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Time courses of the responses of pyruvate dehydrogenase activities to short-term starvation in diaphragm and selected skeletal muscles of the rat

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In the fed state, the percentages of the pyruvate dehydrogenase complex (PDH) in the active form (PDH_a) in diaphragm and a selection of skeletal muscles (adductor longus, soleus, extensor digitorum longus, tibialis anterior, gastrocnemius) ranged from 8% (soleus) to 38% (gastrocnemius). Major decreases in PDH_a activities in all of these muscles were observed after 15 h of starvation, by which time activities were less than 40% of the fed values. In general, the response to starvation was observed more rapidly in muscles of high oxidative capacity. The patterns of changes in skeletal-muscle PDH activities during the fed-to-starved transition are discussed in relation to changes in lipid-fuel supply and oxidation.

INTRODUCTION

The 40% decline in whole-body glucose turnover in rats starved for 48 h results predominantly from decreased glucose utilization in muscles (including heart) which are capable of high rates of lipid oxidation (Issad *et al.*, 1987). This observation lends support to the proposal (Randle *et al.*, 1964) that the oxidation of lipid fuels (circulating NEFA and ketone bodies; endogenous triacylglycerol) may inhibit glucose catabolism in oxidative muscles in starvation. However, starvation-induced decreases in activities of the pyruvate dehydrogenase complex (PDH), which catalyses the physiologically irreversible step in glucose degradation, are not restricted to skeletal muscles containing a high percentage of oxidative fibres (Sugden & Holness, 1989). Furthermore, whereas a major (80%) decline in the concentration of active PDH complex (PDH_a) occurs within 6 h of the initiation of starvation in the heart, by which time only 5% of the complex is in the active form (Holness & Sugden, 1989), PDH in quadriceps (which is composed of a mixture of oxidative and glycolytic fibres) remains more than 16% active (Kruszynska & McCormack, 1989), with a large (73%) decline in PDH_a activity over the period from 6 to 24 h after food withdrawal (Kruszynska & McCormack, 1989). Taken together, these findings suggest that the response of PDH_a activity to an increase in the circulating lipid supply may differ in skeletal muscle and heart.

There have been no detailed studies of the specificity (in terms of fibre type) of the responses of PDH_a activities in individual skeletal muscles to acute starvation. In addition, the role of increased lipid oxidation in effecting the responses of PDH to starvation in individual skeletal muscles has not been assessed. In the present study, we have investigated the time courses of PDH inactivation in diaphragm and a variety of skeletal muscles known to differ in their oxidative capacities. The results are discussed in relation to changes in lipid supply and oxidation.

MATERIALS AND METHODS

Female albino Wistar rats (180–220 g) on a 12 h-light/12 h-dark cycle (light from 08:30 h) were fed *ad libitum* on a standard rodent diet (supplied by E. Dixon and Sons, Ware, Herts., U.K.; 52% digestible carbohydrate, 16% protein, 2% lipid and 30% non-digestible residue, all by weight) before use. Water was provided *ad libitum*. Food was removed at 08:30 h, and rats were sampled after the periods of starvation indicated. 2-Tetradecylglycidate (TDG) (2.5 mg/100 g body wt.), an inhibitor of overt carnitine palmitoyltransferase, was administered by intragastric intubation (see Schofield *et al.*, 1985). PDH kinase was inhibited by the administration of dichloroacetate (DCA) [0.25 ml of a 5% (w/v) solution in 0.9% NaCl, pH 7.4; intraperitoneal injection at 30 min intervals (Holness *et al.*, 1986)]. Lipolysis was inhibited by the administration of 5-methylpyrazole-3-carboxylic acid (MPCA) [0.66 mg/100 g body wt. in 0.2 ml of 0.9% NaCl, pH 7.4, intraperitoneal injection at 90 min intervals (French *et al.*, 1988)]. Rats treated with TDG or DCA were sampled after 2 h. Rats treated with MPCA were sampled after 4.5 h. An artificial elevation in NEFA was achieved by the administration of 5 ml of corn oil and subsequent heparin treatment [see Rennie *et al.* (1976) for details]. The rats were administered corn oil at the time of food removal. After 3 h they were injected with 200 units of sodium heparin, and they were killed after a further 3 h.

Muscles were excised and freeze-clamped while rats were under sodium pentobarbital anaesthesia (5 min; 6 mg/100 g body wt.), and stored in liquid N_2 until extraction. PDH_a and citrate synthase activities were measured in muscle 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 were expressed relative to citrate synthase to correct for variability in the efficiency of mitochondrial extraction, an approach used previously (Caterson *et al.*, 1982; Sugden & Holness, 1989) and recently validated

Abbreviations used: NEFA, non-esterified fatty acids; PDH, the pyruvate dehydrogenase complex; PDH_a, the active form of PDH; TDG, 2-tetradecylglycidate; DCA, dichloroacetate; MPCA, 5-methylpyrazole-3-carboxylic acid.

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(Denyer *et al.*, 1989). Total PDH activities were measured after incubation with PDH phosphatase (Kerbey *et al.*, 1976) and were unchanged by alterations in the nutritional status (results not shown; see also Caterson *et al.*, 1982; Fuller & Randle, 1984; Kruszynska & McCormack, 1989; Denyer *et al.*, 1989). The fibre profiles (fast oxidative glycolytic: fast glycolytic: slow oxidative) of the muscles selected for study are as follows (Ariano *et al.*, 1973): adductor longus, 12:0:88; soleus, 0:0:100; tibialis anterior, 66:32:2; extensor digitorum longus, 59:38:3; gastrocnemius, 37:58:5.

Blood was sampled from the chest cavity at the time of killing. NEFA concentrations were estimated in plasma and ketone-body concentrations in KOH-neutralized $HClO_4$ extracts of whole blood as described previously (Holness *et al.*, 1988).

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.

Sources of materials were as in Holness *et al.* (1988). TDG (McN-3802) was generously provided by McNeill Pharmaceuticals, Spring House, PA, U.S.A. MPCA was a gift from Upjohn Ltd., Crawley, West Sussex, U.K.

RESULTS AND DISCUSSION

Glucose oxidation in individual skeletal muscles

The values for the percentages of the PDH complex in the active dephosphorylated form (PDH_a) in selected muscles in the fed state are shown in Table 1. In diaphragm the value was lower than that reported by Denyer *et al.* (1989) (45% active complex), but exceeded that obtained by Caterson *et al.* (1982) (7% active complex). The percentage of active complex in heart was slightly higher than in diaphragm.

Adductor longus and soleus, postural muscles which are working in the resting state (Penicaud *et al.*, 1987), are composed predominantly of Type I (slow oxidative) fibres. The percentage of active PDH complex in the fed state in adductor longus was comparable with that in heart, whereas that in soleus was considerably lower. The percentages of active PDH complex in tibialis anterior and extensor digitorum longus, non-working muscles containing predominantly Type II (fast-twitch) oxidative fibres, were comparable with those observed in diaphragm and adductor longus. In gastrocnemius, which contains predominantly Type II glycolytic fibres, a relatively high percentage of total PDH was present as active complex in the fed state. This value (38%) compares well with previous reports of the activation state of gastrocnemius (Caterson *et al.*, 1982) or in mixed hindlimb muscles (Hennig *et al.*, 1975; Hagg *et al.*, 1976) in the fed state.

PDH_a activities after inhibition of PDH kinase by DCA

The activation state of the PDH complex is determined by the relative activities of PDH kinase (phosphorylating, inactivating) and PDH phosphatase (dephosphorylating, activating) (reviewed by Randle, 1986). PDH kinase is inhibited by DCA, a non-metabolizable pyruvate analogue (Whitehouse *et al.*, 1973). In the present study, DCA treatment elicited 1.7- and 3.5-fold increases in the percentage of active PDH complex in heart and diaphragm respectively (Table 1): a 2.5-fold increase in cardiac PDH_a activity in response to DCA in the fed state has been noted previously (Sale & Randle, 1982). PDH_a activities in the fed state were increased by the administration of DCA in all the skeletal muscles studied (Table 1). The effects of DCA treatment on skeletal-muscle PDH_a were least in gastrocnemius (a 2.0-fold increase in the percentage of active complex) and greatest in soleus (an 8.3-fold increase; Table 1).

Table 1. PDH activities in heart, diaphragm and skeletal muscles of fed rats

Details of the protocol for measurements of enzyme activities are given in the Materials and methods section. Rats were permitted free access to food and sampled before 10:30 h. One group of rats was treated with DCA to inhibit PDH kinase. Results are means ± S.E.M. of 5–11 rats. Statistically significant effects of DCA are indicated by: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Abbreviation: EDL, extensor digitorum longus.

	DCA	PDH activity (m-units/unit of citrate synthase)		PDH_a activity (% of total)	Citrate synthase activity (units/g wet wt.)
		Initial	Total		
Heart	−	20.2±3.1	64.3±1.9	31.4	90.8±7.8
	+	34.5±3.5**	65.1±3.0	53.0	85.3±3.3
Diaphragm	−	11.7±1.3	60.7±6.7	19.3	29.2±1.5
	+	45.1±3.2**	67.3±2.8	67.0	25.6±1.7
Adductor longus	−	9.8±1.8	37.2±4.9	26.3	10.8±1.4
	+	29.4±2.6***	32.8±2.8	89.6	13.9±3.6
Soleus	−	2.7±0.4	35.8±3.7	7.5	16.2±1.2
	+	22.9±5.0**	36.9±5.7	62.1	17.5±2.2
Tibialis anterior	−	12.6±0.6	55.2±4.9	22.8	22.2±1.6
	+	40.3±5.6***	52.3±7.1	77.1	28.1±5.3
EDL	−	8.8±0.9	48.9±4.3	18.0	14.6±0.8
	+	45.5±4.5***	49.8±6.7	91.4	15.8±3.0
Gastrocnemius	−	20.5±3.2	54.9±5.8	37.5	15.3±1.2
	+	34.3±4.4*	47.4±4.5	74.7	16.9±1.5

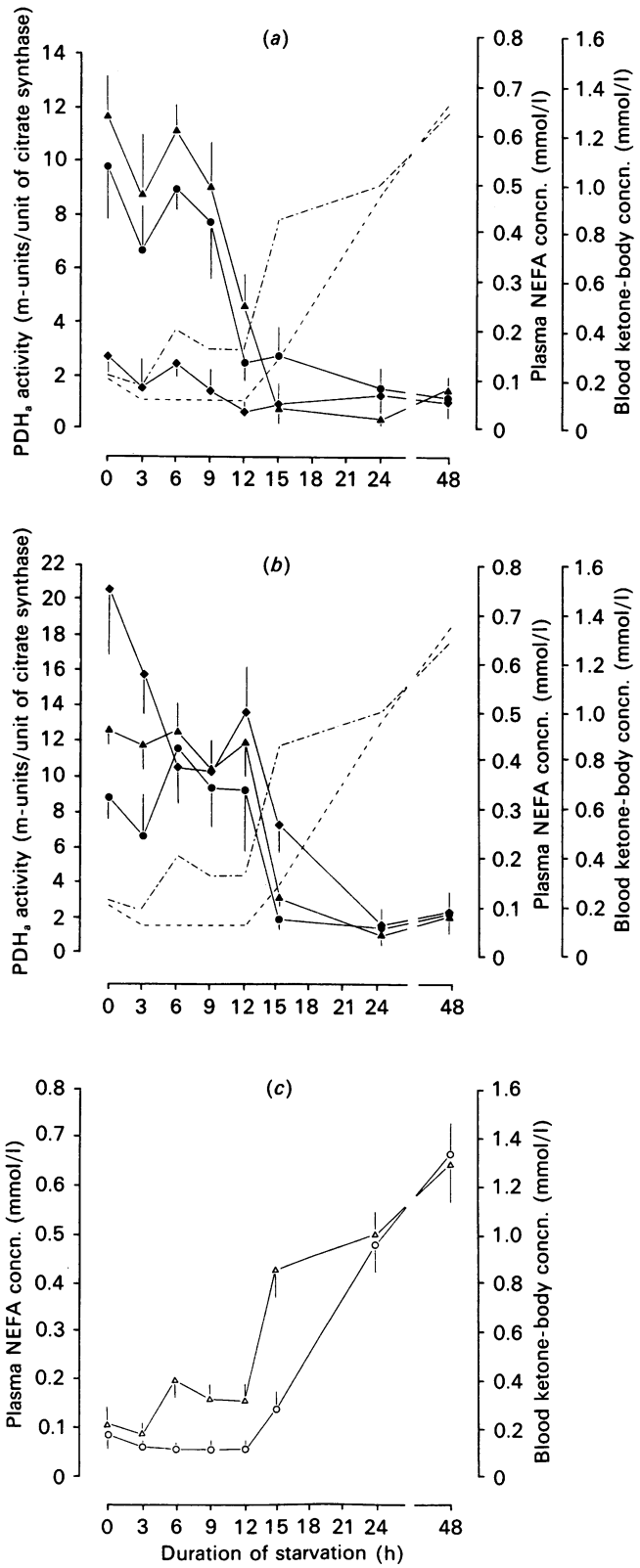

Fig. 1. PDH_a activities in diaphragm and selected skeletal muscles and blood NEFA and ketone-body concentrations during the fed-to-starved transition

Full details of the experimental procedures are given in the Materials and methods section. PDH_a activities are shown for diaphragm (▲), adductor longus (●) and soleus (◆) in (*a*) and for tibialis anterior (▲), extensor digitorum longus (●) and gastrocnemius (◆) in (*b*). NEFA (△) and ketone-body (○) concentrations are shown in (*c*) and as broken lines [NEFA (---); ketone-bodies (----)] in (*a*) and (*b*). Results are means ± S.E.M. of 6–12 determinations. Statistically significant effects of starvation on PDH_a activities were first observed at 6 h for gastrocnemius ($P < 0.05$), at 12 h for diaphragm, adductor longus and soleus ($P < 0.01$ in each case) and at 15 h for tibialis anterior and extensor digitorum longus ($P < 0.001$ in both cases). Statistically significant increases in NEFA and ketone-body concentrations were first observed at 6 h ($P < 0.05$) and 24 h ($P < 0.001$) respectively.

PDH_a activities in diaphragm and skeletal muscles during the fed-to-starved transition

In contrast with the relatively rapid response to starvation in heart (see Holness & Sugden, 1989), PDH_a activities in diaphragm and (except gastrocnemius) skeletal muscles were unchanged during the initial 6 h of fasting (Figs. 1*a* and 1*b*).

PDH_a activities in diaphragm, adductor longus and soleus had significantly decreased between 9 and 12 h after the initiation of starvation (by 61%, 75% and 74% respectively; Fig. 1*a*). Extending the period of starvation beyond 12 h evoked little further response (Fig. 1*a*). In contrast, only limited decreases in PDH_a activities were observed in tibialis anterior and extensor digitorum longus within the first 12 h after initiation of starvation, whereas after 15 h of starvation, activities had significantly declined (by 75% and 77% respectively) (Fig. 1*b*). Only modest further decreases in PDH_a activities occurred between 15 and 24 h after initiation of starvation (Fig. 1*b*). In gastrocnemius, PDH_a decreased by 49% after 6 h of starvation (Fig. 1*b*), at which time PDH_a activity was comparable with that observed in quadriceps after 6 h starvation (Kruszynska & McCormack, 1989). (Quadriceps is composed of four muscles of mixed fibre composition, namely the rectus femoris, vastus intermedius, vastus medialis and vastus lateralis muscles.) PDH_a activity in gastrocnemius was little changed between 6 and 12 h of starvation, but subsequently declined between 15 and 24 h of starvation (Fig. 1*b*). Thus, after 15 h starvation, the PDH complex was relatively inactive in all the muscles studied, the percentage of PDH in the active form in these muscles ranging from 1% to 12% of the total PDH activities.

Relationship between carbohydrate and lipid oxidation during the fed-to-starved transition

Although skeletal-muscle PDH_a activity is decreased by prolonged starvation or experimental diabetes (Hennig *et al.*, 1975; Hagg *et al.*, 1976; Caterson *et al.*, 1982; Fuller & Randle, 1984; Denyer *et al.*, 1989; Kruszynska & McCormack, 1989; Sugden & Holness, 1989), inhibition of the mitochondrial oxidation of long-chain fatty acids by TDG failed to reverse the effects of 48 h starvation under conditions where it reversed the effects in the heart (Caterson *et al.*, 1982). This might be a consequence of a failure to inhibit mitochondrial fatty acid oxidation in skeletal muscle under the conditions employed. Alternatively, inactivation of the PDH complex in skeletal muscle in starvation may be independent of NEFA oxidation. We have therefore employed three independent approaches to investigate the potential importance of increased lipid fuel oxidation in effecting decreases in skeletal-muscle PDH_a activities during the

fed-to-starved transition. First, we have examined the temporal relationship between decreases in PDH_a activities and increases in circulating NEFA and ketone-body concentrations. Secondly, we have measured muscle PDH_a activities after the administration of corn oil and heparin, thereby eliciting an acute elevation of circulating NEFA concentrations during the first 6 h after food withdrawal. Thirdly, we have inhibited either adipose-tissue lipolysis or tissue long-chain fatty acid oxidation by pharmacological means (administration of MPCA or TDG respectively).

The time courses of the changes in circulating NEFA and ketone-body concentrations evoked by acute or more prolonged starvation are shown in Fig. 1(*c*). A 1.8-fold increase in the NEFA concentration was observed between 0 and 6 h of starvation; however, the major increase in the NEFA concentration was observed between 12 and 15 h of starvation. The increment in circulating NEFA was accompanied by increased ketonaemia, but, whereas the major increase in NEFA concentrations occurred between 12 and 15 h of starvation, ketonaemia increased most dramatically during 15–24 h of starvation (Fig. 1*c*). The universal decline in PDH_a activities in skeletal muscle observed between 9 and 15 h of starvation therefore bears a striking correlation with the major elevation of NEFA concentrations (see Figs. 1*a* and 1*b*).

The responses of muscle PDH_a activities to a substantial (4.9-fold) elevation in NEFA concentrations during the first 6 h of starvation are shown in Table 2. PDH_a activities were greatly decreased by this elevation in circulating NEFA concentration in diaphragm (by 74%), adductor longus (by 57%) and soleus (by 67%) (Table 2). The responses were less dramatic in extensor digitorum longus, tibialis anterior and gastrocnemius, where decreases in PDH_a activities were not significant (Table 2). Thus the muscles responding most markedly to an increased circulating fatty acid supply are working muscles containing a high percentage of oxidative fibres.

Although the inhibition of mitochondrial long-chain acyl-CoA uptake by the administration of TDG led to a nearly 4-fold increase in NEFA concentrations (Table 2), a finding which is consistent with suppression of fatty acid utilization, TDG treatment failed to prevent the effects of 15 h starvation to decrease PDH_a activity in diaphragm, soleus or gastrocnemius (Table 2). Modest (not significant) effects of TDG treatment to increase PDH_a activities above control (15 h-starved) values were observed in adductor longus, tibialis anterior and extensor digitorum longus (Table 2). There was, however, a convincing effect of inhibition of adipose-tissue lipolysis by MPCA to minimize or prevent the effects of 15 h starvation. MPCA prevented any increases in plasma NEFA and ketone-body concentrations (Table 2) and, except for the gastrocnemius, PDH_a activities in skeletal muscles of 15 h-starved MPCA-treated rats remained similar to those found in rats fed *ad libitum* (compare values in Tables 1 and 2).

General discussion

In a comparative study of the response of individual skeletal muscles to prolonged (48 h) starvation, we noted that there was no obvious relationship between starvation-induced decreases in PDH_a activities and either fibre composition or contractile activity of the muscles in the resting state (Sugden & Holness, 1989). Similarly, the percentage of total PDH in the active form in the fed state appears to be independent of the fibre-type profiles (the present study). In general, the percentage of active PDH complex in the fed state was between 19 and 38% of total, with the notable exception of the soleus, in which only 8% of total complex was active. The low activation state of PDH in soleus even in the fed state may be presumed, on the basis of results obtained with

Table 2. PDH_a activities in diaphragm and skeletal muscles after manipulation of circulating NEFA and ketone-body concentrations or inhibition of long-chain fatty acid oxidation

Full details are given in the Materials and methods section. Rats were starved for 6 or 15 h as indicated. NEFA concentrations were elevated by the administration of corn oil and heparin, and NEFA and ketone-body concentrations were decreased by inhibition of lipolysis with MPCA. Long-chain fatty acid oxidation was inhibited with TDG. Effects of DCA treatment are shown for comparison. PDH_a activities are expressed as m-units/unit of citrate synthase, and metabolite concentrations as mmol/l. Statistically significant differences from the appropriate control (6 h- or 15 h-starved) values are indicated by: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Abbreviation: EDL, extensor digitorum longus.

	6 h-starved		15 h-starved			
	Control	Elevated NEFA	Control	TDG-treated	MPCA-treated	DCA-treated
PDH_a activity						
Diaphragm	11.2±0.8	2.9±0.8***	3.1±0.4	1.7±0.3*	6.5±2.1	45.9±3.8***
Adductor longus	8.6±0.6	3.7±1.2***	2.8±0.9	4.0±1.1	7.2±1.1**	30.7±2.8***
Soleus	2.4±0.3	0.8±0.1***	1.0±0.6	1.1±0.2	3.3±0.5*	10.1±3.0*
Tibialis anterior	11.6±1.3	10.7±1.5	3.2±0.3	4.2±1.2	14.4±2.6***	42.7±6.0***
EDL	10.8±1.8	5.9±1.8	2.0±0.3	3.1±1.8	14.4±2.9**	34.4±4.1***
Gastrocnemius	10.5±1.8	8.9±3.3	6.7±1.2	5.7±2.5	9.5±3.1	38.6±6.6***
Metabolite concn.						
NEFA	0.20±0.03	0.98±0.14***	0.42±0.05	1.53±0.21***	0.09±0.04***	0.31±0.07
Ketone bodies	0.12±0.01	0.25±0.01***	0.28±0.06	0.16±0.02	0.10±0.02*	0.45±0.08

DCA, to reflect a high PDH kinase/PDH phosphatase activity ratio, although the precise mechanism underlying this is as yet undefined. However, the low activity of PDH_a in soleus is not paralleled by a low rate of glucose uptake and phosphorylation (see Issad *et al.*, 1987), and the possibility therefore exists for concomitant glycolytic degradation of carbohydrate and oxidative degradation of lipid.

From observations that short- and long-chain fatty acids and ketone bodies inhibited pyruvate oxidation in hearts and diaphragms from fed rats, and that oxidation of NEFA derived from endogenous triacylglycerol inhibited utilization of extracellular glucose in isolated muscle preparations from starved rats, it was proposed that the oxidation of lipid fuels might inhibit glucose catabolism in oxidative muscles in starvation (Randle *et al.*, 1964). However, the effects of fatty acids and ketone bodies on glucose utilization in skeletal muscle have been contradictory: some reports have described inhibitory effects (Cuendet *et al.*, 1975; Rennie & Holloszy, 1977; Maizels *et al.*, 1977); others have not (Jefferson *et al.*, 1972; Goodman *et al.*, 1974; Berger *et al.*, 1976; Ruderman *et al.*, 1979; Zorzano *et al.*, 1985). In the present experiments, inhibition of PDH_a activity in response to starvation occurred less rapidly in diaphragm and skeletal muscles than in heart. Nevertheless, the most significant decreases in PDH_a activities evoked by starvation in skeletal muscle occurred concomitantly with marked increases in the circulating concentrations of NEFA, and were not observed if lipolysis was inhibited. Furthermore, an artificial elevation in NEFA concentrations over the first 6 h of starvation led to accelerated PDH inactivation in diaphragm and oxidative (working) skeletal muscles. Taken together with the demonstration of inhibitory effects of increased $NADH/NAD^+$ and acetyl-CoA/CoA concentration ratios on the PDH-complex activity in hind-limb skeletal-muscle mitochondria (Fuller & Randle, 1984), and reversal of the effects of acute (15 h) starvation with DCA (Table 2), there is strong evidence for the operation of the glucose/fatty acid cycle via activation of PDH kinase in oxidative skeletal muscles during the transition from the fed to the starved state.

Considerable heterogeneity is observed in the response of PDH in individual skeletal muscles to re-feeding (chow, *ad libitum*) after prolonged starvation (Sugden & Holness, 1989). PDH re-activation occurred more rapidly in non-working muscles (gastrocnemius, extensor digitorum longus and tibialis anterior) than in oxidative working muscles (diaphragm, adductor longus and soleus) (Sugden & Holness, 1989). In the present study, PDH_a activities in both oxidative and glycolytic skeletal muscles declined within 15 h of starvation, by which time activities were less than 36% of the fed value. However, decreases in activities in oxidative working muscles tended to precede those in non-working muscles by approx. 3 h. Thus those muscles in which PDH was refractory to complete re-activation for the first hours of re-feeding (Holness *et al.*, 1986; Sugden & Holness, 1989) also tended to respond more rapidly to food withdrawal.

The biochemical basis for the heterogeneity of the time courses of the responses to starvation remains to be clearly established. However, it is of interest that the regulation of lipoprotein lipase during nutritional shifts also varies between muscles containing different percentages of oxidative fibres (Kuwajima *et al.*, 1988). An increase in lipoprotein lipase is observed in response to starvation only in oxidative muscles (Kuwajima *et al.*, 1988). This raises the possibility of enhanced utilization of circulating lipoproteins (in addition to NEFA and ketone bodies) by oxidative muscles during the early phase of the fed-to-starved transition. Alternatively, individual skeletal muscles may differ in their abilities to respond to an elevation in the exogenous supply of fatty acids or to utilize endogenous triacylglycerol.

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