A Sparing Effect of Increased Plasma Fatty Acids on Muscle and Liver Glycogen Content in the Exercising Rat

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Increasing plasma free fatty acids decreased the degree of glycogen depletion, and increased the citrate concentration, in slow-red (soleus) and fast-red (deep portion of vastus lateralis) muscle during exercise (approx. 50% depletion of glycogen, as against 75% in control animals). There was no effect in fast-white muscle (superficial portion of vastus lateralis). Glycogen concentration in the liver decreased by 83% in controls, but only by 23% in animals with increased free fatty acids during exercise. The decreased glycogen depletion may be partly explained by the findings that (a) plasma-insulin concentration was two- to three-fold higher in animals with increased plasma free fatty acids and (b) the exercise-induced increase in plasma glucagon was lessened by increased free fatty acids. Blood glucose was higher in the animals with increased free fatty acids after the exercise. The rats with increased plasma free fatty acids utilized approx. 50% as much carbohydrate as did the controls during the exercise.

Glucose uptake, glycolysis, glycogenolysis and pyruvate oxidation are inhibited in isolated beating hearts by the inclusion of long-chain fatty acids in the perfusion medium (Garland *et al.*, 1962, 1964; Newsholme & Randle, 1964; Randle *et al.*, 1964; Neely *et al.*, 1969, 1970). By contrast, studies on voluntary-muscle fibres incubated *in vitro* and on perfused rat hemicorpus or hindquarter preparations have not shown an inhibitory effect of fatty acids on glucose metabolism (Beatty & Bocek, 1971; Goodman *et al.*, 1974; Jefferson *et al.*, 1972; Reimer *et al.*, 1975). These findings led Reimer *et al.* (1975) to conclude that the mechanism proposed by Randle *et al.* (1963), in which availability of fatty acids suppresses carbohydrate utilization, is confined to heart muscle and does not occur in skeletal muscle. However, the inability to demonstrate an effect in these studies may have been due to factors other than a fundamental difference between skeletal and heart muscle. The effects of fatty acids on carbohydrate utilization in the heart appear to depend on their increased oxidation (Neely & Morgan, 1974). Since resting skeletal muscle has a low metabolic rate, increasing the availability of fatty acids may not increase the rate of fatty acid oxidation sufficiently to produce readily measurable effects on glucose utilization. Further, any effect mediated by oxidation of fatty acids might be technically difficult to measure in preparations such as the rat hindquarter, in which approx. 50% of the skeletal-muscle fibres are white (Ariano *et al.*, 1973); white muscle contains few mito-

chondria and has a very low capacity to oxidize fatty acids (Baldwin *et al.*, 1972).

There is, however, indirect evidence that, as in the heart, carbohydrate utilization in contracting red skeletal muscle is subject to regulation by the availability of fatty acids. Red-muscle fibres, which have a high respiratory capacity (Baldwin *et al.*, 1972; Winder *et al.*, 1974), are preferentially recruited, and do most of the work during exercise of light or moderate intensity, whereas the white fibres are not recruited until exercise becomes strenuous (Baldwin *et al.*, 1973b; Armstrong *et al.*, 1974; Henneman, 1974).

During prolonged exercise of moderate intensity there is a progressive increase in plasma free fatty acid concentration (Costill *et al.*, 1971b; Paul, 1971; Rennie & Johnson, 1974) and a decrease in respiratory quotient (Edwards *et al.*, 1934; Christensen & Hansen, 1939; Costill *et al.*, 1971a; Paul, 1971), indicating a change in the carbon source for oxidation from carbohydrate to fat. The decrease in respiratory quotient is evident long before glycogen stores are depleted (Edwards *et al.*, 1934; Ahlborg, 1967; Costill *et al.*, 1971a). These observations suggest that the increase in plasma free fatty acid concentration during exercise results in increased oxidation of fatty acids in the working red muscle fibres and inhibition of carbohydrate utilization.

In the present study we have re-evaluated the effect of increased availability of fatty acids on the utilization of carbohydrate in skeletal muscle. We have used

exercising rats in which plasma free fatty acids were increased by feeding with a fat meal followed by administration of heparin. Information has been obtained on the effect of increased plasma free fatty acids on hepatic glycogen depletion during exercise.

Experimental

Treatment of animals

Male specific-pathogen-free Wistar rats weighing 100–120 g (Hilltop Laboratory Animals, Scottsdale, PA, U.S.A.) were caged in pairs and fed on a diet of Purina chow and water *ad libitum*. The animal room was maintained at a temperature between 18 and 20°C, and was lit between 07:00 and 19:00 h. Beginning with short daily sessions at low speeds, the animals were taught to run on a treadmill (Quinton 42-15, Quinton Instruments, Seattle, WA, U.S.A.) so that within 10 days they became accustomed to running for 10 min daily at 1.3 km/h up a 15% gradient. The animals were then maintained at this work rate, which has previously been found not to cause muscle hypertrophy or to result in any appreciable increase in muscle respiratory capacity (Holloszy, 1967).

After 6 weeks the rats were separated into two groups matched for body weight; at this time the animals' body weights ranged from 280 to 320 g. One group, in which plasma free fatty acid concentrations were to be elevated, was given 5 ml of corn oil by stomach tube at 07:30 h. At 10:30 h, when their plasma was visibly lipaemic, these animals were given 200 units of sodium heparin (Sigma Chemical Co., St Louis, MO, U.S.A.) subcutaneously. The other animals, which served as controls, were given 5 ml of CM-cellulose (Sigma) (which is not digested or absorbed) in an aqueous solution (5%, w/v) by stomach tube at 07:30 h; at 10:30 h these control animals were injected with 0.9% NaCl subcutaneously. Both groups were subdivided into groups of resting and exercising animals. At 10 min after the injection of heparin or NaCl some of the animals were placed on the treadmill, which was then operated for 15 min at 1.3 km/h with a 15% gradient, then for 10 min at 1.6 km/h with a 17.5% gradient, and finally for 5 min at 1.9 km/h with a 20% gradient. Animals were removed from the treadmill after either 15, 25 or 30 min of running.

Sampling of muscle, liver and blood

Immediately after the end of exercise the runners were anaesthetized with sodium pentobarbital (6 mg/100 g body wt.) injected intraperitoneally. Resting animals were anaesthetized either 10 min or 40 min after injection of heparin (elevated free fatty acid group) or NaCl (control group). As soon as the anaesthetic took effect (3–5 min), muscles were ex-

cised from one hindlimb and clamp-frozen between aluminium blocks cooled in liquid N₂ (Wollenberger *et al.*, 1960). The muscles taken were, in order: the soleus, which consists mainly of slow-twitch red fibres; the superficial portion of the vastus lateralis, which consists of fast-twitch white fibres, and the deep portion of the vastus lateralis, which consists predominantly of fast-twitch red fibres (Baldwin *et al.*, 1972; Ariano *et al.*, 1973). The abdominal cavity was opened, and a portion of the liver was clamp-frozen. Samples were stored at –70°C until they were analysed.

Blood was drawn from the aorta into a heparinized syringe. A 0.5 ml portion was deproteinized by addition to 1 ml of ice-cold 10% (w/v) HClO₄; the acid extract was separated by centrifugation and frozen. A 2 ml portion of blood was added to a tube containing 0.1 ml of aprotinin solution (Trasylol, 10000 kallikrein-inactivating units/ml; FBA Pharmaceuticals, New York, NY, U.S.A.), to prevent proteolysis of glucagon. Plasma was separated by centrifugation at 1000 g for 10 min and frozen.

Analytical methods

Glycogen in muscle and liver was measured by the anthrone method (Hassid & Abraham, 1957). HClO₄ extracts of muscle and liver samples were prepared, and neutralized with KOH (Williamson & Corkey, 1969), for determination of citrate (Passonneau & Brown, 1974), lactate (Hohorst, 1965) and glucose 6-phosphate (Slein, 1965).

Samples of the blood extract were used for measurement of glucose (Slein, 1965), lactate (Hohorst, 1965) and 3-hydroxybutyrate (Williamson *et al.*, 1962).

Plasma free fatty acids were determined by a microcolorimetric method (Noma *et al.*, 1973).

Plasma immunoreactive glucagon was assayed by using the 30K antibody (Faloona & Unger, 1974); pig glucagon (Eli Lilly Co., Indianapolis, IN, U.S.A.) was used as the standard. Radio-iodinated glucagon was purchased from Nuclear Medical Laboratories (Dallas, TX, U.S.A.). Plasma immunoreactive insulin was assayed by a single-antibody technique (Herbert *et al.*, 1965); rat insulin (obtained as a gift from Eli Lilly Co.) was used as the standard. Insulin antiserum was obtained from Biotek Labs. (St. Louis, MO, U.S.A.). Radioiodinated insulin was obtained from New England Nuclear Corp. (Boston, MA, U.S.A.). In both assays, dextran-coated charcoal was used for separation of bound and free hormones (Herbert *et al.*, 1965).

Results

Blood metabolites

The responses of plasma free fatty acids, blood glucose and blood lactate concentrations are shown in Table 1.

Table 1. *Effects of exercise and of increased plasma free fatty acids on blood metabolites*

Rats were fed with corn oil and then given heparin 3 h later to increase plasma free fatty acids; control rats were given CM-cellulose and injected with 0.9% NaCl 3 h later (see the Experimental section for details). Blood was taken from some resting animals 10 min after injection of heparin or NaCl (column 3), and from other resting rats 40 min after injection (column 7). The 'exercised' animals began running on the treadmill 10 min after injection of heparin or NaCl. Values are means $\pm$ S.E.M. The number of animals per group is given in parentheses.

		Resting*	15 min exercise	25 min exercise	30 min exercise	Resting†
Plasma free fatty acids (mM)	Control	0.33 $\pm$ 0.05 (5)	0.24 $\pm$ 0.02 (4)	0.60 $\pm$ 0.09 (4)	0.54 $\pm$ 0.06 (13)	0.46 $\pm$ 0.01 (11)
	Raised [free fatty acid]	0.71 $\pm$ 0.04‡ (5)	0.86 $\pm$ 0.05‡ (4)	1.49 $\pm$ 0.32‡ (4)	1.10 $\pm$ 0.13‡ (14)	2.03 $\pm$ 0.34‡ (11)
Blood glucose (μ mol/ml)	Control	5.50 $\pm$ 0.38 (5)	5.75 $\pm$ 0.32 (4)	5.20 $\pm$ 0.44 (4)	3.20 $\pm$ 0.30 (13)	4.97 $\pm$ 0.32 (11)
	Raised [free fatty acid]	5.30 $\pm$ 0.47 (5)	4.90 $\pm$ 0.38‡ (4)	4.75 $\pm$ 0.48 (4)	4.40 $\pm$ 0.45‡ (14)	4.42 $\pm$ 0.26 (11)
Blood lactate (μ mol/ml)	Control	1.97 $\pm$ 0.27 (5)	2.51 $\pm$ 0.44 (4)	3.79 $\pm$ 0.85 (4)	4.40 $\pm$ 0.43 (13)	1.87 $\pm$ 0.18 (11)
	Raised [free fatty acid]	1.89 $\pm$ 0.25 (5)	2.73 $\pm$ 0.92 (4)	3.29 $\pm$ 0.35 (4)	3.50 $\pm$ 0.42‡ (14)	1.85 $\pm$ 0.26 (11)

* Blood was taken from these animals at the time the exercised rats began running.

† Blood was taken from these animals at the same time as from the 30 min exercisers.

‡ Raised [free fatty acid] versus control, $P < 0.01$ (Student's t test).

Free fatty acids. Administration of heparin to the corn-oil-fed animals resulted in an increase in plasma free fatty acid concentration to more than twice the control value after 10 min and to more than four times the control value after 40 min in the resting animals. In the exercising animals, plasma free fatty acid values were more than twice as high in the corn-oil/heparin-treated group as in the controls at all three time-points. However, the exercise prevented plasma free fatty acid concentration from attaining as high a value as that found in the resting animals 40 min after heparin injection (1.1 mM, cf. 2.0 mM, $P < 0.01$).

Glucose. The exercise resulted in a marked fall in blood glucose concentration in the control animals during the last 5 min period of running ($P < 0.01$). Elevation of plasma free fatty acid concentration in the resting, corn-oil/heparin-treated animals resulted in a small (17%) decrease in blood glucose over the 30 min period. The exercise had no additional effect, above that of elevation of free fatty acid concentration alone, on blood glucose in the corn-oil/heparin-treated group. Thus the rats with increased plasma free fatty acids were protected against the hypoglycaemic effect of the exercise and had significantly higher blood glucose values than the controls after the 30 min period of running.

Lactate. The 30 min period of exercise caused a smaller rise in blood lactate concentration in the animals with increased plasma free fatty acids than in the controls.

3-Hydroxybutyrate. Blood concentrations of 3-hydroxybutyrate were not markedly elevated by the corn-oil/heparin treatment, averaging $0.37 \pm 0.11 \mu\text{mol/ml}$ in four resting rats with increased plasma free fatty acids compared with $0.23 \pm 0.06 \mu\text{mol/ml}$ in five resting control rats. After 30 min of exercise, 3-hydroxybutyrate concentration averaged $0.25 \pm 0.04 \mu\text{mol/ml}$ in four rats with increased plasma free fatty acids compared with $0.26 \pm 0.05 \mu\text{mol/ml}$ in four control rats.

Muscle metabolites

The responses of muscle glycogen, citrate and lactate are shown in Table 2.

Glycogen. There was no change in muscle glycogen concentration in the resting control animals during the 30 min period when the exercised animals were running on the treadmill. Elevation of plasma free fatty acid concentration in the resting corn-oil/heparin-treated animals had no significant effect on muscle glycogen concentration over the 30 min period. The 30 min period of running resulted in depletion of roughly three-quarters of the glycogen in all three types of muscles in the control animals. Glycogen concentration in white muscle decreased slowly during the first 25 min and then more rapidly during the last 5 min of the exercise. Since contractile activity results in rapid glycogen depletion in white muscle (Edström & Kugelberg, 1968; Armstrong *et al.*, 1974),

Table 2. *Effects of exercise and of increased plasma free fatty acids on muscle metabolites*

For a description of the experimental procedures, see Table 1 and the Experimental section. Muscle samples were obtained from some resting animals at the beginning of the exercise period and from others 30 min later; as no significant differences were seen between muscles from the two resting groups, the results were combined under the heading 'Resting'. Values are expressed per g wet wt. of muscle and are means $\pm$ S.E.M. The number of animals per group is given in parentheses.

		Glycogen (μ mol of glucose/g)		Lactate (μ mol/g)		Citrate (nmol/g)	
		Resting	30 min exercise	Resting	30 min exercise	Resting	30 min exercise
Red vastus	Control	43.4 $\pm$ 1.5 (18)	11.7 $\pm$ 0.9 (12)	2.41 $\pm$ 0.23 (13)	3.29 $\pm$ 0.35 (13)	179 $\pm$ 16 (13)	240 $\pm$ 26 (13)
	Raised [free fatty acid]	45.5 $\pm$ 1.7 (16)	21.6 $\pm$ 1.6* (14)	2.25 $\pm$ 0.22 (12)	2.65 $\pm$ 0.37* (13)	240 $\pm$ 26* (13)	310 $\pm$ 25* (13)
Soleus	Control	22.7 $\pm$ 2.7 (16)	5.1 $\pm$ 0.8 (12)	2.28 $\pm$ 0.33 (7)	2.88 $\pm$ 0.37 (7)	180 $\pm$ 25 (8)	215 $\pm$ 36 (7)
	Raised [free fatty acid]	21.4 $\pm$ 1.6 (16)	11.2 $\pm$ 2.0* (13)	2.04 $\pm$ 0.27 (6)	2.59 $\pm$ 0.47 (8)	220 $\pm$ 21 (11)	326 $\pm$ 34* (8)
White vastus	Control	43.9 $\pm$ 3.8 (11)	15.1 $\pm$ 3.0 (7)	3.09 $\pm$ 0.50 (8)	7.30 $\pm$ 0.96 (6)	161 $\pm$ 22 (5)	148 $\pm$ 59 (5)
	Raised [free fatty acid]	46.0 $\pm$ 3.3 (11)	19.4 $\pm$ 1.8 (8)	2.68 $\pm$ 0.20 (6)	6.23 $\pm$ 1.11 (6)	168 $\pm$ 37 (5)	163 $\pm$ 58 (5)

* Raised [free fatty acid] versus control, $P < 0.01$.

this finding provides evidence that the white portion of the vastus lateralis contracted infrequently during the first 25 min of exercise when work intensity was mild to moderate.

Elevation of plasma free fatty acid concentration decreased the degree of depletion of glycogen in the fast-red and slow-red types of muscle, so that after 30 min of running there was twice as much glycogen remaining in the soleus and in red portion of the vastus lateralis muscle of the corn-oil/heparin-treated animals as in those of the controls. The raised plasma free fatty acid concentrations did not affect glycogen depletion in the white portion of the vastus lateralis.

Lactate. The concentration of lactate rose only slightly in the red portion of the vastus lateralis and in the soleus during the exercise, but more than doubled in the white portion of the vastus lateralis. Elevation of plasma free fatty acid concentration did not significantly affect this increase in lactate in the white fibres. These findings suggest that the capacity for oxidative metabolism of carbohydrate was exceeded in white muscle but not in the red types of muscle. Most of the lactate accumulation in white muscle (from 3.28 to 6.76 μ mol/g; mean values for both groups combined) occurred during the last 5 min of exercise, when the work rate was highest.

Citrate. Exercise alone and elevation of plasma free fatty acid concentration alone resulted in small increases in citrate concentration in the soleus and the red portion of the vastus lateralis. Increased plasma free fatty acid concentrations accentuated the

effects of exercise, so that citrate concentrations were significantly higher in the red muscles of the corn-oil/heparin-treated animals than in those of the controls, after the exercise.

Liver metabolites

The responses of muscle glycogen, lactate and citrate are shown in Table 3.

Glycogen. The 30 min of exercise caused an 83% decrease in liver glycogen content in the control rats. Elevation of plasma free fatty acid concentration decreased the depletion of liver glycogen to only 23% in the corn-oil/heparin-treated animals during the exercise.

Lactate. The exercise resulted in increases in lactate concentration in the liver in both groups of animals. This increase was greater in the controls, but the difference between the two groups was not statistically significant.

Citrate. The concentration of citrate in the liver was approximately twice as high in the animals with increased plasma free fatty acids as in the controls after the exercise.

Determination of total carbohydrate utilization during the 30 min exercise period

The rats used in the present study, which had an average body weight of 308 $\pm$ 5 g, had livers weighing approx. 10 g and had muscle in their front and hind

Table 3. *Effects of exercise and of increased plasma free fatty acids on liver metabolites*

For a description of the experimental procedures, see Table 1 and the Experimental section. Liver samples were obtained from some resting animals at the beginning of the exercise period and from others 30 min later; as no significant differences were seen between livers from the two groups, the results were combined under the heading 'Resting'. Values are expressed per g wet wt. of liver and are means $\pm$ S.E.M. The number of animals per group is given in parentheses.

	Glycogen (μ mol of glucose/g)		Lactate (μ mol/g)		Citrate (nmol/g)	
	Resting	30 min exercise	Resting	30 min exercise	Resting	30 min exercise
Control	181 $\pm$ 17 (16)	30 $\pm$ 8 (12)	0.30 $\pm$ 0.06 (5)	1.33 $\pm$ 0.82 (5)	179 $\pm$ 14 (16)	186 $\pm$ 17 (14)
Raised [free fatty acid]	192 $\pm$ 19 (16)	148 $\pm$ 14* (14)	0.31 $\pm$ 0.07 (5)	0.85 $\pm$ 0.11 (6)	210 $\pm$ 24 (15)	383 $\pm$ 43* (13)

* Raised [free fatty acid] versus control, $P < 0.01$.

Table 4. *Effects of exercise and of increased plasma free fatty acids on insulin and glucagon concentrations in plasma*

For a description of the experimental procedures, see Table 1 and the Experimental section. Values are means $\pm$ S.E.M. The number of animals per group is given in parentheses.

		Resting*	15 min exercise	25 min exercise	30 min exercise	Resting†
Plasma insulin (ng/ml)	Control	20.2 $\pm$ 5.8 (5)	11.1 $\pm$ 0.9 (4)	12.0 $\pm$ 1.5 (4)	5.2 $\pm$ 2.5 (5)	21.5 $\pm$ 4.2 (3)
	Raised [free fatty acid]	24.0 $\pm$ 5.2 (5)	34.6 $\pm$ 7.0‡ (4)	22.3 $\pm$ 6.7‡ (4)	16.7 $\pm$ 6.2‡ (6)	37.1 $\pm$ 6.0‡ (3)
Plasma glucagon (pg/ml)	Control	126 $\pm$ 23 (5)	252 $\pm$ 13 (4)	358 $\pm$ 26 (4)	505 $\pm$ 71 (6)	118 $\pm$ 18 (3)
	Raised [free fatty acid]	131 $\pm$ 25 (5)	158 $\pm$ 22‡ (4)	223 $\pm$ 11‡ (4)	335 $\pm$ 36‡ (6)	127 $\pm$ 34 (3)

* Blood was taken from these animals at the time the exercised rats began running.

† Blood was taken from these animals at the same time as from the 30 min exercisers.

‡ Raised [free fatty acid] versus control, $P < 0.01$.

limbs weighing about 50 g. On the basis of published information (Baldwin *et al.*, 1972; Ariano *et al.*, 1973), we estimate that this muscle mass is composed of about 40% fast-twitch red fibres (such as are found in the red portion of the vastus lateralis), 50% fast-twitch white fibres (such as are found in the white portion of the vastus lateralis), and 10% slow-twitch red fibres (such as are found in the soleus). On the basis of the assumption that the responses of the muscle samples that we analysed are representative of what happens in the three types of muscle fibre in all the muscles of the front and hind limbs, it can be estimated that the control animals used muscle glycogen equivalent to about 1450 μ mol of glucose, whereas the animals with increased plasma free fatty acids used about 1200 μ mol of glucose during the exercise.

Total liver glycogen depletion averaged 1610 μ mol of glucose in the control animals and only 440 μ mol of glucose for the animals with elevated free fatty acid concentration during the exercise. Thus the total

amount of muscle plus liver glycogen used by the control animals during the 40 min period of exercise was about 3060 μ mol of glucose, compared with a value of 1640 μ mol for the animals with increased plasma free fatty acids.

Plasma hormones

The responses of plasma insulin and glucagon are shown in Table 4.

Insulin. The exercise resulted in a marked decrease in plasma-insulin concentration in the control animals, so that after 30 min of running, plasma-insulin concentration was decreased by 75%. Elevating plasma free fatty acid concentration had the effect of increasing insulin concentration. For example, 40 min after the injection of heparin, plasma-insulin concentration in the resting corn-oil-fed animals was almost twice as high as in resting controls. As a result, at no time during the exercise did insulin values fall

significantly below the resting control concentration in the rats with increased plasma free fatty acids. After 30 min of exercise, the plasma-insulin concentration was threefold greater in the rats with a high plasma free fatty acid concentration than in the control animals.

Glucagon. Plasma glucagon was increased fourfold in the control animals by the 30 min period of exercise. The exercise-induced increase in glucagon was partially inhibited by the increase in plasma free fatty acids. As a result, the plasma glucagon concentration was significantly lower in the corn-oil/heparin-treated animals than in the controls throughout the exercise.

Discussion

The intact exercising animal has a number of advantages as a model for studying the regulation of carbohydrate utilization in skeletal muscle. The rates of glucose uptake, glycogenolysis, glycolysis, and respiration are increased manifold during contractile activity, magnifying the effects of experimental procedures on substrate utilization and making them more readily detectable than in resting muscle. The delivery of O_2 and substrates by the blood is closely geared to the metabolic rate of the working muscles by the normal functioning of the cardiovascular system under metabolic and neural control. This model can also provide information about the integrated control of carbohydrate utilization in the working muscles, and about glucose production by the liver under the influence of a normally functioning endocrine system.

An important consideration in studies of muscle metabolism is that mammalian skeletal muscles are mixtures of three fibre types which differ in their biochemical and physiological properties. In rodents there are: the fast-twitch white muscle fibres, which have a low respiratory capacity, a high glycogenolytic capacity and high myosin ATPase (adenosine triphosphatase) activity, and which fatigue quickly; the fast-twitch red fibres, which have a high respiratory capacity, a high glycogenolytic capacity and high myosin ATPase activity, and which are fatigue-resistant; and the slow-twitch red fibres, which have a moderately high respiratory capacity, a low glycogenolytic capacity and low myosin ATPase activity, and are fatigue-resistant (Edström & Kugelberg, 1968; Baldwin *et al.*, 1972, 1973a; Barnard *et al.*, 1971; Peter *et al.*, 1972; Ariano *et al.*, 1973; Winder *et al.*, 1974). Red muscle fibres, which have small motor neurons that require less excitatory input for discharge than the larger motor neurons innervating white fibres, are preferentially recruited during work of light and moderate intensity (Baldwin *et al.*, 1973b; Armstrong *et al.*, 1974; Henneman, 1974). White fibres are recruited either when the excitatory input into the motor neurons increases to high values during

very strenuous work or when red fibres become fatigued.

The liver glycogen concentration decreases whereas the blood-glucose concentration stays constant or decreases in exercising rats (Baldwin *et al.*, 1973b, 1975). Therefore if the liver-glycogen content remains constant in resting animals during the same time-interval, the decreases in liver glycogen and blood glucose can be used as a rough measure of the amount of glucose taken up by the muscles during a bout of exercise. In the present study, raising the concentration of plasma free fatty acids had a powerful effect against depletion of hepatic glycogen stores during exercise. Clearly, this inhibition of hepatic glycogenolysis would have rapidly resulted in the development of hypoglycaemia in the exercising rats with increased plasma free fatty acids if the rate of glucose transport into their muscle cells had increased as much as it apparently did in the control runners. However, blood glucose concentration was higher in the animals with increased free fatty acids than in the controls at the end of the exercise. This inhibitory effect of increased availability of fatty acids on glucose uptake by skeletal muscle is particularly impressive when one considers that plasma insulin was two to three times higher in the animals with increased free fatty acid, than in the controls.

Glycogen depletion during the exercise was slower in leg muscles of the animals with increased free fatty acids than in the controls. This inhibitory effect on glycogenolysis was seen only in the red types of muscle. It seems likely that the inhibitory effect of increased availability of fatty acids on the uptake and utilization of glucose by skeletal muscle is also limited to the red types of fibre, because the capacity of white muscle to oxidize fatty acids is very low (Baldwin *et al.*, 1972).

White muscle, which is primarily dependent on glycogenolysis for its energy supply, is recruited infrequently during exercise of the intensity used during the first 25 min of the exercise in the present study (Baldwin *et al.*, 1973b; Armstrong *et al.*, 1974). This was evidenced in the present study by the findings that (a) lactate concentration increased minimally in white muscle (from 2.88 to 3.28 $\mu\text{mol/g}$; average values for both groups combined), and (b) glycogen depletion occurred slowly in white muscle during the first 25 min of exercise. Contractile activity results in very rapid glycogen depletion in white muscle (Edström & Kugelberg, 1968; M. J. Rennie & J. O. Holloszy, unpublished work). Thus white muscle fibres probably contributed little to the total increase in substrate utilization in our exercising rats.

Since the animals in the two groups were matched for body weight and performed the same exercise, total energy expenditure must have been similar for the animals with increased free fatty acids and the controls. Therefore the finding that total glycogen

stores in the liver plus the muscles decreased approximately twice as much in the controls indicates that the animals with increased free fatty acids must have obtained a proportionally greater percentage of their energy from oxidation of fatty acids during the exercise.

The present results clearly support the interpretation that the mechanism proposed by Randle *et al.* (1963), in which availability of fatty acids inhibits carbohydrate utilization, is operative in the red types of skeletal muscle. There are two probable reasons why previous studies on preparations of voluntary muscle did not reveal this effect. One is that resting muscle was used (Beatty & Bocek, 1971; Jefferson *et al.*, 1972; Goodman *et al.*, 1974; Reimer *et al.*, 1975). However, this is probably not the entire explanation, because some investigators (Garland *et al.*, 1964; Randle *et al.*, 1964) were able to demonstrate small but significant inhibitory effects of free fatty acids on carbohydrate utilization in resting isolated rat diaphragms; other investigators (Schonfeld & Kipnis, 1968) could not show an inhibition of glucose uptake by free fatty acids in diaphragm. The rat diaphragm is a specialized red muscle with a high respiratory capacity which, in contrast with voluntary skeletal muscle, normally contracts continually. Thus it may be possible under some circumstances to demonstrate an effect of free fatty acids on glucose utilization in a preparation of resting muscle with a high respiratory capacity. However, it would be much more difficult to demonstrate such an effect in a resting preparation of mixed skeletal muscle such as the perfused rat hindquarter (Jefferson *et al.*, 1972; Goodman *et al.*, 1974; Reimer *et al.*, 1975), in which a large proportion of the fibres are white. In such a preparation, a considerable portion of the total glucose uptake would be by the white muscle fibres, and therefore would not be subject to the inhibitory effects of increased fat oxidation. By contrast, during exercise of mild to moderate intensity most of the increase in carbohydrate utilization probably occurs in the red fibres, and is therefore subject to inhibition by increased availability of fatty acids.

There is extensive evidence that, in the heart, increased oxidation of fatty acids results in the accumulation of citrate (Parmeggiani & Bowman, 1963; Garland *et al.*, 1963; Garland & Randle, 1964), which inhibits phosphofructokinase (Garland *et al.*, 1963; Passonneau & Lowry, 1963) and results in the accumulation of fructose 6-phosphate and glucose 6-phosphate (Newsholme *et al.*, 1962; Garland *et al.*, 1963; Newsholme & Randle, 1964). Free glucose also accumulates, indicating an inhibition of hexokinase, probably by glucose 6-phosphate (Randle *et al.*, 1964).

The concentrations of citrate in fast-red and slow-red muscle samples were elevated after exercise in the animals with increased plasma free fatty acids. This

finding is compatible with the interpretation that, as in the heart, the inhibition of carbohydrate utilization in red skeletal muscle by free fatty acids may be mediated in part by inhibition of phosphofructokinase by citrate. However, our results are inconclusive on this point. There is a delay of 3–5 min between the time when an animal stops running and the time at which its muscles can be excised and frozen, as it takes this long for the anaesthesia to take effect. This delay does not interfere with the evaluation of exercise-induced changes in glycogen concentration, because glycogen increases relatively slowly in muscle and liver after depletion by exercise (Terjung *et al.*, 1974). However, the concentrations of various co-factors and metabolites that change during contractile activity return to resting values by the time that the muscle can be frozen. These include ATP and creatine phosphate (Terjung *et al.*, 1972). Thus a detailed analysis of the control mechanisms involved is not possible by using the exercising animal.

Hepatic glycogenolysis and glucose release are closely matched to glucose utilization by muscle during exercise. This is evident from the finding that, except for a slight initial decrease that is often seen, blood glucose is maintained reasonably constant during exercise until liver glycogen stores are low (Baldwin *et al.*, 1973b, 1975; Rosell & Saltin, 1973). Achou & Hetenyi (1974) concluded from cross-circulation experiments that the rate at which glucose is utilized by the tissues regulates the rate of hepatic-glucose release, but the mechanism by which this control is mediated is not clear. However, although the overall control process is still not understood, it seems reasonable that the increased production of glucose by the liver during exercise is, at least in part, hormonally mediated. As shown in the present and previous studies, plasma insulin concentration decreases (Devlin, 1963; Rennie & Johnson, 1974), and glucagon increases (Böttger *et al.*, 1972), during exercise. Glucagon has a strong glycogenolytic effect on the liver (Unger & Eisentraut, 1967), whereas insulin inhibits hepatic-glucose release (Mortimore *et al.*, 1967; Exton *et al.*, 1970). Elevation of plasma free fatty acid concentration stimulates insulin release (Seyffert & Madison, 1967; Balasse & Ooms, 1973; Crespin *et al.*, 1973) and decreases glucagon secretion (Seyffert & Madison, 1967; Edwards & Taylor, 1970). In the present study, an increase in plasma free fatty acids inhibited the exercise-induced decrease in plasma insulin while lessening the increase in glucagon, and could, by these mechanisms, have slowed hepatic glycogenolysis. In addition, the finding that citrate concentration was elevated in the livers of the exercised animals with increased free fatty acids suggests that increased availability of fatty acids may also have a more direct effect on hepatic carbohydrate metabolism. There is evidence that increased citrate concentrations can inhibit glucose

6-phosphatase, and thus glucose production by liver (Nordlie & Lygre, 1966).

In conclusion, the present results show that increased availability of fatty acids inhibits carbohydrate utilization in the red types of skeletal muscle and slows hepatic glycogen depletion during exercise.

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