

Non-esterified Long-chain Fatty Acid Metabolism in Fed Sheep at Rest and During Exercise

D. W. Pethick,^A N. Harman^A and J. K. Chong^{A,B}

^A Division of Veterinary Biology, School of Veterinary Studies, Murdoch University, Murdoch, Western Australia, 6150.

^B Present address: Division of Animal Production, CSIRO, P.O. Wembley, Western Australia, 6014.

Abstract

The role of circulating, non-esterified, long-chain fatty acids (NEFA) as a source of energy for the whole animal and skeletal muscle was investigated in fed non-pregnant sheep at rest and during exercise. Infusion of tracer quantities of [$1-^{14}\text{C}$]oleic or [$1-^{14}\text{C}$]stearic acid was combined with the use of arteriovenous difference studies on fed sheep at rest or during a 2 h period of exercise on a belt treadmill moving at 4.5 km h^{-1} . At rest all parameters of NEFA metabolism indicated a minimal role for oxidation. Thus the concentration in plasma ($0.07 \pm 0.01 \text{ mmol l}^{-1}$), entry rate ($0.08 \pm 0.02 \text{ mmol h}^{-1} \text{ kg}^{-1} \text{ body wt}$), contribution to whole animal oxidation ($1.2 \pm 0.3\%$) and utilization of NEFA by skeletal muscle ($0.046 \pm 0.008 \text{ mmol h}^{-1} \text{ kg}^{-1} \text{ muscle}$) were all low. Exercise prompted a shift to lipolysis and accordingly the above parameters increased markedly some 13-24-fold. The circulating concentration of ketone bodies showed only a small increase during exercise and consequently the role of ketone bodies as an energy source during exercise was minimal. Glucose utilization by skeletal muscle was considerable in animals at rest and it represented the most significant potential fuel of skeletal muscle. Exercise resulted in a sustained increase of 3-4-fold in the utilization of glucose by skeletal muscle. Thus the traditional view that NEFA and not glucose is a predominant fuel of skeletal muscle of fed sheep should be appraised.

Introduction

Studies of metabolite utilization by the sheep hind limb using the arteriovenous difference technique have been used to quantify the utilization of acetate, glucose and ketone bodies by the skeletal muscle of fed non-pregnant sheep both at rest and during exercise (Jarrett *et al.* 1976; Bird *et al.* 1981). In addition these workers have measured the net arteriovenous difference of circulating, non-esterified, long-chain fatty acids (NEFA) across the hind limb. However, these measurements have contributed little to the understanding of NEFA utilization by skeletal muscle since isotopic techniques are necessary to measure the true uptake of NEFA. This is because of a simultaneous uptake and release of NEFA by skeletal muscle in sheep and other species (Pethick *et al.* 1983). The results of Pethick *et al.* (1983) obtained using fasted pregnant ewes suggested that NEFA might be a trivial fuel of skeletal muscle in fed non-pregnant sheep. Such a conclusion is in direct contrast to studies in other species where NEFA is thought to be a predominant fuel for skeletal muscle in most physiological states (Zierler 1976).

Consequently the overall aim of this work was to investigate the role of circulating NEFA as an energy source for the whole animal and skeletal muscle of fed non-pregnant sheep both at rest and during exercise. In addition the study was

designed to determine the time-dependent changes in the utilization of glucose, lactate, pyruvate and ketone bodies by the skeletal muscle of fed sheep during an exercise period of 2 h. Finally, this is the first study to report the utilization of nutrients by skeletal muscle in sheep during exercise using the arteriovenous technique simultaneously reported by Faichney and Hales (1974) and Domanski *et al.* (1974). This technique ensures that the venous blood being sampled is derived largely from the circulation of muscle tissue in the hind limb.

Some of the results have been presented in preliminary form (Pethick *et al.* 1984; Pethick 1985).

Materials and Methods

Animals and Diet

Sixteen non-pregnant Merino ewes weighing 35–45 kg and aged between 3 and 4 years were used. The ewes were fed 800 g of chopped lucerne (*Medicago sativa*) hay. The diet was fed semi-continuously as 12 equal portions at intervals of 2 h.

Animal Preparation

Carotid artery and jugular vein catheters were inserted under general anaesthesia at least 14 days prior to experimentation. The carotid artery was cannulated through a purse string suture at the level of the third cervical vertebra. The cannula was passed 25 cm so as the tip lay near the anterior aorta. The recurrent tarsal vein was cannulated under local anaesthesia 12–24 h before experimentation and the tip passed to the deep femoral vein. This was routinely performed by passing the cannulae to a distance of the ischiatic tuber, usually 25 cm. Immediately after experimentation the recurrent tarsal cannula was removed so as to prevent irreversible thrombosis of the vein. This allowed a second successful cannulation of the vessel. Polyethylene catheters (SP55, Dural Plastics and Engineering, Dural, N.S.W., 1.2 mm ext. diam., 0.8 mm int. diam.) were used throughout and maintained patent with heparin saline (100 i.u. ml⁻¹ in 0.9% w/v NaCl).

All sheep were housed in the room where experiments occurred for at least 3 weeks prior to experimentation and were handled regularly so that they were accustomed to the experimental procedure. Experiments involving exercise were performed on a moving-belt treadmill (not inclined) which was housed in the same room. Once trained to walk on the treadmill sheep were exercised twice weekly at 4.5 km h⁻¹ for 1 h.

Experimental Procedure

Experiments to study the metabolism of NEFA in fed sheep at rest were performed on eight animals and began at 1000 h. The protocol was similar to that described by Pethick *et al.* (1983). Briefly, approximately 8 GBq of [1-¹⁴C]-labelled fatty acid (stearic or oleic acid) was infused into the jugular vein for 5 h. Simultaneous blood samples were taken half hourly from carotid arterial and recurrent tarsal vein cannulae for metabolite determination. Hourly samples were taken for 5–7 h following the infusion.

Experiments to study the metabolism of NEFA in fed sheep during exercise were performed on the same eight animals at least 10 days after resting experiments and were begun at 1100 h. Approximately 8 BGq of [1-¹⁴C]-labelled fatty acid (stearic or oleic acid) was infused into the jugular vein for a period of 2 h during an exercise period on the belt treadmill at 4.5 km h⁻¹. Simultaneous blood samples were taken every 15 min from the carotid arterial and recurrent tarsal vein cannulae for metabolite determination. Hourly samples were taken for 5–7 h following the infusion.

The rate of blood flow in hind limb skeletal muscle was measured using the diffusion equilibrium technique with tritiated water as the marker (Oddy *et al.* 1981). The blood flow determination during exercise began 50 min after the onset of exercise and lasted for 30 min. A separate group of eight similar sheep were exercised for 4 h at 4.5 km h⁻¹. At 15, 75, 135 or 195 min after the onset of exercise the rate of blood flow in hind limb skeletal muscle was measured in order to investigate changes in blood flow with time. During any one experiment only three blood flow determinations were made so as to limit the amount of blood taken and isotope administered.

The protocol for blood-sampling was similar to that described by Pethick *et al.* (1983). Thus whole blood was collected into heparinized syringes for the analysis of carbon dioxide concentration and

radioactivity and oxygen concentration. Whole blood was also collected into tubes containing ethylenediaminetetraacetic acid (1 mg ml^{-1}) and then centrifuged immediately to allow collection of plasma for the determination of plasma NEFA concentration and radioactivity. A further 2 ml of blood was promptly added to 3 ml of 5% (v/v) perchloric acid for the determination of glucose, L-lactate, pyruvate, D-3-hydroxybutyrate and acetoacetate concentration. All blood samples were immediately placed on ice.

Chemical Methods

The concentration and specific radioactivity of metabolites, carbon dioxide and oxygen were determined as described in Pethick *et al.* (1981, 1983) and Pethick and Lindsay (1982). [^{14}C]-labelled fatty acids and tritiated water were purchased from Amersham (Australia) Pty Ltd: catalogue numbers CFA 24, CFA 243 and TRS 3.

Calculations

The calculations used in this study have been described in detail by Pethick *et al.* (1983). In summary they are as follows:

Whole animal

(a) If R is the entry rate of NEFA (mmol h^{-1}), then

$$R = (I/F_{SA}) \times (C_N/C_I),$$

where I is the infusion rate (dpm h^{-1}) of [^{14}C]-labelled fatty acid (stearic or oleic acid) and F_{SA} is the specific radioactivity of the infused fatty acid (dpm mmol^{-1}) in arterial blood and C_I and C_N represent the arterial concentration mmol l^{-1} of the infused fatty acid and NEFA respectively. The calculation assumes that the infused tracer fatty acid was representative of all NEFA.

(b) The fraction of circulating carbon dioxide derived from the oxidation of the infused fatty acid was determined as the ratio of the area under the arterial specific activity curves (dpm matom^{-1} carbon) of circulating carbon dioxide and the infused fatty acid. The fraction of circulating carbon dioxide derived from NEFA was calculated assuming that infused tracer fatty acid was representative of all NEFA.

Skeletal muscle

(a) If N = net fractional extraction of a metabolite (%), then

$$N = 100 \{([A] - [V])/[A]\},$$

where $[A]$ and $[V]$ are the concentrations (mmol l^{-1}) of the metabolite in carotid arterial and recurrent tarsal vein blood respectively. In the case of NEFA the concentrations were those determined in plasma.

(b) If G = gross fractional extraction of NEFA (%), then

$$G = 100[(^{14}\text{C}_A - ^{14}\text{C}_V)/^{14}\text{C}_A],$$

where $^{14}\text{C}_A$ and $^{14}\text{C}_V$ are the amounts of radioactive fatty acids (dpm l^{-1}) in carotid arterial and recurrent tarsal vein plasma respectively.

(c) If U_N = net metabolite utilization by skeletal muscle ($\text{mmol h}^{-1} \text{ kg}^{-1}$ muscle), then

$$U_N = N \times [A] \times Q,$$

where Q is blood flow ($\text{l h}^{-1} \text{ kg}^{-1}$ muscle).

(d) If U_G = gross utilization of NEFA by skeletal muscle ($\text{mmol h}^{-1} \text{ kg}^{-1}$ muscle), then

$$U_G = (^{14}\text{C}_A - ^{14}\text{C}_V)/F_{SAV} \times Q_P \times (C_N/C_I),$$

where F_{SAV} is the specific radioactivity (dpm mmol^{-1}) of the infused fatty acid in recurrent tarsal vein plasma, Q_P is plasma flow in skeletal muscle ($\text{l h}^{-1} \text{ kg}^{-1}$ muscle) and the calculation assumes the tracer fatty acid was representative of all NEFA.

(e) If M = the maximum contribution of a metabolite to the oxidation of skeletal muscle (%), then

$$M = 100\{([A] - [V]) \cdot Q_u\} / ([A] - [V])O_2,$$

where $([A] - [V])$ represents the net (or gross) arteriovenous difference (mmol l^{-1}) for the metabolite

and $([A] - [V])O_2$ represents the corresponding blood oxygen arteriovenous difference (mmol l^{-1}). The oxygen quotient Q_u represents the moles of oxygen needed for the complete oxidation of 1 mol of the metabolite. The following values were used—glucose 6; D-3-hydroxybutyrate 4.5, NEFA for experiments with sheep at rest 24.75; NEFA for experiments with sheep during exercise 24.74. The values for the oxygen quotients of NEFA were calculated from the average molar fatty acid composition found for NEFA at rest and during exercise (Table 2).

(f) If F = the fraction of utilized NEFA directly oxidized, then

$$F = ({}^{14}\text{C}_V - {}^{14}\text{C}_A)\text{CO}_2 / ({}^{14}\text{C}_A - {}^{14}\text{C}_V)\text{NEFA},$$

where $({}^{14}\text{C}_V - {}^{14}\text{C}_A)\text{CO}_2$ and $({}^{14}\text{C}_A - {}^{14}\text{C}_V)\text{NEFA}$ represent the cumulative blood radioactive arteriovenous differences (dpm ml^{-1}) for carbon dioxide and NEFA respectively. The value for NEFA was corrected to a blood concentration using the packed cell volume (i.e. it is assumed that NEFA in red cells makes no contribution).

Statistics

Means were compared using the Student *t*-test or the one-way analysis of variance as described by Steel and Torrie (1981).

Results

Oxygen Uptake by Skeletal Muscle

The packed cell volume, blood flow in skeletal muscle and oxygen utilized by skeletal muscle are shown in Table 1. The uptake of oxygen increased 5.4 times in response to exercise. This increase was in part attributable to a 3.1-fold increase in blood flow through skeletal muscle. In addition the fractional extraction of oxygen by skeletal muscle was increased 1.7 times further contributing to the increased utilization of oxygen found during exercise.

Table 1. Packed cell volume, blood flow in skeletal muscle and oxygen utilization by skeletal muscle in fed sheep at rest and during exercise

Values are mean \pm s.e.m. of seven sheep at rest and six sheep during exercise

	Packed cell volume (%)	Blood flow in muscle ($\text{l h}^{-1} \text{kg}^{-1}$)	Oxygen utilization by muscle ($\text{mmol h}^{-1} \text{kg}^{-1}$)
Rest	30.1 ± 1.1	4.3 ± 0.21	10.6 ± 1.6
Exercise	33.2 ± 1.2^A	13.5 ± 1.32^B	56.9 ± 1.1^A

^A Values represent the mean of the 2 h exercise period.

^B A single determination of blood flow was made during exercise between 50 and 80 min after the beginning of exercise.

In separate experiments, 4 h of continuous exercise produced a constant blood flow through skeletal muscle. Thus the rate of blood flow determined in a 30-min interval beginning at 15, 75, 135 and 195 min after the onset of exercise was 16.6 ± 1.9 ($n = 9$), 14.9 ± 1.7 ($n = 6$), 19.8 ± 2.3 ($n = 6$), 17.5 ± 4.9 ($n = 4$) $\text{l h}^{-1} \text{kg}^{-1}$ muscle respectively. These values were not significantly different (one-way analysis of variance).

Metabolism of NEFA by the Whole Animal

The molar proportions of the individual fatty acids contributing to the concentration of NEFA in plasma are shown in Table 2. Palmitic, stearic and oleic acids accounted for 86.4 and 90.5% of the total NEFA concentration at rest

and during exercise respectively. Quantitatively, the most substantial change in composition related to the ratio of the major unsaturated fatty acid, oleic acid, to the major saturated fatty acids, palmitic and stearic acids. The ratio changed from 0.38 at rest to 0.71 during exercise.

Table 2. Molar composition of arterial plasma non-esterified long-chain fatty acids in fed sheep at rest and during exercise

Values are means \pm s.e.m. for eight sheep. Values with similar superscripts represent a significant difference (paired Student's *t*-test) between rest and exercise

	Molar composition						
	C _{14:0}	C _{16:0}	C _{16:1}	C _{18:0}	C _{18:1}	C _{18:2}	C _{18:3}
Rest	1.5 \pm 0.1 ^a	26.7 \pm 1.9 ^b	4.0 \pm 0.3	35.7 \pm 1.9	23.9 \pm 3.5 ^c	5.2 \pm 0.3 ^d	2.9 \pm 0.1
Exercise ^A	0.8 \pm 0.1 ^a	20.8 \pm 0.7 ^b	3.8 \pm 0.5	32.1 \pm 1.7	37.6 \pm 1.7 ^c	3.4 \pm 0.6 ^d	1.5 \pm 0.3
	<i>P</i> < 0.001	<i>P</i> < 0.025			<i>P</i> < 0.01	<i>P</i> < 0.05	

^A Values represent the mean of the 2 h exercise period.

The data summarizing the whole animal metabolism of NEFA both at rest and during exercise are shown in Table 3. The concentration and entry rate of NEFA and the contribution of NEFA to circulating carbon dioxide were consistently lower in those animals where stearic acid was used as the tracer fatty acid. These differences were significant for the concentration and entry rate of NEFA during exercise. Exercise prompted a 15-fold increase in the mean concentration

Table 3. Plasma concentration, entry rate and the contribution to circulating carbon dioxide of non-esterified long-chain fatty acids (NEFA) in fed sheep at rest and during exercise in experiments using either [1-¹⁴C]oleic acid or [1-¹⁴C]stearic acid as the tracer fatty acid

Values are mean \pm s.e.m. of four sheep for both [1-¹⁴C]oleic acid and [1-¹⁴C]stearic acid as the tracer fatty acid. Values with similar superscripts represent a significant difference (Student's *t*-test) between experiments utilizing the different tracer fatty acids

Tracer fatty acid	Plasma concn of NEFA (mmol l ⁻¹)		Entry rate of NEFA (mmol h ⁻¹ kg ⁻¹ body wt)		Contribution of NEFA to circulating CO ₂ (%)	
	Rest	Exercise ^A	Rest	Exercise ^A	Rest	Exercise ^A
Oleic acid	0.08 \pm 0.01	1.44 \pm 0.17 ^a	0.08 \pm 0.01	1.29 \pm 0.13 ^b	1.32 \pm 0.24	23.68 \pm 5.82
Stearic acid	0.05 \pm 0.02	0.67 \pm 0.09 ^a	0.07 \pm 0.03	0.72 \pm 0.08 ^b	1.05 \pm 0.26	21.07 \pm 4.20
Overall mean	0.07 \pm 0.01	1.05 \pm 0.13	0.08 \pm 0.02	1.00 \pm 0.10	1.18 \pm 0.25	22.38 \pm 5.41
		<i>P</i> < 0.01		<i>P</i> < 0.01		

^A Values represent the mean of the 2 h exercise period.

of NEFA in blood, a 13-fold rise in the mean entry rate of NEFA and a 19-fold elevation in the contribution of NEFA to the carbon dioxide production by the whole animal. However, the magnitude of the response showed considerable variation. Thus the maximum concentration of NEFA reached after 120 min of exercise was 2.1 mmol l⁻¹ while the minimum concentration was 0.6 mmol l⁻¹.

The changes in concentration and entry rate of NEFA found during exercise are shown in Fig. 1. Both increased steadily for 75–90 min and tended to remain constant or decline during the last 30 min of exercise.

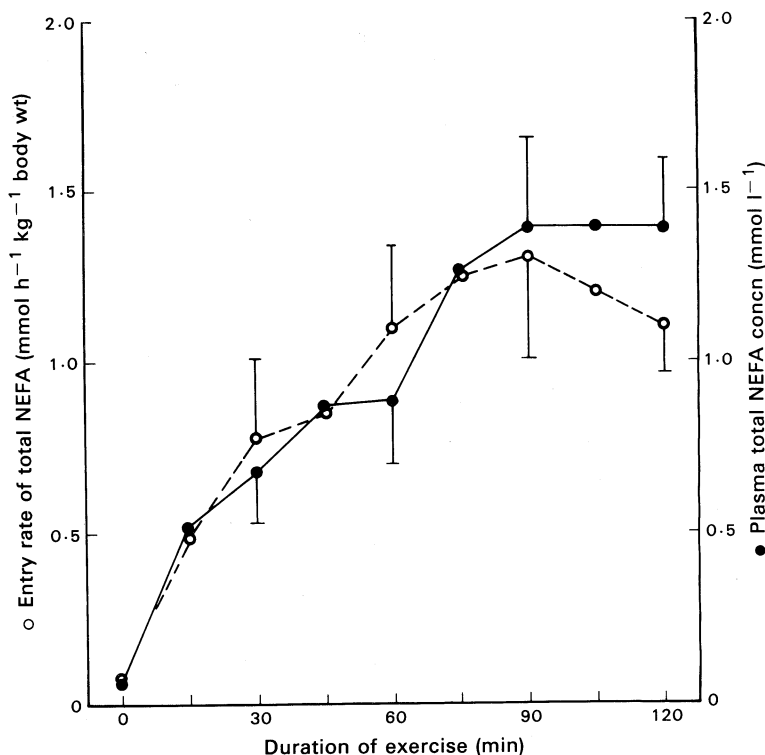


Fig. 1. Entry rate ($\text{mmol h}^{-1} \text{kg}^{-1}$ body weight) of non-esterified fatty acids (○) and the concentration (mmol l^{-1}) of non-esterified fatty acids (●) in the plasma of fed sheep at rest and during a 2-h exercise period. Values are mean \pm s.e.m. of eight sheep.

Metabolism of NEFA by Skeletal Muscle

The net and gross fractional extraction and proportion of the tracer NEFA promptly oxidised by skeletal muscle are shown in Table 4. The net fractional

Table 4. Net fractional extraction of non-esterified, long-chain fatty acids (NEFA), gross fractional extraction of the tracer fatty acid and the proportion of tracer fatty acid promptly oxidised (%) by skeletal muscle of fed sheep at rest and during exercise in experiments using either [^{14}C]oleic acid or [^{14}C]stearic acid as the tracer fatty acid

Values are mean \pm s.e.m. of three sheep for both [^{14}C]oleic acid and [^{14}C]stearic acid as the tracer fatty acid. Values with similar superscripts represent a significant difference (Student's *t*-test) between experiments utilizing the different tracer fatty acids

Tracer fatty acid	Net extraction of NEFA (%)		Gross extraction of the tracer fatty acid (%)		Proportion of tracer fatty acid promptly oxidized (%)	
	Rest	Exercise ^A	Rest	Exercise ^A	Rest	Exercise ^A
Oleic acid	1.1 \pm 5.5	6.1 \pm 4.4	18.2 \pm 5.4	8.5 \pm 2.0	36.8 \pm 9.5	73.7 \pm 8.7 ^a
Stearic acid	3.8 \pm 4.6	6.4 \pm 4.5	19.9 \pm 3.9	11.5 \pm 2.0	43.9 \pm 11.2	99.8 \pm 5.2 ^a
Overall mean	2.4 \pm 3.3	6.2 \pm 3.2	19.0 \pm 4.5	10.0 \pm 2.0	40.2 \pm 6.7	86.7 \pm 8.9

P < 0.05

^A Values represent the mean of the 2 h exercise.

extraction of NEFA was not significantly different from zero in ewes at rest or during exercise. In contrast there was a similar and significant gross fractional extraction of both oleic and stearic acid. The gross fractional extraction of oleic and stearic acid was significantly less during exercise. However, the mean (\pm s.e.m.) utilization increased from 0.046 ± 0.008 at rest to 0.99 ± 0.11 mmol h⁻¹ kg⁻¹ muscle during exercise. At rest 40% of the NEFA utilized by skeletal muscle was promptly oxidized and this increased to nearly 90% during exercise. The estimated proportion of NEFA promptly oxidized by skeletal muscle during exercise was significantly greater when stearic acid was the tracer NEFA.

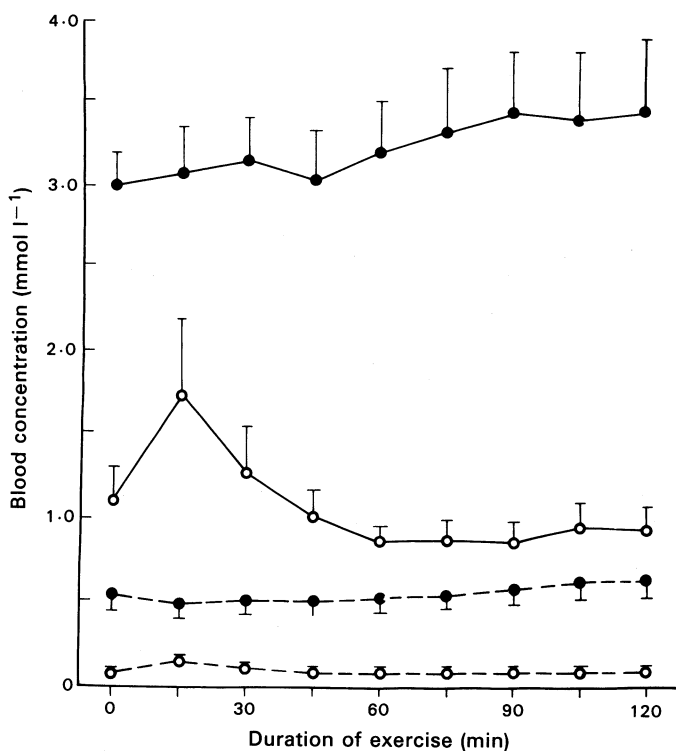


Fig. 2. Whole blood concentration (mmol l⁻¹) of glucose (●—●), L-lactate (○—○), D-3-hydroxybutyrate (●---●) and pyruvate (○---○) in fed sheep at rest and during a 2-h exercise period. Values of means \pm s.e.m. of eight sheep.

Metabolism of Glucose, L-Lactate, Pyruvate and Ketone Bodies

The changes in the concentration of glucose, L-lactate, pyruvate and D-3-hydroxybutyrate induced by exercise are shown in Fig. 2. The concentrations of glucose and D-3-hydroxybutyrate tended to increase during exercise so that after 2 h the concentrations had significantly increased by 16 and 17% respectively when compared to resting values (Students *t*-test, $P < 0.05$). The concentration of acetoacetate was always low and never exceeded 0.05 mmol l⁻¹. L-Lactate concentration increased by 55% in the first 15 min of exercise but then declined

to a concentration below the pre-exercise value. Pyruvate concentration showed a similar trend increasing by 83% after 15 min of exercise but then declined to near the pre-exercise level.

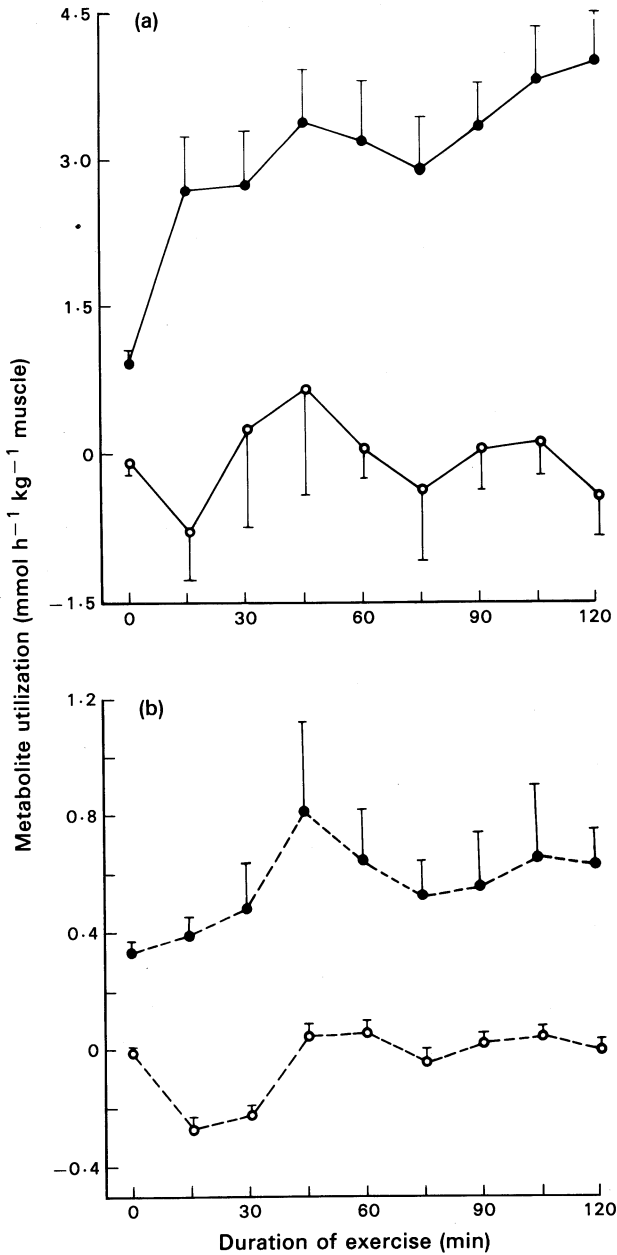


Fig. 3. Metabolite utilization (mmol h⁻¹ kg⁻¹ muscle) by skeletal muscle of fed sheep at rest and during a 2-h exercise period. Values are mean \pm s.e.m. of seven animals at rest and six animals during exercise. (a) Utilization of glucose (●—●) and L-lactate (○—○). (b) Utilization of D-3-hydroxybutyrate (●---●) and pyruvate (○---○).

The net utilization of glucose, lactate, pyruvate and D-3-hydroxybutyrate by skeletal muscle at rest and during exercise are shown in Fig. 3. Glucose utilization increased 2.9 times within 15 min of exercise and tended to increase further so that after 2 h it was 4.3 times greater than the resting value. Changes in the net utilization of D-3-hydroxybutyrate were smaller although after 120 min of exercise the uptake had increased significantly by 1.8 times. Acetoacetate utilization was low in animals at rest (0.04 ± 0.006 mmoles $\text{h}^{-1} \text{kg}^{-1}$ muscle) and there was no significant uptake detectable during exercise. The net utilization of L-lactate by skeletal muscle was variable and at no time was it significantly different from zero. However, there was a tendency for L-lactate release at 15 min of exercise. Net pyruvate utilization was not significantly different from zero at rest or after 30 min of exercise but there was a significant release of pyruvate at 15 and 30 min of exercise.

Table 5. Maximum contribution of metabolites to the oxidation of skeletal muscle in fed sheep at rest and during exercise

Values are means \pm s.e.m. of eight sheep except for non-esterified fatty acids which are for six sheep

Metabolite	Contribution (%) during rest	Contribution (%) during exercise in the interval	
		15-60 min	75-120 min
Non-esterified fatty acids	10 ± 1	30 ± 3	54 ± 2
Glucose ^A	50 ± 10	32 ± 3	36 ± 3
Ketone bodies	14 ± 1	5 ± 1	5 ± 0.2

^A Corrected for L-lactate and pyruvate release or uptake.

The maximum contribution of glucose, ketone bodies and NEFA to the oxidation of skeletal muscle both at rest and during exercise is shown in Table 5. At rest glucose is potentially the largest contributor to the energy requirements of skeletal muscle while NEFA make only a small contribution. During exercise the contribution made by NEFA increased such that in the latter half of exercise it was the major energy source of skeletal muscle. The contribution made by glucose during exercise was relatively constant throughout the exercise period. D-3-Hydroxybutyrate could maximally supply 14% of the energy requirements of skeletal muscle at rest and this value declined to 5% during exercise.

Discussion

Metabolism of Individual NEFA

In this study the metabolism of NEFA has been estimated by infusing either [$1-^{14}\text{C}$]stearic or [$1-^{14}\text{C}$]oleic acid and assuming that either was representative of all NEFA. Pethick *et al.* (1983) established that palmitic, stearic and oleic acids were metabolized similarly in fasted pregnant ewes when differences in arterial concentration were accounted for. Results from the present study generally support the earlier finding although the significantly lower entry rate of NEFA found during exercise when stearic acid was used as the tracer NEFA (Table 3) might appear to be contradictory. However, the discrepancy is largely explained by a significantly lower concentration of total NEFA in the group of sheep used for the [$1-^{14}\text{C}$]stearic

acid infusions. The significant difference found for the oxidation of oleic acid in exercising muscle cannot be similarly explained. It may therefore represent a true difference in the rates of oxidation and esterification of different fatty acid species in a contracting skeletal muscle. Some caution in interpretation is warranted since only small numbers of animals were used and no similar difference was found for resting muscle or for whole animal oxidation.

In summary, most of the major parameters of NEFA metabolism (i.e. entry rate, whole animal oxidation and muscle uptake) are similarly estimated by using either radiolabelled stearic or oleic acid.

Metabolism at Rest

The entry rate of NEFA found in this study represents 21 g of lipid per day for a 40 kg fed, non-pregnant sheep at rest. Other estimates are higher. Leat and Ford (1966) estimated an entry rate of 80 g per day while an entry rate of 50 g per day can be calculated from the data of Leng and West (1969). These differences are probably due to the higher concentration of NEFA reported in both the previous studies (0.5 and 0.2 mmol l⁻¹ respectively) since the entry rate of NEFA is proportional to the circulating concentration (Pethick *et al.* 1983). The low concentration of NEFA reported in this study for fed sheep at rest indicate experimental conditions that minimize lipolysis and so there is little doubt that the entry rate of NEFA is low and subsequently the contribution to oxidative metabolism is minimal. Using the rate of respiratory carbon dioxide production obtained in earlier work (14.1 mmol h⁻¹ kg⁻¹ body wt, Pethick *et al.* 1981) then the entry rate of NEFA found in this study could account for 10% of the respiratory carbon dioxide production assuming complete oxidation. In fact only 1% of the respiratory carbon dioxide was accounted for by NEFA oxidation in fed ewes at rest. Therefore only about 12% of this lipid was promptly oxidized. Other studies have shown that NEFA is not completely oxidized, although the estimates of oxidation are usually higher at 30–40% (Leat and Ford 1966; Annison *et al.* 1967; Pethick *et al.* 1983).

It is tempting to suggest that in this study circulating NEFA is derived largely from the breakdown of dietary derived lipoprotein triacylglycerol. Harrison and Leat (1972) using sheep fed a similar diet measured the appearance of lipid from intestinal lymph and found that 16 g of triacylglycerol entered the blood per day strongly suggesting that much of the 21 g of NEFA entering the circulation per day is ultimately derived from the diet. Furthermore Bergman *et al.* (1971) showed that circulating NEFA can be derived from lipoprotein-bound, esterified, long-chain fatty acids particularly as lipoproteins are hydrolysed by the extrahepatic tissues. Another source of NEFA would be hydrolysis of lipoprotein triacylglycerol of hepatic origin. This pathway is thought to be of low activity because of the very low rate of hepatic fatty acid and triacylglycerol synthesis found in sheep liver (Bell 1981). The third source of NEFA would be from lipolysis in adipose tissue. However, in this study it seems plausible that the animals used were under minimal stress and consequently lipolysis in adipose tissue was minimal; this may not have been so in earlier studies in which entry rates of NEFA were measured.

This study re-emphasizes the need to use isotopic measurements to determine the true uptake of NEFA by skeletal muscle since no net fractional extraction was detected. The gross extraction of NEFA by skeletal muscle in fed sheep at

rest was more than twice that found for fasted pregnant ewes (Pethick *et al.* 1983). Despite this greater potential for utilization of NEFA in non-pregnant fed sheep, they make only a small contribution to the energy metabolism of skeletal muscle as predicted by the earlier work. Thus, even if completely oxidized, NEFA could at best account for 10% of the oxidation in skeletal muscle. Since only 40% of the NEFA was promptly oxidized, this reduces the estimate to 4%.

It is overwhelmingly clear that alternative fuels are utilized by skeletal muscle of fed sheep at rest. This study and others (Pethick *et al.* 1983; Oddy *et al.* 1985) point to glucose being an important contributor to the energy metabolism of skeletal muscle in fed non-pregnant sheep at rest and this is in contrast to the findings in non-ruminant animals (Zierler 1976). Further work is needed to clarify the extent to which glucose is oxidized by skeletal muscle. The low rate of L-lactate and pyruvate release by skeletal muscle indicates a low activity of the Cori cycle, consistent with glucose oxidation. Felig (1975) has suggested that glucose carbon can be transferred to alanine thus preventing the oxidation of glucose. In fed sheep the release of alanine is small (Lindsay *et al.* 1976) and less than 1% of the glucose utilized by skeletal muscle could be accounted for by this pathway. Finally, if the maximum contribution to oxidation of the major nutrients utilized by skeletal muscle is summed, i.e. NEFA 10% (this study), glucose 50% (this study), ketone bodies 14% (this study and Pethick *et al.* 1983) and acetate 35% (Pethick *et al.* 1981) then 109% of the energy metabolism is accounted for. Clearly there is not a gross overestimate of total oxidation, again implying that much of the glucose which is utilized must be oxidized in the long term. However, the ultimate pathways of oxidation may well be indirect since Oddy *et al.* (1985) found greater than 70% of the glucose utilized was not promptly oxidized by skeletal muscle.

Metabolism during Exercise

Exercise prompted a sustained shift toward fat mobilization. The entry rate of NEFA reached considerable proportions by the end of the 2 h exercise period so that it was twice that seen in resting fasted pregnant ewes (Pethick *et al.* 1983). Using the rate of CO₂ production found for a similar intensity of exercise by Judson *et al.* (1976) then the entry rate of NEFA found after 2 h of exercise could have accounted for nearly 65% of the oxidative metabolism. The actual contribution of NEFA to the oxidative metabolism in the whole animal for the exercise period was much lower at 22%. However, this value represents an average contribution throughout the entire exercise period and still emphasizes the important role played by NEFA in maintaining the fuel homeostasis during exercise.

Some caution is needed in the interpretation of entry and oxidation rates of NEFA in studies such as this where steady state conditions are not apparent. Brockman and Halvorson (1981) made similar studies of glucose metabolism in the whole animal of sheep during exercise. They argued that the errors were tolerable at around 10% for glucose biokinetics. It is likely that the errors for assessing NEFA metabolism are even smaller due to the very rapid turnover time of NEFA in the circulation (2 min, Leat and Ford 1966) and considering that the rate of change of the NEFA entry rate was always less than 2% per minute during the sampling times.

Indirect oxidation of NEFA to ketones is not a major pathway for fatty acid oxidation during exercise in fed sheep. This can be implied from the lack of a

substantial increase in the circulating concentration and utilization of ketone bodies by skeletal muscle. These results agree with those of Jarrett *et al.* (1976) and show that hepatic ketogenesis is controlled by factors in addition to the availability of NEFA. A plausible mechanism may be inhibition of hepatic fatty acid oxidation by methyl malonyl CoA inhibition of carnitine palmitoyltransferase (EC 2.3.1.21) which has been shown by Brindle *et al.* (1985). Methylmalonyl CoA concentration should respond to rates of propionate uptake by the liver which may not change greatly during exercise. In addition, stressful situations leading to increased activity of the sympathetic nerves may directly inhibit hepatic ketogenesis (Beuers *et al.* 1986).

In skeletal muscle several adaptations were seen in response to exercise. The increased arterial concentration of NEFA prompted by exercise in addition to an increased rate of blood flow resulted in a substantial increase with time in the utilization of NEFA by skeletal muscle. Blood flow remained constant between 15 and 240 min of exercise and this agrees with work in humans where blood flow increases rapidly at the onset of exercise and then is maintained at a constant rate throughout the exercise period (Ahlborg *et al.* 1974). NEFA will thus be utilized in proportion to the arterial concentration as found by previous work in sheep (Pethick *et al.* 1983) and other species (Zierler 1976). The role of NEFA as a fuel is unequivocal since most was promptly oxidized by skeletal muscle.

The increasing importance of NEFA as a fuel during exercise is not at the expense of other circulating metabolites utilized by muscle. Thus glucose uptake by muscle increased considerably over the resting state, confirming the findings of Bird *et al.* (1981) and Jarrett *et al.* (1976). Significantly the increased glucose utilization is sustained throughout the exercise period similarly to that found in man (Ahlborg *et al.* 1974). Again the glucose consumed is likely to be completely oxidized since only during the initial stages of exercise was there a tendency toward L-lactate or pyruvate release. The extent of the glucose-alanine cycle during exercise in sheep is unknown but it is unlikely to be large since it is not substantial even in fasted sheep (Lindsay *et al.* 1977). Thus glucose appears as a significant fuel during exercise in addition to its important role in the resting animal.

The control of glucose utilization by skeletal muscle during exercise warrants further investigation. Thus contracting muscle is one of the few tissues where there is a large and simultaneous increase in the utilization of both glucose and NEFA. Classically the two are thought to be mutually exclusive since it is believed that fatty acids actually inhibit glucose utilization by skeletal muscle (Newsholme 1976).

It remains to be determined just which fuels are replaced as NEFA oxidation increases in skeletal muscle during exercise. However, it is probable that endogenous fuels within the muscle fibre are spared. Evidence for this hypothesis can be obtained by summing the contribution to oxidation made by the major fuels available for oxidation and assuming that endogenous fuels make up the difference to achieve 100% of the oxidative metabolism. Between 15 and 60 min of exercise the total maximum contribution to oxidation made by glucose, ketone bodies and NEFA was 67%, to which can be added an 8% contribution made by acetate, assuming conditions are as in the studies of Bird *et al.* (1981). That is, only 75% of the total oxidation in skeletal muscle can be accounted for, implying that 25% of the oxidation is of endogenous origin. The same calculation for the interval between 75 and 120 min of exercise results in 103% of the oxidation being

accounted for, that is there is no requirement for endogenous fuels. This calculation is consistent with a role of circulating NEFA to spare endogenous substrates in muscle as exercise progresses. The endogenous substrate utilized is probably glycogen since contraction and the breakdown of glycogen in muscle are synchronous because inorganic phosphate, a product of muscle contraction, and calcium, the stimulus for contraction, both activate glycogenolysis (McGilvery and Goldstein 1983). However, intramuscular triacylglycerol cannot be excluded as a significant fuel (Essen *et al.* 1977).

In conclusion, this study has pointed for a need to reappraise the traditional review that fatty acids and not glucose are the predominant fuel of skeletal muscle in fed sheep. This phenomenon is not restricted to sheep since in the fed steer Bell and Thompson (1979) showed that NEFA were not an important fuel of the hind limb while the same laboratory (Bell *et al.* 1975) established glucose could make a large contribution to oxidative metabolism.

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