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Glucose utilization in heart, diaphragm and skeletal muscle during the fed-to-starved transition

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The progressive effects of starvation on muscle glucose utilization were studied in the conscious resting rat. High rates of glucose uptake and phosphorylation in constantly working cardiothoracic (heart, diaphragm) and postural skeletal muscles (soleus, adductor longus) were maintained for at least 9 h of starvation. A rapid decline in cardiac glucose utilization was observed during the period 9–24 h of starvation, but for the other muscles the decline was more gradual. Consequently, even after 24 h, rates of glucose utilization in these muscles remained quantitatively significant. In both cardiothoracic and working (postural) skeletal muscle, glucose uptake and phosphorylation and activity of the active form of pyruvate dehydrogenase exhibited differential sensitivities to starvation and also to acute elevation of fatty acid concentrations during acute (4–9 h) starvation, such that pyruvate oxidation was more rapidly suppressed than glucose uptake and phosphorylation. The results are discussed in relation to the role of the glucose/fatty acid cycle in glucose conservation during the fed-to-starved transition.

INTRODUCTION

Starvation invokes adaptive mechanisms for glucose production and conservation (reviewed by Sugden *et al.*, 1989). Glucose derived from dietary carbohydrate is replaced by the generation of glucose via the mobilization of glycogen and/or gluconeogenesis, and irreversible glucose disposal is restricted. Glucose conservation in individual tissues may be achieved at the level of inhibition of glucose uptake and phosphorylation, which blocks both glucose degradation and storage (use as a precursor for glycogen synthesis), or by inhibition of the pyruvate dehydrogenase complex (PDH), which specifically blocks irreversible net loss of carbohydrate by oxidation.

Heterogeneity exists between individual tissues in terms of the time course of PDH inactivation after food withdrawal (Holness & Sugden, 1989; Holness *et al.*, 1989). Cardiac PDH is particularly susceptible to inhibition, with significant inactivation within 4–6 h (Holness & Sudgen, 1989). Inactivation of cardiac PDH_a (active form of PDH) is reversed by inhibition of mitochondrial fatty acid oxidation (Holness & Sugden, 1989). PDH in diaphragm and the skeletal-muscle mass (the major site of glucose utilization in the body) is more refractory to inactivation in response to starvation, with inactivation after 9–15 h of starvation (Holness *et al.*, 1989). However, as in the heart, inhibition of PDH activity in starvation is secondary to an increase in lipid fuel oxidation: inhibition of lipolysis attenuates the response (Holness *et al.*, 1989).

Studies *in vivo* have demonstrated that there is a major decline in glucose utilization after prolonged (48 h) starvation in heart, diaphragm and working skeletal muscles (Issad *et al.*, 1987). By analogy with the response of muscle PDH to starvation, this decline in glucose utilization has been attributed to the preferential use of fatty acids and ketone bodies as oxidative substrates (Issad *et al.*, 1987). However, increases in circulating concentrations of fatty acids and ketone bodies are observed as early as 9–12 h after food withdrawal (Holness *et al.*, 1989), at which time skeletal-muscle PDH_a activity exhibits its major decline (Holness *et al.*, 1989). The effects of more short-term starvation on muscle glucose uptake and phosphorylation have not been defined. Furthermore, prolonged (48 h) starvation appears to have a differential effect on individual pathways of glucose utilization in non-working muscles, with significant inhibition of PDH_a activities (and, by implication, carbohydrate oxidation) (Holness *et al.*, 1989), but minor decreases in the already relatively low rates of glucose uptake and phosphorylation (Issad *et al.*, 1987). In the present paper we have investigated the possibility that a selective or differential response of glucose uptake and phosphorylation and pyruvate oxidation may also be observed in working skeletal muscles and, in addition, whether the pattern of inhibition of glucose utilization varies between oxidative and non-oxidative pathways. Consequently, we have defined the precise time course of the response of glucose uptake and phosphorylation to starvation in heart, diaphragm and a selection of individual working or non-working skeletal muscles. Changes in rates of glucose uptake and phosphorylation *in vivo* (assessed by the accumulation of 2-deoxy[1-^{3}H]glucose 6-phosphate) are correlated with changes in PDH_a activities and circulating concentrations of fatty acids and ketone bodies.

MATERIALS AND METHODS

Sources of materials were as in Holness *et al.* (1988).

Animals

Female albino Wistar rats (200–250 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) Ltd.; 52% digestible carbohydrate, 16% protein, 2% lipid and 30% non-digestible residue, all by weight] before use. Water was provided *ad libitum*.

Measurement of tissue glucose utilization indices *in vivo*

The study of changes in rates of glucose utilization *in vivo* were conducted in conscious unrestrained rats each fitted with an indwelling cannula (for details see Ferré *et al.*, 1985; Issad *et al.*, 1987). Rats were sampled at 5–7 days after cannulation to permit recovery from surgery. In brief, a tracer dose of 2-deoxy-[^{3}H]glucose (30 μCi) was injected through the indwelling cannula, and serial blood samples were taken through the same cannula at 1, 3, 5, 10, 20, 40 and 60 min after the 2-deoxy[^{3}H]glucose

Abbreviations used: PDH, pyruvate dehydrogenase complex; PDH_a, active form of PDH; NEFA, non-esterified fatty acids.
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injection. Rats were killed at 1 h after the administration of radiolabel by injection of sodium pentobarbitol (60 mg/kg body wt.) via the indwelling cannula. Blood and muscles (heart ventricle, diaphragm and hind-limb skeletal muscles) were treated as described in Ferré *et al.* (1985). Glucose utilization was calculated as described by Issad *et al.*, (1987) by dividing the radioactivity (d.p.m.) of 2-deoxy[^{3}H]glucose 6-phosphate in the tissues by the calculated integral of blood 2-deoxy[^{3}H]-glucose/[glucose]. As in Issad *et al.* (1987), the value obtained was not corrected by the discrimination factor for 2-deoxyglucose in glucose metabolic pathways (see Ferré *et al.*, 1985). The results are therefore expressed as glucose utilization indices (see Issad *et al.*, 1987).

Food was removed at 08:30 h (the end of the dark phase of a 12 h-dark/12 h-light cycle) on the day of the experiment. One group of rats was sampled immediately. These rats are referred to as the controls (0 h-starved): they are still in the absorptive phase and hepatic glycogen concentrations are high (see Holness & Sugden, 1989). Other groups of rats were sampled after 6, 9, 12, 24, 30 and 48 h of starvation as indicated: the 6 h period of starvation corresponds to the nutritional state termed 'post-absorptive' in the study by Issad *et al.* (1987). In some experiments an artificial elevation of non-esterified fatty acid (NEFA) concentrations was achieved by the administration of 5 ml of corn oil at the time of food removal, followed by heparin treatment [for details see Rennie *et al.* (1976); French *et al.* (1988)]. Rats were injected with 200 units of sodium heparin at 3 h after the administration of corn oil, and were killed after a further period as indicated.

Measurements of enzyme activities

Heart, diaphragm and hind-limb skeletal muscles were excised and freeze-clamped while rats were under sodium pentobarbital anaesthesia (intraperitoneal injection of 60 mg/kg body wt.; 5 min exposure; see Sugden & Holness, 1989). Muscles were stored in liquid N_2 until extraction. The fibre profiles (fast oxidative glycolytic:fast glycolytic:slow oxidative) of the hind-limb 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. PDH activities (active and total) were measured in muscle extracts as described by Caterson *et al.* (1982). PDH_a activities were expressed as a percentage of total activities, which were measured after incubation of tissue extracts with PDH phosphatase. Total PDH activities in individual skeletal muscles are unchanged by alterations in the nutritional status (Fuller & Randle, 1984; Kruszynska & McCormack, 1989; Denyer *et al.*, 1989; Holness *et al.*, 1989).

Blood metabolite measurements

Glucose was measured by using glucose oxidase in the serial blood samples obtained via the indwelling cannula during the 2-deoxy[^{3}H]glucose experiments as described by Ferré *et al.* (1985). At the end of the 2-deoxy[^{3}H]glucose experiments, a larger blood sample was taken. A portion was used for determination of plasma NEFA concentrations (with a WAKO C-test kit). A further portion of whole blood was deproteinized in 6% (w/v) $HClO_4$ and the neutralized supernatant was used for the determination of blood ketone-body (3-hydroxybutyrate + acetoacetate) concentrations (Williamson *et al.*, 1962).

Statistics

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

RESULTS

Time course of changes in the glucose utilization indices in muscles of conscious resting rats

Values obtained for glucose utilization indices in muscles of progressively starved rats are shown in Table 1. Values obtained in post-absorptive (6 h-starved) and 48 h-starved rats were comparable with those reported previously by Issad *et al.* (1987). As demonstrated by Issad *et al.* (1987), the highest glucose utilization indices in post-absorptive rats were observed in oxidative muscles working in the resting state (i.e. heart, diaphragm, soleus, adductor longus): these muscles also exhibited the greatest absolute diminution of glucose uptake and phosphorylation after starvation for 48 h.

Cardiothoracic muscles. Glucose utilization indices in heart and diaphragm were high in the fed state and were maintained for at least the first 9 h of starvation. After this period of starvation, cardiac glucose utilization rates declined relatively rapidly, such that after starvation for 24 h cardiac glucose utilization was only 8% of the fed or immediately post-absorptive value. A further modest decline in cardiac glucose utilization was observed if the starvation period was extended from 24 to 48 h (Table 1). Glucose utilization indices in diaphragm were consistently lower than those of the heart. A moderate (32%; $P < 0.05$) decline in glucose utilization was observed over the first 6 h of starvation, and subsequently a steady (rather than rapid) decline in glucose utilization continued to be observed over the remaining 42 h period. Consequently, in marked contrast with the response of the heart, glucose utilization by the diaphragm remained sub-

Table 1. Glucose utilization indices in individual muscles of conscious rats during progressive starvation

Experimental details are given in the Materials and methods section. Results are means ± S.E.M. for 5–14 determinations. Statistically significant effects of starvation are indicated by: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Abbreviation: EDL, extensor digitorum longus.

Duration of starvation (h)	Glucose utilization index (ng of glucose/min per mg wet wt.)							Metabolite concn. (mM)	
	Heart	Diaphragm	Soleus	Adductor longus	Tibialis anterior	EDL	Gastrocnemius	NEFA	Ketone bodies
0	78.4 ± 13.0	40.2 ± 3.8	37.2 ± 6.2	29.8 ± 3.9	4.8 ± 0.5	5.1 ± 0.6	3.9 ± 0.7	0.14 ± 0.03	0.15 ± 0.03
6	71.6 ± 5.7	27.2 ± 4.7*	26.3 ± 5.6	22.7 ± 3.7	5.3 ± 0.7	4.4 ± 0.5	3.1 ± 0.5	0.20 ± 0.02	0.13 ± 0.01
9	85.9 ± 10.3	31.3 ± 7.2	29.4 ± 6.5	14.5 ± 2.8*	5.5 ± 0.7	4.2 ± 0.5	4.0 ± 0.5	0.15 ± 0.01	0.10 ± 0.03
12	53.0 ± 10.5	29.2 ± 3.2*	17.2 ± 6.1*	19.1 ± 2.4*	3.3 ± 0.2*	3.8 ± 0.4	2.4 ± 0.2	0.16 ± 0.02	0.11 ± 0.02
24	6.1 ± 1.1***	15.3 ± 3.1**	22.0 ± 2.0*	12.5 ± 1.8**	4.6 ± 0.9	3.6 ± 0.5	2.5 ± 0.4	0.59 ± 0.06***	0.71 ± 0.09***
30	8.9 ± 2.2***	12.5 ± 1.6***	20.6 ± 3.4*	7.6 ± 1.3***	4.1 ± 0.7	4.3 ± 0.6	2.3 ± 0.3	0.62 ± 0.04***	0.93 ± 0.10***
48	3.6 ± 1.5***	4.0 ± 1.4***	8.3 ± 2.1**	3.5 ± 1.0***	2.4 ± 0.6*	1.9 ± 0.6**	1.2 ± 0.5*	0.63 ± 0.03***	1.47 ± 0.19***

stantial (approx. 73 % of those observed in the fed state) even after 24 h starvation, still greatly exceeding (by approx. 4.5-fold) rates of glucose uptake and phosphorylation in non-working muscles in the fed state (Table 1).

Working skeletal muscles. The postural muscles soleus and adductor longus have been selected as examples of skeletal muscles which are working in the resting state. The soleus is characterized by one of the highest rates of glucose utilization both in the fed state and after 6 h starvation (Table 1), and its rate of glucose utilization decreases by over 80 % after starvation for 48 h (Issad *et al.*, 1987). However, remarkably, high rates of glucose utilization (55 % of fed values) were maintained for the first 30 h of starvation (Table 1). The most rapid decline in glucose utilization was observed over the period 30–48 h of starvation (Table 1). A steady decline in glucose utilization in adductor longus was observed throughout the period of starvation, and, again, rates of glucose utilization after 24 h starvation were substantial, approx. 55 % of the initial value (Table 1).

Non-working skeletal muscles. All three muscles studied (extensor digitorum longus, tibialis anterior and gastrocnemius) exhibited low rates of glucose uptake and phosphorylation in the fed state, only approx. 10 % of those rates observed in working oxidative muscles (Table 1). Therefore, although rates of glucose utilization in these non-working muscles declined by as much as 50–69 % after prolonged starvation, suppression of glucose utilization in these muscles can make only a very limited contribution to glucose conservation in starvation.

Using empirical curve-fitting for the data shown in Table 1, we have calculated the extent to which glucose utilization is spared for each of the working muscles over consecutive 12–24 h periods of starvation (Table 2). In the heart, total utilization of exogenous (i.e. blood-borne) glucose over the first 12 h of starvation, during which period NEFA and ketone-body concentrations remain relatively low (Table 1), is as much as 97 % of that which would be observed if initial rates of exogenous glucose utilization were to be maintained. However, because of the substantial decline in glucose utilization between 12 and 24 h of starvation, over which period NEFA and ketone-body concentrations significantly increase (Table 1), total cardiac glucose utilization over the first day of starvation is decreased by 30 %. Over 48 h, total sparing of glucose is much more considerable (approx. 61 % suppression of utilization). This arises because of almost complete (91 %) suppression of glucose utilization over the period 24–48 h of starvation. Rates of glucose utilization in diaphragm, soleus and adductor longus are decreased only by 20–34 % over the first 12 h of starvation (Table 2). As in heart, glucose sparing is enhanced as the duration of starvation is extended. Mean suppression of glucose utilization in these muscles amounted to approx. 34 % over the period 0–24 h of starvation, and approx. 70 % over the period 24–48 h of starvation. The percentage decline in glucose utilization in these working muscles observed after starvation for 48 h (70–90 %) exceeds the percentage decline in whole-body glucose turnover observed after starvation for 48 h (approx. 40 %; Issad *et al.*, 1987). The results indicate that tissues other than oxidative skeletal muscles make a significant contribution to continued glucose utilization after prolonged starvation.

Changes in glucose uptake and phosphorylation in working muscles in relation to glucose oxidation

Inactivation of PDH in the heart (Holness & Sugden, 1989) and a range of individual skeletal muscles (Holness *et al.*, 1989) is reversed by suppression of fat oxidation, indicating that

Table 2. Glucose utilization in individual working muscles during the fed-to-starved transition

Absolute glucose utilization (μg of glucose utilized/mg wet wt.) in individual muscles during 12, 24 and 48 h of starvation was estimated from curves fitted using the data given in Table 1. The results are expressed as percentages of that which would be expected if the rate of glucose utilization were to be maintained at the fed value throughout the period studied. Values during cumulative, as well as consecutive, periods of starvation are shown.

Duration of starvation (h)	Glucose utilization			
	Heart	Diaphragm	Soleus	Adductor longus
0–12	97.0	66.4	79.5	73.9
12–24	42.4	71.3	54.2	51.0
24–48	9.3	24.5	41.7	23.3
0–24	69.7	68.9	66.8	62.5
0–48	39.5	46.7	54.3	42.9

increased fat oxidation underlies the response. Furthermore, artificial elevation of NEFA concentrations over the first 6 h of starvation accelerates PDH inactivation in diaphragm and oxidative skeletal muscles (Holness *et al.*, 1989). These findings strongly support a role for the operation of the glucose/fatty acid cycle (Randle *et al.*, 1964) *in vivo* at the level of pyruvate oxidation in skeletal muscle in the resting state.

The results shown in Table 1 demonstrate that, although glucose utilization in oxidative muscles declines with starvation, significant rates of glucose utilization persist in skeletal muscle for as long as 12–24 h after food withdrawal. In contrast, the capacity for pyruvate oxidation in heart and skeletal muscle is suppressed within only 6 h and 15 h of starvation respectively, decreases in PDH_a activities being observed over a relatively short period (Holness & Sugden, 1989; Holness *et al.*, 1989). Therefore, either the signal for suppression of glucose uptake and phosphorylation is not identical with that of glucose oxidation, or alternatively glucose uptake/phosphorylation and pyruvate oxidation exhibit differential sensitivities to a common regulatory signal, such that pyruvate oxidation is more sensitive to suppression than is glucose utilization.

It has been suggested (Issad *et al.*, 1987) that diminished glucose utilization in oxidative working muscles after prolonged (48 h) starvation is a direct consequence of the increased availability of NEFA and ketone bodies, the concentrations of which are considerably increased after starvation for 48 h (Table 1). We therefore investigated whether glucose uptake/phosphorylation and pyruvate oxidation exhibit differential sensitivity to an accelerated increase in lipid fuel availability at the onset of starvation. Rates of glucose utilization and PDH_a activities in cardiac and skeletal muscles were measured after an acute elevation of circulating NEFA concentrations was evoked by the administration of a lipid meal followed by injection of heparin after 3 h. Results obtained after a further 3 h (corresponding to a starvation period of 6 h) are shown in Table 3. Glucose utilization was significantly suppressed (by 49 %) in the heart. However, the same period of exposure to an elevated NEFA supply failed to suppress significantly rates of glucose utilization in oxidative working skeletal muscles. This failure contrasts markedly with the decline in PDH_a activities observed over the same period in oxidative working skeletal muscles (Table 3).

The effects of more acute or more prolonged exposure to an elevated NEFA concentration are shown in Table 4. If rats were sampled at 1–2 h after the injection of heparin (denoted Group

Table 3. Glucose utilization indices and PDH_a activities in individual muscles of 6 h-starved rats after an artificial elevation of fatty acid concentrations

Experimental details are given in the Materials and methods section. Elevated NEFA concentrations were achieved by the administration of corn oil and heparin. Rats were sampled at 3 h after the injection of heparin (6 h after the removal of food). Fatty acid concentrations were measured in plasma, obtained via the indwelling cannula, as described in Holness *et al.* (1988). Fatty acid concentrations were 0.18±0.03 mM in the control (6 h-starved) group and 2.94±0.62 mM in the elevated-NEFA group. Results are means±S.E.M. for 5–10 determinations. Statistically significant effects of elevated fatty acid concentrations are indicated by: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

	Glucose utilization index (ng of glucose/min per mg wet wt.)		PDH_a activity (% of total)	
	Control	Elevated NEFA	Control	Elevated NEFA
Heart	73.8±6.5	38.0±1.3***	7.2±2.7	6.5±0.3
Diaphragm	30.4±5.7	26.4±2.2	18.8±1.8	6.7±1.5***
Soleus	27.5±3.2	22.3±4.5	6.7±1.7	2.1±0.5*
Adductor longus	25.1±3.2	33.8±3.2	25.3±3.5	10.4±2.3**
Tibialis anterior	4.6±0.6	4.3±0.7	22.1±4.9	18.5±2.8
Extensor digitorum longus	4.1±0.4	3.9±0.8	23.5±6.3	12.1±3.7
Gastrocnemius	3.2±0.6	3.2±1.0	18.0±5.1	18.7±6.0

Table 4. Glucose utilization indices and PDH_a activities in individual muscles of starved rats after artificial elevation of fatty acid concentrations

Experimental details are given in the Materials and methods section. Control rats were starved for either 4–5 h (Group I) or 7–9 h (Group II). Artificial elevation of NEFA concentrations was achieved by the administration of corn oil plus heparin. Rats in Group I were sampled at 1–2 h after heparin injection (corresponding to a starvation period of 4–5 h), whereas rats in Group II were sampled at 4–6 h after heparin injection (corresponding to a starvation period of 7–9 h). Fatty acid concentrations were 2.87±0.62 mM in Group I (4–5 h-starved) and 2.08±0.54 mM in Group II (7–9 h-starved). Results are means±S.E.M. for 5–8 determinations. Statistically significant effects of fatty acid concentrations are indicated by: *$P < 0.05$; *** $P < 0.001$.

	Glucose utilization index (ng of glucose/min per mg wet wt.)			
	Group I		Group II	
	Control	Elevated NEFA	Control	Elevated NEFA
Heart	72.4±7.2	76.1±6.5	88.3±4.4	17.2±0.9***
Diaphragm	33.1±3.5	41.4±6.6	27.7±2.9	18.3±2.7*
Soleus	30.4±3.8	28.2±1.7	27.5±4.6	14.5±3.2*
Adductor longus	25.3±3.0	31.3±4.0	14.8±3.2	17.8±1.9
Tibialis anterior	4.7±0.7	3.5±0.4	4.3±0.4	2.6±0.2***
Extensor digitorum longus	4.5±0.4	3.8±0.7	4.0±1.2	2.6±0.2
Gastrocnemius	4.2±0.7	4.1±0.8	4.3±0.7	2.5±0.4*

I), rates of cardiac glucose utilization were not significantly decreased. However, in contrast, a decrease in cardiac PDH_a activity was observed within this period [to 11.4±2.1% of total PDH ($n = 7$); see also Jin *et al.* (1988); French *et al.* (1988)]. If rats were sampled at 4–6 h after injection of heparin (Group II), modest suppression of glucose utilization was observed in oxidative working and non-oxidative non-working skeletal muscles as well as in heart, although differences did not always attain statistical significance (see Table 4).

Mechanisms by which differential suppression of glucose utilization and pyruvate oxidation may be achieved

When hearts are perfused with fatty acids or ketone bodies, the glycolytic rate is decreased but the combined output of lactate and pyruvate is not diminished (Randle *et al.*, 1964); furthermore, in spite of impaired glucose phosphorylation, glucose 6-phosphate and fructose 6-phosphate accumulate. Randle *et al.* (1964) therefore suggested that the degree of impairment among the various steps affected by lipid fuel oxidation was (from greatest to least): pyruvate oxidation, fructose 6-phosphate phosphorylation and glucose phosphorylation. These authors further suggested that this might be of importance to allow for the provision of glycolytic intermediates without overall loss of glucose by oxidation, and that the pyruvate and lactate not required by the heart might be converted into glucose by the liver. In the present study, a decrease in cardiac PDH_a activity was detected before significant diminution of glucose uptake and phosphorylation was observed. This study therefore provides clear evidence for the operation of differential regulation of cardiac glucose utilization by fatty acids *in vivo*. The pattern of inhibition of glucose utilization and pyruvate oxidation in starvation, with suppression of pyruvate oxidation preceding suppression of glucose utilization, resembles that observed in response to artificial elevation of fatty acid concentrations. It is therefore reasonable to suggest that the effects of starvation on both glucose utilization and pyruvate oxidation are mediated via the operation of the glucose/fatty acid cycle.

The results shown in Tables 3 and 4 provide evidence that a differential response of glucose utilization and pyruvate oxidation to exposure to an elevated fatty acid supply also exists in skeletal muscle *in vivo*. A more sustained increase in fat oxidation is required to evoke suppression of glucose uptake and

phosphorylation than to inhibit pyruvate oxidation. As in heart, the pattern of suppression of glucose metabolism resembles that occurring during progressive starvation. The most marked sparing of the utilization of exogenous glucose by skeletal muscle occurs over the period 24–48 h of starvation, where high concentrations of NEFA and ketone bodies have been sustained for a considerable period (> 9 h, Table 1; see also Holness *et al.*, 1989). The findings are consistent with the concept that suppression of glucose metabolism in skeletal muscle in the resting state in response to starvation is secondary to increased lipid fuel oxidation.

DISCUSSION

An important finding of the study is that the acute restriction of the irreversible disposal of carbohydrate via PDH is not closely paralleled by a restriction of the uptake and phosphorylation of circulating glucose. Thus within each individual muscle the time course of inhibition of glucose utilization varies between oxidative and non-oxidative pathways. In particular, working muscles continue to exhibit relatively high rates of glucose uptake during early starvation at times when PDH_a activities are markedly decreased. As a consequence, there is a distinct metabolic transition where there is clear suppression of glucose oxidation in the absence of diminished glucose uptake. This period may persist for as long as 12 h.

Stringent control of PDH activity is obligatory for carbohydrate homoeostasis, because (under physiological conditions) acetyl-CoA cannot be re-converted into glucose. In contrast, although maintenance of glycaemia in the absence of dietary glucose is facilitated by restricted utilization of blood-borne glucose, the uptake and phosphorylation of glucose does not lead to net loss of carbohydrate if PDH is inactive. The potential exists for use of glucose 6-phosphate for glycogen or triacylglycerol synthesis (Jenkins *et al.*, 1988), or perhaps more importantly, for glucose cycling via glycolysis, lactate production and hepatic gluconeogenesis. Hepatic gluconeogenesis from available C_3 precursors is favoured by early suppression of hepatic PDH_a activity (Holness & Sugden, 1989) and lipid synthesis (Holness & Sugden, 1990) within 6 h of the initiation of starvation. It is therefore likely that, if glycolysis is active in oxidative working muscles, there is cycling of lactate to glucose to maintain glycaemia. More rapid inactivation of carbohydrate oxidation (via PDH) than suppression of glucose uptake and phosphorylation may therefore be envisaged as a mechanism to prevent the further degradation of the product of glycolysis *in situ* and to minimize the use of exogenous (blood-borne) lactate as an oxidative substrate.

More rapid suppression of glucose utilization in response to increased NEFA availability is observed in the heart than in oxidative skeletal muscles. This is reminiscent of the response of skeletal-muscle PDH_a activities to starvation (Holness *et al.*, 1989). It has been demonstrated (Jenkins *et al.*, 1988) that the response of glucose utilization to the administration of exogenous lipid is critically dependent on the insulin status in skeletal muscle, though not in heart. Thus the acute administration of NEFA during hyperinsulinaemic clamp may actually increase glucose uptake and phosphorylation in skeletal muscle, while depressing that in heart (Jenkins *et al.*, 1988). In man, it has been demonstrated that prior exposure to an elevated circulating insulin concentration renders the body refractory to the actions of lipid on glucose oxidation: if triacylglycerol is infused after prior exposure to high insulin concentrations, its inhibitory effects on non-oxidative glucose disposal are abolished and inhibition of glucose oxidation is attenuated (Bonadonna *et al.*, 1989). Thus, although the present results provide direct support for the operation of the glucose/fatty acid cycle at the level of glucose uptake and phosphorylation during the fed-to-starved transition, it is not excluded that a decrease in blood insulin may also be necessary to facilitate or permit a high rate of oxidation of exogenous lipid fuels to be established in skeletal muscle.

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