Effects of re-feeding after prolonged starvation on pyruvate dehydrogenase activities in heart, diaphragm and selected skeletal muscles of the rat

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We investigated the capacity for pyruvate oxidation in skeletal muscle, diaphragm and heart after starvation and re-feeding. Starvation for 48 h decreased pyruvate dehydrogenase (PDH) activity in soleus (by 47 %), extensor digitorum longus (64 %), gastrocnemius (86 %), diaphragm (87 %), adductor longus (90 %), tibialis anterior (92 %) and heart (99 %). Chow re-feeding increased PDH activity in all muscles to 43–78 % of the fed value within 2 h. However, complete re-activation was not observed for at least 4–6 h, during which time hepatic glycogen was replenished. We discuss the importance of muscle PDH activity in relation to sparing carbohydrate for hepatic glycogen synthesis.

INTRODUCTION

Randle *et al.* [1] have proposed that the oxidation of NEFA and ketone bodies entering the circulation in starvation might inhibit glucose catabolism in oxidative muscles, thereby sparing carbohydrate (the glucose/fatty acid cycle). There is convincing evidence for the operation of this cycle in prolonged starvation. For example, the 40 % decline in glucose turnover in rats starved for 48 h is predominantly the result of decreased glucose utilization in muscles with a large potential capacity for lipid oxidation [2]. However, on re-feeding after prolonged starvation, although the exogenous carbohydrate supply is increased and NEFA and ketone-body concentrations decline [3], rates of glucose degradation may remain relatively low. Complete re-activation of cardiac PDH is not observed until 8–24 h after re-feeding [4,5], and in both heart and certain skeletal muscles concentrations of fructose 2,6-bisphosphate, an activator of 6-phosphofructo-1-kinase, remain lower than in the fed state [6].

Despite the quantitative importance of the skeletal-muscle mass for glucose disposal, and the considerable heterogeneity between the responses of individual muscles to starvation in terms of glucose uptake/phosphorylation [2], there have been no detailed studies of the response of PDH in individual skeletal muscles to starvation or to re-feeding after prolonged starvation. The latter studies are important, because it is plausible that a restriction of the capacity for pyruvate oxidation in skeletal muscle may spare carbohydrate carbon for use by the liver as a glycogenic precursor during the starved-to-fed transition [3,7]. It is known that flux through the PDH reaction and the concentration of active PDH complex (PDH_a) is diminished in rat skeletal muscle by prolonged starvation [8,9], and that the regulatory characteristics of hind-limb skeletal-muscle PDH resemble those of heart [9]. However, whereas a glucose load fails to increase significantly cardiac or mixed (hind-limb) skeletal muscle PDH_a within 2 h [7], a significant

increase in PDH_a has been observed in quadriceps muscles of 48 h-starved rats after a 2.5 h glucose clamp [10].

In the present study, we have defined the response of PDH_a in individual skeletal muscles to re-feeding after prolonged starvation. We have selected a variety of muscles known to differ in their oxidative capacities and contractile activities in the resting state, and have used the physiological stimulus of re-feeding with a mixed diet (chow) *ad libitum*.

EXPERIMENTAL

Female albino Wistar rats (180–220 g) subjected to a 12 h-light/12 h-dark cycle (light from 08:30 h) were fed *ad libitum* on a standard rodent diet (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. Rats were starved thereafter. Some rats were allowed unrestricted access to chow after starvation for 48 h. Chow intakes were monitored, and did not differ significantly from those in ref. [3].

Rats were sampled while under sodium pentobarbital anaesthesia (5 min; 6 mg/100 g body wt.). Muscles were rapidly freeze-clamped, and stored in liquid N₂ until extraction. PDH_a and citrate synthase activities were measured in muscle extracts as described in [11]. PDH_a activities are expressed relative to citrate synthase to correct for possible variation in the efficiency of mitochondrial extraction (see [11]). Total PDH activities were not measured, as total activity is unchanged by alterations in the nutritional status [8,10,11]: changes in measured activities thus represent changes in the percentage of the enzyme in the active form of the complex. Mean citrate synthase activities (units/g wet wt.) were: heart, 74.6 ± 2.5 (55); diaphragm, 23.4 ± 0.7 (53); gastrocnemius, 13.9 ± 0.6 (54); soleus, 13.0 ± 0.5 (54); adductor

Abbreviations used: NEFA, non-esterified fatty acids; PDH, pyruvate dehydrogenase complex; PDH_a, active form of PDH.

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longus, 9.0 ± 0.4 ; extensor digitorum longus, 14.0 ± 0.4 (55); and tibialis anterior, 20.9 ± 0.5 (54). One unit of enzyme activity is that which converts $1 \mu\text{mol}$ of substrate into product/min at 30°C .

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

RESULTS AND DISCUSSION

PDH_a activities in heart, diaphragm and gastrocnemius muscles on re-feeding after prolonged starvation

Kerbey & Randle [4] examined the time course of cardiac PDH re-activation in response to re-feeding in rats previously starved for 48 h. After starvation for 48 h

Fig. 1. PDH_a activities in heart, diaphragm and skeletal muscles on re-feeding after 48 h starvation

Full details of the procedures are given in the Experimental section. Results are means $\pm$ S.E.M. for 6–12 determinations, and are expressed as percentages of the value observed in rats fed *ad libitum*. Muscles: ■, heart; ▨, diaphragm; □, gastrocnemius; ▤, adductor longus; ▥, extensor digitorum longus; □, tibialis anterior.

Table 1. Cardiac, diaphragm and skeletal-muscle PDH_a activities and hepatic glycogen concentrations on re-feeding after prolonged starvation

Full details are given in the Experimental section. Results are means $\pm$ S.E.M. for 5–12 rats. PDH_a activities are expressed as m-units/unit of citrate synthase. Hepatic glycogen concentrations are given as mg/g wet wt. of liver. Significant effects of re-feeding after 48 h starvation are shown by: **P* < 0.05; ***P* < 0.01; ****P* < 0.001. Significant effects of 48 h starvation are shown by: †*P* < 0.05; ††*P* < 0.01; †††*P* < 0.001. Abbreviation: EDL, extensor digitorum longus.

	48 h starved	2 h re-fed	4 h re-fed	6 h re-fed	Fed <i>ad libitum</i>
PDH _a activity					
Heart	0.6 ± 0.1 †††	1.4 ± 0.3 ***	5.3 ± 0.4 ***	6.7 ± 0.8 ***	20.2 ± 2.6
Diaphragm	1.4 ± 0.2 †††	4.7 ± 0.7 ***	5.9 ± 0.9 ***	5.8 ± 1.1 **	10.8 ± 1.8
Gastrocnemius	2.8 ± 0.7 †††	10.0 ± 1.6 ***	8.6 ± 1.1 ***	9.9 ± 2.3 **	19.8 ± 2.5
Soleus	1.2 ± 0.5	1.3 ± 0.5	2.0 ± 0.5	1.2 ± 0.4	2.4 ± 0.6
Adductor longus	1.0 ± 0.5 †††	4.9 ± 0.8 **	5.1 ± 1.2 **	5.3 ± 0.7 **	10.6 ± 2.0
EDL	2.4 ± 0.3 †††	4.8 ± 0.7 **	7.5 ± 1.7 *	6.7 ± 2.0 *	9.6 ± 1.2
Tibialis anterior	2.4 ± 0.7 †††	10.5 ± 1.1 ***	9.0 ± 1.5 **	13.4 ± 1.2 ***	13.5 ± 0.9
Hepatic glycogen	0.7 ± 0.3 †††	19.1 ± 3.2 ***	35.6 ± 2.4 ***	55.7 ± 2.4 ***	68.1 ± 3.4

activity declined to approx. 1 % of the fed value ([4,5]; Table 1). At 6–8 h after re-feeding, the activity of the complex was only approx. 30 % of that observed in the fed state [4]. We have confirmed these findings ([5]; see also Table 1). The extent of PDH inactivation in prolonged starvation and the pattern of PDH re-activation after re-feeding in another constantly contracting muscle, the diaphragm, are shown in Table 1. After 48 h starvation, the activity was 13 % of that in the fed state, and, although a 3–4-fold increase in activity was observed in response to re-feeding, the response of PDH_a in diaphragm, as in heart, was initially incomplete (Table 1). Thus, even after 6 h of re-feeding, PDH_a activity, and hence the capacity for pyruvate oxidation, was only 54 % of the value observed in the fed state (Fig. 1).

In the initial studies of the response of skeletal muscle to starvation and re-feeding, we chose to investigate PDH_a activity changes in gastrocnemius muscle, which we considered to be reasonably representative of the skeletal-muscle mass of the body, being of mixed fibre composition [12]. After 48 h starvation, the activity was approx. 14 % of the fed value (Fig. 1). A similar marked decrease in the capacity for pyruvate oxidation in response to starvation has been observed previously in perfused hind-limbs [13]. As in diaphragm, there was a substantial increase in PDH_a activity in response to re-feeding, with a 3-fold increase in activity within 2 h of the provision of chow (Table 1). However, there was no further increase in PDH_a activity if the period of re-feeding was extended to 6 h (Table 1). The mean activity during the first 6 h of re-feeding therefore approximated to only 50 % of the fed value (Fig. 1).

Glucose oxidation in individual skeletal muscles

In a study of glucose utilization rates in individual skeletal muscles, it was observed that the most significant responses to 48 h starvation occurred in working oxidative muscles (namely soleus and adductor longus) [2]. In contrast, little or no effect of starvation on glucose utilization was observed in non-working muscles (namely extensor digitorum longus and tibialis anterior) [2] with a lower percentage of oxidative fibres [12]. In the present study, we have therefore compared the responses of PDH_a in these specific muscles to starvation and re-feeding.

(i) Soleus and adductor longus. These muscles are characterized by high rates of glucose uptake/

phosphorylation in the fed state and a marked decline in rates of glucose uptake/phosphorylation in response to 48 h starvation [2]. Soleus-muscle PDH_a activity declined by approx. 50 % after 48 h starvation (Table 1). PDH_a activity in adductor longus fell to 10 % of the fed value after 48 h starvation (Table 1). Re-feeding failed to restore PDH_a activity to the fed value during the first 6 h in either muscle (Table 1).

(ii) Extensor digitorum longus and tibialis anterior.

Although these two muscles exhibit only minor (non-significant) decreases in glucose uptake/phosphorylation in response to prolonged starvation [2], decreases in PDH_a activities of 64 and 92 % respectively were observed after 48 h starvation (Table 1). Both muscles responded to re-feeding with a marked elevation in PDH_a activity (Table 1). In extensor digitorum longus, a 2-fold increase in activity was observed within 2 h (Table 1), and the activity was 78 % of that found in the fed state within 4 h (Fig. 1). In tibialis anterior, PDH re-activation was substantial within 2 h (at which time activity was 78 % of the fed value), and complete within 6 h (Table 1; Fig. 1).

General discussion

PDH_a activities in both working and non-working muscles declined with fasting. This uniform response contrasts with the effects of starvation to decrease rates of glucose uptake/phosphorylation specifically in working muscles. To facilitate comparison with previous rates of glucose utilization, which have been made in post-absorptive rats [2], PDH_a activities were measured in skeletal muscles of 6 h-starved rats, and results have been expressed in terms of glucose equivalents oxidized. In working oxidative muscles, PDH_a activities are relatively low compared with rates of glucose uptake/phosphorylation (Table 2). As observed rates of glucose uptake/phosphorylation exceed the capacity for pyruvate oxidation, it is implied that a significant proportion of the glycolytic product is released as lactate, rather than oxidized. This is particular notable for soleus, where pyruvate oxidation can account for only a relatively small percentage of total glucose uptake/phosphorylation (Table 2). In contrast, the capacity for glucose oxidation exceeds the rate of glucose uptake/phosphorylation in extensor digitorum longus and tibialis anterior in the post-absorptive state (Table 2). Thus the marked inactivation of PDH which is observed in starvation is

Table 2. Comparison of PDH_a activities and glucose-utilization indices in working and non-working skeletal muscles of post-absorptive rats

Full details of the protocol for measurements of PDH_a activities are given in the Experimental section. Glucose-utilization indices (GUI) measured *in vivo* have been taken from ref. [2]. No correction has been made for measurements of PDH_a activities at 30 °C. Abbreviation: EDL, extensor digitorum longus.

	PDH _a activity		GUI		PDH _a /GUI (%)
	(m-units/unit of citrate synthase)	(μ mol/min per g wet wt.)	(mg/min per g wet wt.)	(μ mol/min per g wet wt.)	
Soleus	2.5 $\pm$ 0.4	17.6 $\pm$ 2.8	44.0 $\pm$ 7.5	244 $\pm$ 42	7.2
Adductor longus	9.0 $\pm$ 0.8	31.8 $\pm$ 2.8	28.0 $\pm$ 3.0	155 $\pm$ 17	20.5
EDL	11.6 $\pm$ 2.5	80.6 $\pm$ 17.4	5.3 $\pm$ 0.7	29 $\pm$ 4	274.2
Tibialis anterior	12.5 $\pm$ 1.8	126.9 $\pm$ 18.3	5.9 $\pm$ 1.0	33 $\pm$ 6	386.8

obligatory to diminish irreversible carbohydrate disposal in these muscles.

On the basis that complete re-activation of hepatic, cardiac and hind-limb skeletal-muscle PDH is not observed within 2 h of glucose re-feeding [9], and that accelerated PDH re-activation inhibits hepatic glycogenesis [9], we proposed that uncoupling of glycolysis from glucose oxidation might spare C_3 derivatives of glucose for use as precursors for hepatic glycogenesis via the indirect pathway [9,14]. The present data demonstrate that PDH_a activities in a range of skeletal muscles are not fully restored to the fed values in the period during which hepatic glycogen synthesis is repleted (Table 1). Although it is as yet unknown whether glycolytic flux from exogenous glucose is restored after re-feeding (reviewed in [15]), a restriction of the capacity for pyruvate oxidation in some skeletal muscles may be important as a mechanism to facilitate the indirect pathway of hepatic glycogen replenishment by sparing lactate, generated either *in situ* or in other glycolytic tissues. Incomplete re-activation of skeletal-muscle PDH can be taken to reflect a continuation of carbohydrate sparing during the starved-to-fed transition.

This study shows that the decline in the capacity for pyruvate oxidation which is elicited by starvation differs between individual muscles. The response bears no obvious relationship with either fibre composition or contractile activity in the resting state. However, a close examination of the patterns of the responses of the individual muscles to re-feeding suggests that there may be greater suppression of activity in working (postural or cardiothoracic) muscles than in non-working muscles. Since the former muscles are constantly working, it is unlikely that their energy requirements are less during the starved-to-fed transition than in the fed state. Thus the existence of lower PDH_a activities during the starved-to-fed transition than in the fed state suggests either that the additional ATP requirements can be met by glycolysis (these muscles exhibit high rates of glucose uptake/phosphorylation relative to the capacity for pyruvate oxidation in the post-absorptive state) or that ATP production from glucose is supplemented by the oxidation of non-carbohydrate substrates (these muscles

contain a high percentage of oxidative fibres). The possible fibre-type specificity of the response of skeletal-muscle PDH_a activity to re-feeding contrasts with that of skeletal-muscle glycogen metabolism, where the rate of glycogen deposition after re-feeding is dependent on the extent of glycogen depletion observed during starvation, rather than on the fibre type [16].

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