
	Starved	890.3 $\pm$ 19.9†††	143.1 $\pm$ 18.5††	23.7 $\pm$ 1.2†††	5.65 $\pm$ 0.73†††	0.16 $\pm$ 0.02††
MPCA	Fed	908.7 $\pm$ 26.2†††	138.8 $\pm$ 14.1††	15.2 $\pm$ 1.3†	6.17 $\pm$ 0.75††	0.15 $\pm$ 0.02††
	Starved	896.9 $\pm$ 25.8†††	99.9 $\pm$ 18.0†††	19.6 $\pm$ 0.9*†††	8.18 $\pm$ 1.18†††	0.11 $\pm$ 0.02†††
MPCA + glucose	Starved	900.7 $\pm$ 32.3†††	79.5 $\pm$ 26.5†††	18.6 $\pm$ 2.9	9.26 $\pm$ 0.64†††	0.09 $\pm$ 0.03†††

Table 3. Effects of modulation of fuel supply on cardiac glycogen, Fru-2,6- P_2 and citrate concentrations and the activity of cardiac PDH complex in fed or 48 h-starved rats

Results are given as means $\pm$ S.E.M. for six to ten rats. Treatment of rats was as in the legend to Table 1, with sampling after 2 h. Metabolites and PDH_a were measured in extracts of freeze-clamped tissue. PDH_a is expressed relative to citrate synthase, in order to correct for variation in efficiencies of mitochondrial extraction. Statistically significant effects of starvation are shown by: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Statistically significant effects of other treatments are shown by: † $P < 0.05$; †† $P < 0.01$; ††† $P < 0.001$.

Treatment	Nutritional status	Glycogen (mg/g wet wt.)	Fru-2,6- P_2 (nmol/g wet wt.)	Citrate (μ mol/g wet wt.)	PDH _a (munits/unit of citrate synthase)	Citrate synthase (units/g wet wt.)
Control	Fed	4.88 $\pm$ 0.34	1.34 $\pm$ 0.20	0.27 $\pm$ 0.04	13.3 $\pm$ 0.5	125 $\pm$ 3
	Starved	6.47 $\pm$ 0.36**	0.64 $\pm$ 0.06*	0.45 $\pm$ 0.03**	0.2 $\pm$ 0.1***	124 $\pm$ 4
Glucose feeding	Fed	4.83 $\pm$ 0.45	1.60 $\pm$ 0.28	0.11 $\pm$ 0.02†	14.2 $\pm$ 1.6	127 $\pm$ 2
	Starved	6.15 $\pm$ 0.35**	0.73 $\pm$ 0.07*	0.24 $\pm$ 0.04*†	0.7 $\pm$ 0.2***†	126 $\pm$ 2
Glucose + insulin	Fed	4.66 $\pm$ 0.50	1.70 $\pm$ 0.36	0.12 $\pm$ 0.02†	11.8 $\pm$ 0.3	126 $\pm$ 1
	Starved	8.64, 7.56	0.54, 0.52	0.16, 0.25	0.8 $\pm$ 0.2***†	128 $\pm$ 1
MPCA	Fed	4.82 $\pm$ 0.31	1.68 $\pm$ 0.11	0.09 $\pm$ 0.02†	21.8 $\pm$ 2.4	129 $\pm$ 1
	Starved	2.41 $\pm$ 0.39††	0.50 $\pm$ 0.14**	0.08 $\pm$ 0.01†††	3.5 $\pm$ 0.3***†††	125 $\pm$ 2
MPCA + glucose	Starved	5.07 $\pm$ 0.70	0.72 $\pm$ 0.18	0.05 $\pm$ 0.01†††	3.7 $\pm$ 0.2†††	127 $\pm$ 1

Cardiac carnitine concentrations and PDH activities after the administration of glucose or glucose plus insulin

In fed rats, the provision of glucose alone had little effect on cardiac carnitine concentrations, except that free (non-esterified) carnitine concentrations were slightly increased (by 11 %; see Table 2). After the administration of glucose in combination with insulin, more marked increases in the concentration of free (non-esterified) carnitine were apparent. These could be largely accounted for by significant decreases in short-chain acylcarnitine (Table 2). Decreased short-chain acylcarnitine concentra-

tions may be secondary to decreased lipid oxidation or to accelerated acylcarnitine utilization. The activity of cardiac PDH was not increased by insulin (Table 3).

Starvation resulted in increased concentrations of acylated carnitine, with a proportional decrease in free carnitine (Table 2). The ratio of short-chain acylcarnitine to free carnitine was increased, indicating cardiac oxidation of long-chain fatty acids (Pearson & Tubbs, 1967). Since, in the heart, short-chain acylcarnitine is predominantly acetylcarnitine and the activity of carnitine acetyltransferase is high, it may be inferred that the acetyl-CoA/CoA concentration ratio is increased [see Pearson & Tubbs (1967) for discussion]. Caterson *et al.*

(1982) have demonstrated that this is partly responsible for the inactivation of cardiac PDH which is observed in starvation (Table 3).

Cardiac Fru-2,6- P_2 , citrate and glycogen concentrations after the administration of glucose or glucose plus insulin

In Langendorff-perfused hearts from fed rats, increases in glycolytic rate in response to increased glucose concentrations or the provision of insulin can be correlated with increases in Fru-2,6- P_2 (Rider & Hue, 1984; Lawson & Uyeda, 1987). However, Lawson & Uyeda (1987) have indicated that effects of glucose or insulin are not accompanied by increased Fru-2,6- P_2 in working hearts. In the present experiments carried out *in vivo*, the administration of glucose (alone or in combination with insulin) caused significant decreases in cardiac citrate concentrations in fed rats (Table 3), but we were unable to demonstrate acute increases in cardiac Fru-2,6- P_2 either in response to a glucose load or in response to exogenous insulin (Table 3). However, clear decreases in cardiac Fru-2,6- P_2 concentrations were observed in response to 48 h starvation (Table 3). At the same time citrate and glycogen concentrations were increased.

Although the administration of glucose to 48 h-starved rats restored the cardiac carnitine profile to that found in the fed state, and (consistent with suppression of fat oxidation) citrate concentrations declined, Fru-2,6- P_2 concentrations remained depressed and PDH activity remained low (Table 3). Glycogen concentrations remained elevated (Table 3). Differences between cardiac glycogen and Fru-2,6- P_2 concentrations and in PDH activities in the fed and starved states were maintained even after the administration of insulin. The retarded reactivation of cardiac PDH may be secondary to starvation-induced increases in PDH-kinase activator protein, which are not immediately reversed by re-feeding (Kerbey & Randle, 1982). The failure to decrease glycogen concentration suggests that glycolysis from glycogen may also be restricted after re-feeding. Although the distribution of citrate between the subcellular compartments is unknown, it would appear unlikely, in view of the low concentrations, that such a restriction is exerted via citrate inhibition of PFK.

Cardiac metabolite concentrations and PDH activities after pharmacological modulation of fat fuel supply

In fed rats, inhibition of lipolysis with MPCA significantly elevated the cardiac ratio of free to acylated carnitine (Table 2), decreased citrate concentrations (Table 3) and markedly increased the activity of cardiac PDH_a (Table 3) [this last observation contrasts with a previous report where inhibition of long-chain fatty acid oxidation by 2-tetradecylglycidate in fed rats had no effect (Caterson *et al.*, 1982), but in that previous study the period of exposure to inhibitor was less than that in our work]. The changes in carnitine and citrate concentrations and in PDH activities are compatible with the idea that available lipid fuels are utilized preferentially by the heart, even in the fed state, and, when not subject to long-term inhibition (as after prolonged starvation), cardiac PDH can readily respond to decreases in fat fuel availability. However, Fru-2,6- P_2 concentrations were unaffected by MPCA.

In 48 h-starved rats, MPCA considerably decreased

the long-chain acylcarnitine concentrations and elevated the cardiac ratio of free to acylated carnitine (Table 2). The cardiac concentration of acylated carnitine after treatment with MPCA was lower in starved rats than in fed rats. MPCA treatment did not elicit changes in cardiac Fru-2,6- P_2 concentrations, but it significantly decreased citrate concentrations and depleted cardiac glycogen. As glucose-6-phosphatase is not present in heart, this could be taken to indicate glycolytic flux from glycogen. Depletion of glycogen was not observed when glucose was provided in addition to MPCA, suggesting a glycogen-sparing effect of exogenous carbohydrate.

Although from the decreased cardiac concentrations of glycogen, citrate and acylcarnitine it could be inferred that there is increased glucose oxidation and decreased fatty acid oxidation, PDH_a increased to only 25% of the control (fed) value. Therefore, either this decreased activity of PDH_a is adequate to support control rates of glucose oxidation (which would appear unlikely), or fatty acid oxidation continues, or cardiac oxygen consumption declines.

Lawson & Uyeda (1987), using purified cardiac PFK, have demonstrated that increasing Fru-2,6- P_2 concentrations over a range of values similar to those shown in Table 3 can oppose the inhibitory effect of citrate. Thus the ratio of the concentrations of these two effectors may be critical for determining flux through PFK *in vivo*. An analysis of the results obtained by Lawson & Uyeda (1987) at near-physiological concentrations of fructose 6-phosphate and adenine nucleotides indicated that increases in [Fru-2,6- P_2]/[citrate] ratio from 2.5×10^{-3} to 10×10^{-3} increased the PFK activity ratio ($v/V_{\max}$). Although the subcellular distribution of citrate and Fru-2,6- P_2 is unknown, it is interesting that, under the physiological conditions selected for the present series of experiments (fed, starved, glucose re-fed), the [Fru-2,6- P_2]/[citrate] ratio varied from 1.4×10^{-3} (starved), to 3.0×10^{-3} (starved plus glucose), 5.0×10^{-3} (fed) and 14.5×10^{-3} (fed plus glucose). Such variation may contribute to the marked changes in glucose utilization observed in the heart in response to changes in nutritional status *in vivo* (Issad *et al.*, 1987).

Metabolite concentrations in skeletal muscle in response to changes in substrate supply

Bosca *et al.* (1985) have demonstrated that, in isolated epitrochlearis, increases in glycolysis in response to insulin are paralleled by increases in Fru-2,6- P_2 . In the present experiments, the administration of insulin to fed rats *in vivo* was accompanied by increases in Fru-2,6- P_2 in both gastrocnemius and soleus (Table 4). The increases in concentration were not simply a consequence of decreased lipid fuel supply, as they were not observed in response to inhibition of lipolysis. The failure to observe significant increases in Fru-2,6- P_2 in fed rats at 2 h after the administration of glucose probably arises because at this time of sampling glucose (and presumably, insulin) concentrations have returned to basal values. The responsiveness of skeletal-muscle Fru-2,6- P_2 concentration to exogenous insulin in the fed state contrasts with the failure to observe increased Fru-2,6- P_2 in hearts sampled from the same animals (Table 3).

It has been demonstrated that starvation markedly decreases glucose phosphorylation (and by inference glucose utilization) in certain skeletal muscles of the rat (Issad *et al.*, 1987). As the effects of starvation are more

Table 4. Effects of modulation of fuel supply on skeletal-muscle glycogen and Fru-2,6- P_2 concentrations in fed or 48 h-starved rats

Results are given as means $\pm$ S.E.M. for three to six rats. Treatment of rats was as in the legend to Table 1, with sampling after 2 h. The muscles were excised while rats were under pentobarbital anaesthesia and motor activity had ceased. Statistically significant effects of starvation are shown by: * P < 0.05; ** P < 0.01; *** P < 0.001. Statistically significant effects of other treatments are shown by: † P < 0.05; †† P < 0.01; ††† P < 0.001.

Treatment	Muscle ... Nutritional status	Gastrocnemius		Soleus
		Glycogen (mg/g wet wt.)	Fru-2,6- P_2 (nmol/g wet wt.)	Fru-2,6- P_2 (nmol/g wet wt.)
Control	Fed	8.10 $\pm$ 0.35	1.31 $\pm$ 0.09	1.14 $\pm$ 0.07
	Starved	5.68 $\pm$ 0.54**	0.73 $\pm$ 0.07***	0.43 $\pm$ 0.08***
Glucose feeding	Fed	8.30 $\pm$ 0.21	1.55 $\pm$ 0.29	1.31 $\pm$ 0.23
	Starved	8.08 $\pm$ 0.49††	0.54 $\pm$ 0.09**	0.59 $\pm$ 0.10***
Glucose + insulin	Fed	8.91 $\pm$ 1.05	3.18 $\pm$ 0.55††	2.66 $\pm$ 0.25†††
	Starved	8.72 $\pm$ 0.60††	0.47 $\pm$ 0.04***††	0.71 $\pm$ 0.25***
MPCA	Fed	7.96 $\pm$ 1.05	1.73 $\pm$ 0.21	1.06 $\pm$ 0.14
	Starved	5.15 $\pm$ 0.19*	0.65 $\pm$ 0.12**	0.49 $\pm$ 0.10**
MPCA + glucose	Starved	7.64 $\pm$ 0.94	0.59 $\pm$ 0.10	0.54 $\pm$ 0.05

Table 5. Effects of increased plasma fatty acids on Fru-2,6- P_2 concentrations in heart and skeletal muscles of fed rats

In order to increase NEFA, fed rats were given corn oil by intragastric tube. Heparin was given 3 h later, with sampling after a further 40 min (see Rennie *et al.*, 1976). Values are means $\pm$ S.E.M. for three to six rats. Statistically significant differences between control fed rats and fed rats treated with corn oil and heparin are indicated: *** P < 0.001.

Treatment	Fru-2,6- P_2 (nmol/g wet wt.)			NEFA (mequiv./l)
	Heart	Gastrocnemius	Soleus	
Control	1.50 $\pm$ 0.38	1.32 $\pm$ 0.13	1.15 $\pm$ 0.11	0.18 $\pm$ 0.01
Increased fatty acids	2.09 $\pm$ 0.34	1.45 $\pm$ 0.10	1.20 $\pm$ 0.18	0.68 $\pm$ 0.18***

marked in muscles possessing a high capacity for oxidizing fatty acids and ketone bodies, it was proposed that decreases in glucose utilization were secondary to increased lipid fuel availability, rather than to a lower plasma insulin concentration. Although PDH is inactivated in skeletal muscle in response to starvation (Caterson *et al.*, 1982; Fuller & Randle, 1984; Holness *et al.*, 1986a), Zorzano *et al.* (1985) have queried the operation of the glucose-fatty acid cycle in skeletal muscle in starvation, partly on the basis that starvation does not increase citrate concentrations in skeletal muscle. We confirmed that skeletal-muscle citrate concentrations were not elevated in response to starvation (results not shown). However, we were able to demonstrate substantial decreases in Fru-2,6- P_2 in response to starvation in both gastrocnemius and soleus (Table 4).

The question arises as to the extent to which the decrease in skeletal-muscle Fru-2,6- P_2 observed in starvation is secondary to decreased glucose uptake and phosphorylation. The depletion of skeletal-muscle glycogen occurring in response to starvation (Sugden *et al.*, 1976; Zorzano *et al.*, 1985; Table 4) is reversed by the administration of glucose, either alone or in combination with insulin or MPCA (Table 4). Since skeletal-muscle glycogen synthesis mainly utilizes exogenous glucose as precursor, it may be inferred that there is glucose uptake

and phosphorylation after re-feeding. Nevertheless Fru-2,6- P_2 concentrations remain low.

As glycolytic flux was not measured directly, we are unable to comment on the partitioning of available glucose 6-phosphate between glycogen synthesis and glycolysis in skeletal muscle after re-feeding. However, it would seem reasonable to suggest that a restraint may be imposed on skeletal-muscle glycolysis both in the starved state and immediately after re-feeding through the maintenance of low Fru-2,6- P_2 concentration.

Muscle Fru-2,6- P_2 concentration after chow re-feeding

It might be argued that the response to an intragastric glucose load might be distinct from that to a mixed meal taken *ad libitum*. However, cardiac and skeletal-muscle Fru-2,6- P_2 were not significantly increased at 2 h, or even at 8 h, after the provision of chow to 48 h-starved rats (Fig. 1). Restoration of the fed value was observed by 24 h (Fig. 1).

Effects of an increased fatty acid supply in the fed state

To determine the extent to which the decreases in Fru-2,6- P_2 observed in starvation could be attributed to an increased supply of lipid-derived substrates, cardiac and skeletal-muscle Fru-2,6- P_2 concentrations were measured in fed rats after acute increases in plasma fatty acids were

Fig. 1. Fru-2,6-P₂ concentrations in heart (a), gastrocnemius (b) and soleus (c) after chow re-feeding

Rats were starved for 48 h. They were then provided with chow *ad libitum*, and sampled at the times indicated. Chow intakes are given in the Materials and methods section. Values for rats fed *ad libitum* are shown for comparison. Results are means $\pm$ S.E.M. for three to six rats. Significant effects of re-feeding are indicated: * $P < 0.001$.

achieved by the administration of corn oil and heparin, an approach used successfully to demonstrate a sparing effect of increased fatty acids on muscle glycogen concentration in the exercising rat (Rennie *et al.*, 1976). Acute exposure to fatty acid concentrations comparable with those observed in the starved state did not lead to a significant decrease in Fru-2,6-P₂ in either heart or skeletal muscle (Table 5). Thus neither acute increases in fatty acid supply in the fed state, nor acute decreases in fatty acid supply in the starved state, cause changes in muscle Fru-2,6-P₂ concentrations *in vivo*.

Speculations on muscle fuel supply after re-feeding

Neither cardiac (Kerbey & Randle, 1982; Holness *et al.*, 1986a) nor skeletal-muscle (Holness *et al.*, 1986a; see also Caterson *et al.*, 1982) PDH is re-activated immediately after re-feeding. Consequently energy requirements must be met by the non-oxidative metabolism of carbohydrate or the utilization of alternative fuels. It would appear probable that flux through PFK is less than that in the fed state, because of the maintenance of low Fru-2,6-P₂ concentrations. It is therefore suggested that alternative substrates are utilized.

Denton & Randle (1967) have demonstrated that cardiac and skeletal-muscle (gastrocnemius) triacylglycerol concentrations are increased under conditions where the exogenous NEFA supply is increased. It is assumed that the high plasma fatty acid content increases triacylglycerol synthesis. In hearts perfused with glucose but not NEFA, endogenous triacylglycerol can be utilized to spare glucose oxidation, even in the presence of insulin (Denton & Randle, 1967). Therefore, after re-feeding, the decreased NEFA concentration may permit mobilization of endogenous triacylglycerol, which may function as oxidative substrate until total PDH re-activation is effected. As decreases in acylcarnitine and citrate concentrations are nonetheless observed (see also Holness *et al.*, 1986b), it is suggested that, before the establishment of a new steady state, the rate of β -oxidation exceeds the rate of acylcarnitine formation and the rate of disposal of citrate exceeds the rate of formation of acetyl-CoA. This situation contrasts with that found in the starved state, and is essentially a reversal of the metabolic changes observed after switching from the oxidation of glucose to the oxidation of fatty acids (Randle *et al.*, 1970).

Although the failure of MPCA to elicit re-activation of cardiac PDH_a would argue against triacylglycerol utilization, it has not been firmly established whether MPCA blocks lipolysis in muscle; MPCA is an analogue of nicotinic acid, and in dogs nicotinate blocks adipocyte, but not muscle, lipolysis (Carlson *et al.*, 1966). Complete re-activation of cardiac PDH is achieved after treatment of starved rats with 2-tetradecylglycidate (Caterson *et al.*, 1982), which would be expected to block the mitochondrial oxidation of NEFA derived from both exogenous and endogenous triacylglycerol.

General conclusions

There is now evidence that chronic changes in hepatic enzyme complement may limit liver functional capacity during the first few hours after nutrient provision (see, e.g., Sugden *et al.*, 1983; Spence & Koudelka, 1985; Kuwajima *et al.*, 1986). Thus the administration of carbohydrate to 48 h-starved rats, while decreasing the availability of fatty acids and increasing the insulin/glucagon ratio, does not lead to an immediate increase in

hepatic glycolytic flux and pyruvate oxidation. A failure to restore Fru-2,6- P_2 concentrations to fed values limits flux through PFK (Claus *et al.*, 1984); gluconeogenesis (Sugden *et al.*, 1983; Newgard *et al.*, 1984) and fat oxidation (Holness *et al.*, 1987) continue, and PDH remains inactive (Holness *et al.*, 1986a,b, 1987). The possibility of long-term adaptive changes in carbohydrate utilization in muscle has not been considered. However, in both heart and skeletal muscle, where PDH subserves a purely energetic function, re-activation of the complex is also not immediate (Kerbey & Randle, 1982; Holness *et al.*, 1986a,b). The present experiments indicate that restoration of muscle Fru-2,6- P_2 concentrations in response to re-feeding after 48 h starvation is also delayed, possibly as a consequence of altered response to insulin. As a response of cardiac Fru-2,6- P_2 to insulin is observed after overnight starvation (Rider & Hue, 1984), the biochemical basis and the physiological significance of the hysteresis induced by more prolonged starvation warrants further investigation.

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