Effects of starvation and exercise on concentrations of citrate, hexose phosphates and glycogen in skeletal muscle and heart

Evidence for selective operation of the glucose–fatty acid cycle

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1. Concentrations of citrate, hexose phosphates and glycogen were measured in skeletal muscle and heart under conditions in which plasma non-esterified fatty acids and ketone bodies were physiologically increased. The aim was to determine under what conditions the glucose–fatty acid cycle might be operative in skeletal muscle *in vivo*. 2. In keeping with the findings of others, starvation increased the concentrations of glycogen, citrate and the fructose 6-phosphate/fructose 1,6-bisphosphate ratio in heart, indicating that the cycle was operative. In contrast, it decreased glycogen and had no effect on the concentration of citrate or the fructose 6-phosphate/fructose 1,6-bisphosphate ratio in the soleus, a slow-twitch red muscle in which the glucose–fatty acid cycle has been demonstrated *in vitro*. 3. In fed rats, exercise of moderate intensity caused glycogen depletion in the soleus and red portion of gastrocnemius muscle, but not in heart. In starved rats the same exercise had no effect on the already diminished glycogen contents in skeletal muscle, but it decreased cardiac glycogen by 25–30%. 4. After exercise, citrate and the fructose 6-phosphate/fructose 1,6-bisphosphate ratio were increased in the soleus of the starved rat. Significant changes were not observed in fed rats. 5. The data suggest that in the resting state the glucose–fatty acid cycle operates in the heart, but not in the soleus muscle, of a starved rat. In contrast, the metabolite profile in the soleus was consistent with activation of the glucose–fatty acid cycle in the starved rat during the recovery period after exercise. Whether the cycle operates during exercise itself is unclear.

INTRODUCTION

Non-esterified fatty acids and ketone bodies can inhibit glycolysis and glucose oxidation and enhance glycogen synthesis in heart (Randle *et al.*, 1963, 1964; Newsholme & Randle, 1964), a series of events that in part constitute the glucose–fatty acid cycle. Metabolite analyses and flux measurements indicate that lipid fuels inhibit glycolysis at phosphofructokinase, owing to an accumulation of citrate, and that they inhibit glucose oxidation at pyruvate dehydrogenase by causing increases in acetyl-CoA and the [NADH]/[NAD⁺] ratio (Newsholme & Randle, 1964; Randle, 1966; Adrouny, 1969; Randle *et al.*, 1978). Glycogen synthesis is presumably enhanced owing to an increase in the concentration of glucose 6-phosphate (secondary to the block at phosphofructokinase), which activates some forms of glycogen synthase (Kato & Bishop, 1972; Nakai & Thomas, 1974; Roach, 1981) and inhibits phosphorylase *b* (Morgan & Parmegiani, 1964).

The quantitative importance of the glucose–fatty acid cycle in glucose homeostasis *in vivo* depends on whether it operates in skeletal muscle, which comprises approx. 40% of body mass in man and is the major site at which exogenous glucose is taken up (reviewed by Katz & McGarry, 1984). Initial studies suggested that lipid fuels do not inhibit glucose utilization in incubated or perfused muscle (Beatty & Bocek, 1971; Houghton & Ruderman, 1971; Jefferson *et al.*, 1972; Goodman *et al.*, 1974;

Reimer *et al.*, 1975; Berger *et al.*, 1976), with the possible exception of diaphragm (reviewed by Ruderman *et al.*, 1969), but that they inhibit glucose oxidation at the pyruvate dehydrogenase step (Berger *et al.*, 1976; Hagg *et al.*, 1976). More recently, inhibition of glucose utilization and glycolysis by fatty acids and ketone bodies has been demonstrated in rat soleus muscles incubated *in vitro* (Maizels *et al.*, 1977; Pearce & Connett, 1980); however, contradictory findings have been reported in the isolated perfused, well-oxygenated, rat hindquarter (Rennie & Holloszy, 1977; Ruderman *et al.*, 1980; Richter *et al.*, 1982b). Likewise, Rennie *et al.* (1976) have observed increased [citrate] and a lesser degree of glycogen depletion in red muscles of the rat after treadmill exercise *in vivo*, when plasma non-esterified fatty acid concentrations had been artificially raised. Overall, these observations suggest that inhibition of glycolysis and stimulation of glycogen synthesis and/or inhibition of its degradation by lipid fuels occurs in skeletal muscle, but only in selected circumstances.

To examine this notion, metabolite and glycogen profiles in the soleus and heart of intact rats were determined under a variety of conditions. The soleus was selected because it is primarily composed of red fibres, it is easily freeze-clamped *in situ*, and lipid fuels have been shown to inhibit glycolysis and enhance glycogen synthesis *in vitro*. Muscles were taken from fed, 48 h-starved and diabetic rats at rest and from the fed and starved rats during the recovery period after exercise. The

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results suggest that the glucose–fatty acid cycle operates in the soleus muscle of the starved rat after exercise, but not at rest, whereas it operates in the heart under both situations.

METHODS

Animals

Male Sprague–Dawley rats weighing 200–250 g were obtained from Charles River Breeding Laboratories (Wilmington, MA, U.S.A.). They were either fed on Purina rat chow *ad libitum* or starved for 48 h before a study. In most of the exercise studies the rats ran on a treadmill. To accustom them to the latter, they were run for 5 min at a rate of 18 m/min on the week before the experiment. On the morning of the experiment, they were divided into exercise and non-exercise groups. The exercised rats ran on the treadmill at a rate of 18 m/min for 43 min, then at 36 m/min for 2 min (moderate intensity). In separate experiments 48 h-starved rats were studied after 1 h of swimming in a barrel (40 cm water depth, 33 cm diameter) with a weight (4% of body weight) tied to their tails. Two rats were placed in each barrel and the water temperature was maintained at 33–35 °C. Triton was added to the water to diminish the air in their fur.

Experimental protocols and tissue sampling

Samples of blood and tissue were taken from non-exercised fed, starved and diabetic rats and from fed and starved exercised rats after intraperitoneal anaesthesia with pentobarbital (5 mg/100 g body wt.). Diabetes was induced by intravenous administration of streptozotocin (65 mg/kg body wt.) 48 h before the experiment. Samples were obtained immediately after the swim or at 0 (actually it took 3–4 min for the animals to be anaesthetized), 30, 60 or 120 min after the end of the treadmill exercise. Non-exercised rats were interspersed with the exercised groups to diminish the effect of small differences owing to time of sampling. During the post-exercise period, water but not food was provided. In the swimming studies and in experiments with diabetic animals, the soleus was frozen *in situ* with clamps cooled in liquid N₂, and then blood was drawn from the abdominal aorta. In all other experiments the soleus and white portion of the gastrocnemius were freeze-clamped *in situ* and the red portion of the gastrocnemius was excised and then freeze-clamped as described previously (Richter *et al.*, 1982a). Blood was then drawn from the abdominal aorta and placed in heparinized tubes. In some experiments liver and heart were subsequently freeze-clamped *in situ* for glycogen determination. To determine metabolite concentrations in the heart, separate experiments were performed in which only the heart was removed. Since we wanted to study the heart in the immediate post-exercise state, it was not possible to wait 30 min for hexose phosphates concentrations to equilibrate (Rider & Hue, 1984) or to adrenalectomize the animals (Newsholme & Randle, 1964) to prevent the increase in catecholamines during the collection procedure. As a result, concentrations of glucose 6-phosphate and fructose 6-phosphate were higher than those reported previously by Newsholme & Randle (1964) and by Adrouny (1969).

Analyses

The deproteinized filtrates of plasma were neutralized with 0.5 M-KOH/triethanolamine. Glucose, β -hydroxybutyrate and lactate were determined by standard enzymic methods as previously described (Goodman *et al.*, 1974). Plasma samples were used for determination of non-esterified fatty acids (Novak, 1965) and insulin (Micromedic Systems, Horsham, PA, U.S.A.). Frozen muscle was homogenized in 3 ml of ice-cold 6% (w/v) HClO₄ and the deproteinized extract neutralized with 0.5 M-KOH/triethanolamine. Glucose 6-phosphate (Hohorst, 1963a), fructose 6-phosphate (Hohorst, 1963a), fructose 1,6-bisphosphate (Williamson & Corkey, 1969), citrate (Start & Newsholme, 1968), pyruvate (Williamson & Corkey, 1969), lactate (Hohorst, 1963b), phosphocreatine (Lamprecht & Stein, 1963) and ATP (Lamprecht & Trautschold, 1963) were determined by standard enzymic methods (Goodman *et al.*, 1974), with a double-beam spectrophotometer (Perkin Elmer, model 356). Muscle (Karlsson *et al.*, 1971) and liver glycogen were determined as described previously (Goodman *et al.*, 1974). Statistical analysis utilized a Student's *t* test when two groups were compared or a one-way analysis of variance and further comparisons with the Scheffe test.

RESULTS

Effects of sampling method on muscle metabolite and glycogen contents

Earlier studies suggest that the glucose–fatty acid cycle operates in fast-twitch and slow-twitch red muscle, but not in white muscle (Rennie *et al.*, 1976; Maizels *et al.*, 1977; Pearce & Connett, 1980). Some fast-twitch red muscles such as the red portion of the gastrocnemius have to be excised before freeze-clamping. To determine the effect of cutting the muscle on its metabolic profile, we compared metabolite contents in the gastrocnemius freeze-clamped *in situ* with those of the same muscle freeze-clamped immediately after it had been excised. The contents of glucose 6-phosphate (205 ± 57 and 621 ± 32 nmol/g *in situ* and after excision respectively), fructose 6-phosphate (37 ± 11 and 161 ± 21 nmol/g), pyruvate (37 ± 5 and 138 ± 22 nmol/g) and lactate (1021 ± 79 and 1575 ± 245 nmol/g) were significantly increased in the excised muscle, suggesting an acceleration of both glycogenolysis and glycolysis. In contrast, concentrations of citrate, ATP, phosphocreatine and the [lactate]/[pyruvate] ratio were unchanged. On the basis of these findings, metabolite concentrations were determined only in the soleus, a slow-twitch red muscle that can be freeze-clamped *in situ* without cutting its fibres.

Effects of starvation and diabetes in non-exercised rats

Starvation and diabetes cause substantial increases in plasma ketone bodies and non-esterified fatty acids, and in both situations the glucose–fatty acid cycle has been demonstrated in heart (Randle *et al.*, 1964; Newsholme & Randle, 1964). The effects of 48 h of starvation on the metabolite profile of the soleus and of the heart are shown in Table 1. In keeping with the findings of others (see the Introduction), starvation caused significant increases in the concentrations of glycogen and citrate, a decrease in fructose 1,6-bisphosphate and an increase in the fructose 6-phosphate/fructose 1,6-bisphosphate ratio in the heart.

Table 1. Effect of 48 h of starvation on metabolite and glycogen concentrations in soleus muscle and heart

Values are means $\pm$ S.E.M. for five to ten observations per group. Data for soleus muscle and in heart were obtained in different experiments. Results for glycogen, ATP and phosphocreatine are in $\mu\text{mol/g}$; all others are nmol/g . *Value significantly different from that of the fed group, at $P < 0.05$.

Metabolite	Soleus		Heart	
	Fed	Starved	Fed	Starved
Glycogen	23 $\pm$ 1	15 $\pm$ 1*	26 $\pm$ 2	36 $\pm$ 3*
Citrate	177 $\pm$ 20	151 $\pm$ 17	245 $\pm$ 21	466 $\pm$ 75*
Glucose 6-phosphate	137 $\pm$ 19	95 $\pm$ 11*	445 $\pm$ 30	426 $\pm$ 32
Fructose 6-phosphate	17 $\pm$ 3	9 $\pm$ 2*	64 $\pm$ 9	68 $\pm$ 6
Fructose 1,6-bisphosphate	11 $\pm$ 1	9 $\pm$ 1	29 $\pm$ 5	17 $\pm$ 2*
Fructose 6-phosphate/fructose 1,6-bisphosphate ratio	1.6 $\pm$ 0.3	1.0 $\pm$ 0.2	2.2 $\pm$ 0.3	5.4 $\pm$ 0.9*
ATP	3.4 $\pm$ 0.1	3.0 $\pm$ 0.3	3.4 $\pm$ 0.2	3.4 $\pm$ 0.2
Phosphocreatine	9.6 $\pm$ 0.6	9.3 $\pm$ 0.5	4.3 $\pm$ 0.4	3.5 $\pm$ 0.4

For reasons noted in the Methods section, glucose 6-phosphate and fructose 6-phosphate contents were artefactually high. In contrast, in the soleus (Table 1), starvation failed to increase citrate or glycogen; in fact, glycogen, glucose 6-phosphate and fructose 6-phosphate concentrations were lower than in fed rats. The fructose 6-phosphate/fructose 1,6-bisphosphate ratio and ATP and phosphocreatine contents were unchanged. A similar pattern was noted in the soleus of rats made diabetic 2 days previously by the administration of streptozotocin (65 mg/kg body wt., intravenously). Glycogen was 22.1 ± 0.8 and $18.5 \pm 1.1 \mu\text{mol/g}$ and citrate 215 ± 10 and $240 \pm 20 \text{ nmol/g}$ in control and diabetic rats respectively. Glucose 6-phosphate, fructose 6-phosphate and fructose 1,6-bisphosphate contents were not significantly altered by diabetes.

Effect of exercise in starved rats: swimming

As there was no evidence for the glucose–fatty acid cycle in resting skeletal muscle, the possibility that it might operate during or after exercise was next examined. In preliminary experiments we assessed the effect of 1 h of swimming on the metabolite pattern and glycogen content of the soleus in 48 h-starved rats. As shown in Table 2, the expected post-exercise increases in β -hydroxybutyrate, non-esterified fatty acids and lactate were quite marked in these rats, whereas plasma glucose was decreased. Concurrent with those changes, the concentrations of citrate, glucose 6-phosphate and fructose 6-phosphate in the soleus were increased 2–3-fold and the concentration of fructose 1,6-bisphosphate was, if anything, diminished. Thus, after exercise in starved rats, intramuscular metabolite changes are consistent with a block at phosphofructokinase. Also, glycogen was not diminished, as we have found it to be in fed rats after a swim (30 ± 2 and $18 \pm 2 \mu\text{mol/g}$ at rest and post-swimming respectively).

Effect of exercise in starved rats: moderately intense treadmill running

Circulating fuels. In an attempt to confirm and extend these findings, starved rats were studied after treadmill running. This type of exercise is more easily standardized than is swimming, and its effects on glycogen depletion and repletion in fed rats have been characterized in this

laboratory (Richter *et al.*, 1982a; Garetto *et al.*, 1984). Fed and starved rats were compared at various times after the completion of moderately intense exercise. As shown in Table 3, in 48 h-starved rats changes in plasma metabolites immediately after running were qualitatively similar to those seen after swimming. The two groups differed in that the increases in non-esterified fatty acids were greater after swimming. Plasma lactate showed a transient increase, as it did after swimming, and plasma insulin a transient decrease. In contrast with these changes in starved rats, in fed rats blood glucose and lactate were not altered even immediately after exercise, and β -hydroxybutyrate was only slightly increased. Insulin concentrations were higher than in the starved rats; but, as in the latter, they tended to be decreased

Table 2. Effect of 1 h swimming on metabolite concentrations in plasma and soleus muscles of 48 h-starved rats

Values are means $\pm$ S.E.M. for six to 20 observations per group. Rats swam for 1 h with a weight (4% of body wt.) tied to their tails in water maintained at 33–35 °C. They were anaesthetized immediately after exercise. Plasma metabolites are expressed in $\mu\text{mol/ml}$ and soleus metabolites in nmol/g , except for ATP and phosphocreatine, which are in $\mu\text{mol/g}$. *Value significantly different from that of the resting group, at $P < 0.05$.

	Rest	Post-exercise
Plasma		
Glucose	5.2 $\pm$ 0.4	4.4 $\pm$ 0.4*
Lactate	1.0 $\pm$ 0.2	2.6 $\pm$ 0.4*
Non-esterified fatty acids	0.37 $\pm$ 0.05	1.89 $\pm$ 0.12*
β -Hydroxybutyrate	0.74 $\pm$ 0.10	1.57 $\pm$ 0.10*
Soleus		
Glycogen	18 $\pm$ 2	17 $\pm$ 3
Citrate	231 $\pm$ 21	440 $\pm$ 20*
Glucose 6-phosphate	90 $\pm$ 20	287 $\pm$ 50*
Fructose 6-phosphate	25 $\pm$ 5	64 $\pm$ 11*
Fructose 1,6-bisphosphate	25 $\pm$ 4	15 $\pm$ 4
ATP	3.1 $\pm$ 0.1	3.5 $\pm$ 0.1
Phosphocreatine	8.8 $\pm$ 0.9	10.3 $\pm$ 1.0

Table 3. Plasma metabolite and insulin concentrations after moderately intense treadmill exercise in fed and 48 h-starved rats

Results are means $\pm$ S.E.M. for five to ten observations per group. Rats ran for 43 min at 18 m/min and then for 2 min at 36 m/min. Metabolites are expressed in μ mol/ml and insulin is in μ units/ml. *Value significantly different from that of the resting group, at $P < 0.05$.

		Time after exercise (min)	
	Rest	0	30
Fed			
Glucose	6.8±0.4	6.8±0.2	6.8±0.2
Lactate	2.4±0.1	2.4±0.1	2.3±0.2
β-Hydroxybutyrate	0.17±0.02	0.22±0.02	0.20±0.05
Non-esterified fatty acids	0.36±0.05	0.38±0.04	0.43±0.06
Insulin	49±7	35±5	40±4
Starved			
Glucose	6.1±0.3	5.4±0.2*	5.8±0.4
Lactate	1.5±0.2	2.3±0.4*	1.8±0.6
β-Hydroxybutyrate	0.45±0.06	1.17±0.25*	0.86±0.24
Non-esterified fatty acids	0.43±0.04	0.56±0.06	0.48±0.10
Insulin	20±2	13±1*	20±4

Table 4. Glycogen concentrations in muscles and liver after moderately intense treadmill exercise in fed and 48 h-starved rats

Results are means $\pm$ S.E.M. for five to ten observations per group. See the legend to Table 3 for details. Tissue glycogen is expressed in μ mol/g. *Value significantly different from that of the resting group, at $P < 0.05$.

		Time after exercise (min)	
	Rest	0	30
Fed			
Soleus	23±1	15±1*	23±1
Red gastrocnemius	28±1	23±1*	27±2
White gastrocnemius	33±2	33±3	30±1
Heart	26±2	32±3	31±3
Liver	209±10	122±18*	154±17*
Starved			
Soleus	15±1	12±1	14±1
Red gastrocnemius	23±1	23±1	27±2
White gastrocnemius	29±2	34±2	32±2
Heart	36±2	26±2*	32±5
Liver	26±2	11±4*	12±4*

somewhat immediately after exercise. In both fed and starved groups, plasma metabolites were not significantly different from resting values 30 min after the cessation of the exercise.

Muscle and liver glycogen. The finding that an artificial increase in plasma non-esterified fatty acids diminishes muscle glycogen depletion during exercise has been used as evidence that the glucose-fatty acid cycle operates in skeletal muscle of exercising humans and rodents (Rennie

Table 5. Metabolite and glycogen concentrations in soleus muscle after moderately intense treadmill exercise

Results are means $\pm$ S.E.M. for four to ten observations. See legends to Tables 3 and 4 for details. Fructose 6-phosphate/fructose 1,6-bisphosphate ratios are calculated only in muscles in which both measurements were available. This accounts for occasional discrepancy between ratio and absolute values in the Table. Results are expressed as nmol/g, except for glycogen, which is in μ mol/g. *Value significantly different from that of the resting groups, at $P < 0.05$.

	Rest	Time after exercise (min)	
		0	30
Fed			
Citrate	177 ± 20	223 ± 5	159 ± 21
Glucose 6-phosphate	137 ± 19	261 ± 45*	192 ± 31
Fructose 6-phosphate	17 ± 3	41 ± 7*	33 ± 8
Fructose 1,6-bisphosphate	11 ± 1	25 ± 7*	19 ± 6
Fructose 6-phosphate/ fructose 1,6-bisphosphate ratio	1.6 ± 0.3	1.6 ± 0.5	2.2 ± 0.7
Starved			
Citrate	151 ± 17	247 ± 15*	208 ± 25*
Glucose 6-phosphate	95 ± 11	134 ± 17*	62 ± 13
Fructose 6-phosphate	9 ± 2	20 ± 3*	8 ± 2
Fructose 1,6-bisphosphate	9 ± 1	12 ± 2	6 ± 2
Fructose 6-phosphate/ fructose 1,6-bisphosphate ratio	1.0 ± 0.2	2.2 ± 0.5*	1.6 ± 0.4

et al., 1976; Costill *et al.*, 1977; Hickson *et al.*, 1977). In keeping with such a notion, treadmill exercise caused significant decreases in the glycogen content of the soleus and the red portion of the gastrocnemius in fed rats, but not in starved rats (Table 4). Almost the reverse of this pattern occurred in the heart, however. Cardiac glycogen was unchanged or slightly increased immediately after exercise in the fed group, but it was significantly diminished in starved rats. The common denominator in these events might be the initial glycogen content, since at rest cardiac glycogen is higher in starved rats, whereas skeletal-muscle glycogen is higher in fed rats.

As noted previously, after treadmill exercise of the intensity used (Richter *et al.*, 1982a; Garetto *et al.*, 1984), glycogen repletion was nearly complete within 30 min of the cessation of the exercise in the soleus and red gastrocnemius of fed rats, and by 2 h glycogen contents were higher than those found in non-exercised rats (results not shown). The white portion of the gastrocnemius showed no decrease in glycogen in either fed or starved rats, presumably because it is not recruited during moderately intense running (Baldwin *et al.*, 1973; Richter *et al.*, 1982a). One tissue in which glycogen decreased during exercise in both fed and starved rats was the liver. In fed rats liver glycogen decreased by nearly 50% (Table 4) and it took longer to become repleted than it did in muscle. Thus, 120 min after the cessation of the run, liver glycogen was still 21% lower than in non-exercised rats (results not shown). Although liver glycogen was already depleted in the 48 h-starved rats

Table 6. Cardiac citrate and glycogen contents before and immediately after moderately intense exercise

Results are means $\pm$ S.E.M. for five to seven observations per group. See legends to Tables 4 and 5 and the Methods section for details.

	Rest	Post-exercise
Fed		
Citrate	245 $\pm$ 21	350 $\pm$ 10*
Glycogen	31 $\pm$ 3	34 $\pm$ 2
Starved		
Citrate	466 $\pm$ 75	749 $\pm$ 78*
Glycogen	41 $\pm$ 4	28 $\pm$ 2*

(12% of that in fed rats), it was decreased by an additional 57% after the treadmill run. Interestingly, repletion to the concentrations found in non-exercised starved rats was completed by 60 min.

Tissue metabolites after exercise: the soleus. In starved rats the metabolite profile of the soleus shortly after the cessation of the treadmill run (zero time) was similar to that observed in starved rats after swimming (Table 5). Thus citrate, glucose 6-phosphate and fructose 6-phosphate were all significantly increased, as was the fructose 6-phosphate/fructose 1,6-bisphosphate ratio. Likewise, both ATP and phosphocreatine were not significantly different from resting values (results not shown). In general, these changes were relatively short-lived, although by 30 min after exercise muscle citrate was still significantly increased.

In fed rats, citrate was increased slightly and insignificantly immediately after exercise, in parallel with increases in circulating lipid fuels (Table 3). By 30 min after exercise, all tissue metabolite contents were identical with those of the non-exercised group. For reasons unknown, tissue concentrations of hexose phosphates showed a biphasic pattern. They increased immediately after exercise, decreased during the next 30–60 min, and then increased again (results not shown). These changes occurred without alteration in the fructose 6-phosphate/fructose 1,6-bisphosphate ratio, suggesting that they were unrelated to inhibition or deinhibition of phosphofructokinase.

Citrate in heart. Owing to the different pattern of glycogen depletion during exercise in the heart and the soleus (Table 4), a second series of experiments was carried out to confirm these findings and to examine the effect of exercise on cardiac citrate content. As shown in Table 6, the pattern of glycogen depletion in heart was identical with that observed in the above study, i.e. glycogen content was unchanged by exercise in fed rats, whereas in starved rats exercise caused a 30% decrease. In contrast, after exercise citrate was increased in hearts of both fed and starved rats, although the absolute increase was 3-fold greater in the starved group. Thus in heart there was a close relationship between plasma concentrations of non-esterified fatty acids and ketone bodies and muscle citrate concentrations in the post-exercise state, but no apparent relationship between plasma lipids and sparing of glycogen during exercise.

DISCUSSION

The object of this study was to determine under what conditions non-esterified fatty acids and ketone bodies inhibit glycolysis and enhance glycogen synthesis in rat skeletal muscle *in vivo*. Our approach was indirect in that rates of glycolysis and glycogen synthesis were not directly measured. On the other hand, changes in tissue contents of citrate, hexose phosphates and glycogen are quite characteristic in heart when the glucose–fatty acid cycle is operative; therefore it seemed warranted to base conclusions on whether similar changes occurred in skeletal muscle.

The first studies were performed in non-exercised rats. When circulating contents of non-esterified fatty acids and ketone bodies were increased by 48 h of starvation or the induction of diabetes with streptozotocin, no evidence for the glucose–fatty acid cycle was found. In contrast, heart muscle from the starved rats displayed a doubling of citrate content, changes in hexose phosphates consistent with inhibition of phosphofructokinase, and increased glycogen. The pattern was very similar to that noted by others in hearts of starved (Randle *et al.*, 1963, 1964; Newsholme & Randle, 1964; Randle, 1966; Neely *et al.*, 1969, 1970) and diabetic animals (Newsholme & Randle, 1964; Chen *et al.*, 1984). The reason for this difference between heart and skeletal muscle is unknown. One possible factor is the greater enzymic capacity of heart to oxidize lipids (Winder *et al.*, 1974; Holloszy *et al.*, 1973). Another is that the heart is a continuously contracting muscle with a high rate of glycolysis (Randle *et al.*, 1964; Randle, 1966) compared with perfused skeletal muscle (Ruderman *et al.*, 1971; Goodman *et al.*, 1974; Berger *et al.*, 1976) and presumably non-exercising muscle *in vivo* (Ruderman *et al.*, 1971; Wahren *et al.*, 1971). In keeping with this notion, the glucose–fatty acid cycle has been demonstrated in brain (Ruderman *et al.*, 1974), mammary tissue (Hawkins & Williamson, 1972; Williamson *et al.*, 1974; Robinson & Williamson, 1977) and submaxillary glands (Thompson & Williamson, 1975), all of which have high rates of glycolysis and glucose oxidation. Likewise it has been observed in the incubated soleus (Maizels *et al.*, 1977; Pearce & Connett, 1980), which, unlike perfused muscle, has a high rate of glycolysis. More direct evidence for the role of glycolysis was presented by Ruderman *et al.* (1978), who found that acetoacetate failed to increase citrate in the incubated soleus when the glycolytic rate was diminished by omitting glucose from the medium. In addition, citrate could be increased by acetoacetate in the absence of glucose if pyruvate was added to the incubation medium. Ruderman *et al.* (1978) hypothesized that the pyruvate generated by glycolysis could allow for increased oxaloacetate formation by enhancing the deamination of amino acids such as aspartate and glutamate and/or that the more reduced cytosolic redox state produced by glycolysis would allow for more rapid entrance of C₄ precursors of citrate into the mitochondria.

The second condition in which the glucose–fatty acid cycle was examined was exercise. As muscle glycogen depletion is associated with the onset of fatigue, previous investigators have examined the effect of artificially increasing plasma non-esterified fatty acids by a fat meal plus heparin on the exercise-induced depletion of glycogen in skeletal muscle. Such procedures have been shown to diminish muscle glycogen depletion and

increase endurance during exercise in both humans and rodents (Rennie *et al.*, 1976; Costill *et al.*, 1977; Hickson *et al.*, 1977). Interpretation of these results is complicated, because such feeding regimens tend to increase plasma insulin. Also they result in the conservation of hepatic glycogen, even though the glucose-fatty acid cycle is not thought to operate in liver (Start & Newsholme, 1968). For this reason we examined the effect of exercise in rats in which plasma non-esterified fatty acid and ketone bodies were increased by starvation. Exercise caused less, if any, glycogen depletion in red muscles of 48 h-starved rats than it did in fed controls, in keeping with the findings of others (Dohm *et al.*, 1983). Pre-exercise glycogen contents were lower in the starved group, however. It has repeatedly been demonstrated that there is an inverse correlation between the concentrations of glycogen and the percentage of glycogen synthase I in muscle (Danforth, 1965; Conlee *et al.*, 1978), probably as a consequence of glycogen-induced inhibition of glycogen synthase phosphatase (Villar-Palasi, 1969). Whether this and/or the glucose-fatty acid cycle is/are responsible for the conservation of muscle glycogen in the starved rats during exercise cannot be determined from the data. If anything, changes in cardiac glycogen content suggest that the glucose-fatty acid cycle is not the dominant factor. Thus, in contrast with skeletal muscle, cardiac glycogen content was low in the fed rat and did not decrease during moderately intense exercise, whereas in the starved rat initial contents of glycogen were 30–40% higher than in the fed group, and exercise caused them to diminish. Thus conservation of glycogen in the heart appeared to be related to its initial glycogen concentration rather than to circulating concentrations of lipids.

The third situation in which the glucose-fatty acid cycle in muscle was examined was the post-exercise state. This is hypothetically a likely time for it to operate, since hyperketonaemia, increased concentrations of non-esterified fatty acids (reviewed by Koeslag, 1982) and rapid repletion of muscle glycogen (Garetto *et al.*, 1984) characterize this period. The metabolite changes in the soleus, particularly in the starved rat, are consistent with inhibition of phosphofructokinase. Cardiac citrate content demonstrated a similar pattern of increase in fed and starved rats. The physiological relevance of these changes remains to be determined, as even in the soleus of the starved rat they were relatively short lived. For this reason it will be of especial interest to repeat these studies after prolonged intense exercise, when glycogen depletion is more marked and plasma contents of non-esterified fatty acids and ketone bodies may be elevated for several hours (Johnson *et al.*, 1969; Poland *et al.*, 1980). Definitive proof that the glucose-fatty acid cycle is operative in the post-exercise state will require simultaneous measurement of glucose uptake and disposition and muscle metabolites *in vivo*.

In man, the role of the glucose-fatty acid cycle in the regulation of glucose homeostasis in general and muscle glucose metabolism in particular is also unclear. Some investigators have observed decreased glucose tolerance after fat infusion in normal subjects (Nestel *et al.*, 1964; Schall & Kipnis, 1965; Balasse & Neef, 1974), but others have not (Pelkonen *et al.*, 1968). Increases in citrate and glucose 6-phosphate in human muscle biopsies have been observed immediately after exercise (Essen, 1977), suggesting activation of the cycle in this situation. Studies in humans by the insulin-glucose clamp technique have

demonstrated that an increase in plasma non-esterified fatty acids inhibits glucose utilization in peripheral tissues (Thiebaud *et al.*, 1982; Ferrannini *et al.*, 1983). Such inhibitory effects of non-esterified fatty acids were not observed in the presence of hypoinsulinaemia, when glycolysis in skeletal muscle would presumably be minimal. Glucose utilization and disposition by muscle were not specifically investigated, however.

In summary, the results indicate that a metabolic profile compatible with operation of the glucose-fatty acid cycle does not occur in the soleus muscle of a starved rat at rest, but it does, albeit transiently, in the post-exercise state. Starvation is associated with a conservation of glycogen in the soleus during exercise; however, it is unclear whether this is due to the glucose-fatty acid cycle, initially lower glycogen contents or other factors. These results contrast with findings in cardiac muscle, in which the glucose-fatty acid cycle is operative in the starved rat both at rest and after exercise. They suggest that the glucose-fatty acid cycle can occur in skeletal muscle *in vivo*, but that it does so under selective conditions.

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