An investigation of arterial insufficiency in the rat hindlimb

Correlation of skeletal muscle bloodflow and glucose utilization *in vivo*

R. A. John CHALLISS,* David J. HAYES and George K. RADDA

Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, U.K.

1. Muscle bloodflow and the rate of glucose uptake and phosphorylation were measured *in vivo* in rats 7 days after unilateral femoral artery ligation and section. 2. Bloodflow was determined by using radiolabelled microspheres. At rest, bloodflow to the gastrocnemius, plantaris and soleus muscles of the ligated limb was similar to their respective mean contralateral control values; however, bilateral sciatic nerve stimulation at 1 Hz caused a less pronounced hyperaemic response in the muscles of the ligated limb, being 59, 63 and 49% of their mean control values in the gastrocnemius, plantaris and soleus muscles respectively. 3. The rate of glucose utilization was determined by using the 2-deoxy[³H]glucose method [Ferré, Leturque, Burnol, Penicaud & Girard (1985) *Biochem. J.* **228**, 103–110]. At rest, the rate of glucose uptake and phosphorylation was statistically significantly increased in the gastrocnemius and soleus muscles of the ligated limb, being 126 and 140% of the mean control values respectively. Bilateral sciatic nerve stimulation at 1 Hz caused a 3–5-fold increase in the rate of glucose utilization by the ligated and contralateral control limbs; furthermore, the rate of glucose utilization was significantly increased in the muscles of the ligated limb, being 140, 129 and 207% of their mean control values respectively. 4. For the range of bloodflow to normally perfused skeletal muscle at rest or during isometric contraction determined in the present study, a linear correlation between the rate of glucose utilization and bloodflow can be demonstrated. Applying similar methods of regression analysis to glucose utilization and bloodflow to muscles of the ligated limb reveals a similar linear correlation. However, the rate of glucose utilization at a given bloodflow is increased in muscles of the ligated limb, indicating an adaptation of skeletal muscle to hypoperfusion.

INTRODUCTION

Recently, we have reported a small animal model of arterial insufficiency (Challiss *et al.*, 1986; Hayes *et al.*, 1985a, 1986) in which we investigated the ability of the muscle mass and associated vasculature to adapt a chronic ischaemia. In these studies we have found a remarkable time-dependent improvement in bloodflow and high energy phosphate utilization of hypoperfused muscle caused to contract (Challiss *et al.*, 1986). This was associated with a rapid and maintained increase in glycolytic capacity in the affected muscle mass, but no alteration in oxidative enzyme capacity was noted (Hayes *et al.*, 1986).

The most common clinical manifestation of peripheral arterial insufficiency is restricted perfusion of the lower limbs resulting in intermittent claudication, rest pain or ischaemic gangrene depending on the severity and operability of the condition (O'Donnell *et al.*, 1977; Clyne *et al.*, 1985). The human condition has a complex aetiology culminating in clinical presentation of claudication as a result of arterial stenosis. The prognosis for the claudicant will depend largely on the site of the stenosis (Clyne, 1980).

A number of conflicting findings have been reported with respect to enzymic adaptation, changes in muscle capillary density and substrate utilization in studies of patients with symptoms of intermittent claudication (Hagenfeldt *et al.*, 1972; Holm *et al.*, 1973; Bylund *et al.*, 1976; Hammarsten *et al.*, 1980; Henriksson *et al.*, 1980; Bylund-Fellenius *et al.*, 1981; Clyne *et al.*, 1982). It is

likely that much of this disagreement relates directly to the heterogenous pool of claudicants from which the data is obtained; however, progress is also hampered by the lack of reliable biochemical data on the effects of chronic ischaemia on skeletal muscle bioenergetics and physiological performance. Although the animal model of arterial insufficiency described previously (Challiss *et al.*, 1986) does not reproduce the clinically encountered human condition, it does allow investigation of the mechanisms by which skeletal muscle adapts to hypoperfusion. Thus, it is hoped that use of the animal model will increase our understanding of the biochemical basis of adaptive mechanisms in ischaemia.

In the present study we report direct evidence for an increased glucose uptake by the hypoperfused muscle *in vivo*. Glucose uptake has been measured by using [³H]deoxyglucose (Ferré *et al.*, 1985) and is correlated with muscle bloodflow (Armstrong & Laughlin, 1983). In addition, a possible role for insulin in the increased glucose uptake by the hypoperfused muscle is investigated indirectly in the isolated soleus muscle *in vitro*.

MATERIALS AND METHODS

Animal preparation

Male Wistar rats (200–240 g) were obtained from Bantin and Kingman, Hull, U.K. and were maintained in the Department's animal house for at least 5 days before use. Under light anaesthesia with ether, the artery–vein–nerve bundle of the left thigh was exposed

* To whom correspondence and reprint requests should be addressed.

and the femoral artery isolated by blunt dissection. Two silk ligatures were tied around the artery 1 cm proximal to the epigastric bifurcation and the artery was cut between the ligatures. Sham-operated controls were treated in a similar manner except that the artery was not ligatured or sectioned. The wound was sutured and animals allowed to recover. Ligated and sham-operated rats were housed together in groups of six to eight with free access to food and water for 7 days prior to study (Challiss *et al.*, 1986). Ligated animals were normally active, grew at rate similar to the sham-operated group and were not observed to spare the affected limb.

Animals were prepared in a similar manner for glucose utilization and bloodflow measurements. Anaesthesia was induced with pentobarbital (60 mg/kg, intraperitoneal) and maintained using 0.5–1% halothane in N₂O/O₂ (1:1) delivered via a face mask. Animals were studied at rest or during steady-state isometric contraction at 1 Hz. For stimulation experiments the left and right sciatic nerves were exposed and pairs of Teflon-coated silver wires were sewn into place in contact with the nerve. Distal tendons of the soleus, plantaris and gastrocnemius muscles were ligated and the limbs fixed to optimize muscle length for maximal isometric contraction (Challiss *et al.*, 1986). Rectal temperature was monitored and maintained at 37 °C.

Glucose utilization studies

For each animal the right jugular vein and tail artery were cannulated. In experiments at rest, a 200 μ l arterial blood sample was drawn from the tail artery and 2-deoxy[2,6-³H]glucose (38 μ Ci; 3.6 Ci/mmol) was immediately injected in 200 μ l of 0.9% NaCl as a bolus into the jugular vein. At various times (1, 6, 11 and 21 min) after deoxyglucose administration further 200 μ l blood samples were drawn from the tail artery. On obtaining the final blood sample the animal was killed by cervical dislocation and the soleus, plantaris and gastrocnemius muscles from each limb were rapidly dissected and freeze-clamped. In experiments performed during bilateral muscle stimulation, a blood sample was taken and isometric contraction at 1 Hz commenced by sciatic nerve stimulation (50 μ s pulse, 40 V). After 4 min of contraction 2-deoxy[2,6-³H]glucose was injected via the jugular vein and blood was sampled 1, 4, 7 and 11 min after administration. On obtaining the last blood sample the animal was killed by cervical dislocation and the muscles processed as above.

Analysis of blood and muscle samples

Blood samples were maintained on ice until the completion of each experiment. Samples were centrifuged (9000 g, 5 min) and the plasma withdrawn for immediate determination of glucose concentration (by glucose oxidase kit; Sigma Chemical Co.) and radioactivity (see below).

Muscle samples were homogenized in 4 vol. of 6% (w/v) HClO₄ (Polytron, setting 6, 25 s) at 4 °C. Homogenates were centrifuged (1500 g, 15 min, 4 °C), the supernatants were neutralized with 3 M-KHCO₃ and the perchlorate precipitate of KClO₄ was removed by centrifugation (1500 g, 15 min, 4 °C). Supernatants were applied to Dowex-2 (formate form) ion exchange columns (Hammerstedt, 1980); after thorough washing with water to remove unchanged deoxyglucose, 2-deoxy[2,6-³H]glucose 6-phosphate was eluted with 0.3 M-

ammonium formate in 1 M-formic acid (Hammerstedt, 1980) in a 6 ml fraction. A sample of this elute was added to 5 vol. of Liquiscint (National Diagnostics, Somerville, NJ, U.S.A.) and counted in a Beckman LS 7500 scintillation counter.

Muscle bloodflow studies

Bloodflow was assessed by a modification of the microsphere method of Armstrong & Laughlin (1983). Anaesthetized animals were studied at rest and during isometric contraction (caused by bilateral sciatic nerve stimulation at 1 Hz).

Pre-mixed microspheres (⁴⁵Sc; 15 μ m NEN-TRACTM microspheres, 10 mCi/g; 400000 beads/animal) were infused at rest or in the fifth minute of bilateral nerve stimulation via a cannula placed in the left ventricle via the right carotid artery. After microsphere administration the cannula was flushed with warm saline and the animal killed 1 min later. Tissue samples were dissected and counted in a Packard Auto-gamma 5320 scintillation spectrometer. Bilateral microsphere distribution was confirmed in each experiment by bead content of the kidneys and testes (Table 1).

Insulin sensitivity experiments

Rats were used 7 days after unilateral femoral artery ligation and section. Soleus muscle strips were prepared and incubated in Krebs–Ringer bicarbonate buffer containing 1.5% defatted bovine serum albumin, 5.5 mM-glucose (0.5 μ Ci of [U-¹⁴C]glucose/ml) and various concentrations of insulin as described previously (Challiss *et al.*, 1983). Rates of lactate formation and glycogen synthesis from [U-¹⁴C]glucose were determined (Challiss *et al.*, 1983). Using the soleus muscle preparation we also determined correction factors for the discrimination against 2-deoxyglucose at physiological (25 μ units/ml) insulin concentrations as described by Ferré *et al.* (1985). For soleus muscle strips from normally perfused and femoral artery ligated limbs of animals 7 days after surgery, correction factors were 0.95 ± 0.04 (6) and 1.02 ± 0.09 (6) respectively. It is not possible to use gastrocnemius or plantaris muscles *in vitro* for determination of correction factors; therefore, extensor digitorum longus muscle [taken from 60–80 g rats, see Lozeman *et al.* (1986)] was used as a muscle of comparable type II fibre composition which retained biochemical viability *in vitro*. Determination of correction factors for intact soleus and extensor digitorum longus muscles *in vitro* taken from 60–80 g rats were 1.00 ± 0.06 (8) and 0.98 ± 0.05 (8) respectively. Therefore, 'lumped constants' were assumed to be not significantly different from unity.

Calculation of rates *in vivo* of glucose utilization and bloodflow

Glucose utilization was calculated as described by Ferré *et al.* (1985). The integral of the arterial plasma [2-deoxy[2,6-³H]glucose]/[glucose] was determined from the experimental time points by using a scientific pocket calculator. The assumptions made in obtaining quantitative information from studies using 2-deoxyglucose to estimate glucose utilization have been fully discussed previously (Sokoloff *et al.*, 1977; Ferré *et al.*, 1985).

In experiments using radiolabelled microspheres to measure bloodflow, our primary purpose was to obtain distribution patterns between muscles of the control and

Table 1. Distribution of microspheres to rat kidney and testis at rest and during bilateral muscle stimulation at 1 Hz

Results are presented as means $\pm$ S.E.M. with the number of observations in parentheses. Microspheres were infused as a single bolus via the right carotid artery into the left ventricle (see the Materials and methods section for experimental details).

	Distribution (beads trapped/g wet wt.)			
	Kidney		Testis	
	Left	Right	Left	Right
Rest	3793 $\pm$ 492 (5)	3780 $\pm$ 309 (5)	341 $\pm$ 69 (5)	329 $\pm$ 68 (5)
Stimulation	4129 $\pm$ 436 (7)	4091 $\pm$ 429 (7)	329 $\pm$ 40 (7)	305 $\pm$ 44 (7)

Table 2. Glucose utilization and bloodflow to gastrocnemius, plantaris and soleus muscles of rats which had undergone unilateral femoral artery ligation 7 days previously

Experiments were performed either at rest or during isometric muscle contraction at 1 Hz (for details, see the Materials and methods section). Results are presented as means $\pm$ S.E.M. with number of observations in parentheses. Glucose utilization is expressed as μ mol/min per 100 g of muscle and bloodflow is expressed as ml/min per 100 g of muscle. Statistical significance was determined using Student's *t*-test for paired observations for muscles of control and affected limbs of unilaterally ligated animals and is indicated as **P* < 0.05, ***P* < 0.01.

	Gastrocnemius		Plantaris		Soleus	
	Control	Ligated	Control	Ligated	Control	Ligated
Rest						
Glucose utilization	9.3 $\pm$ 1.9 (7)	11.5 $\pm$ 1.8 (7)*	10.5 $\pm$ 1.2 (7)	12.9 $\pm$ 1.9 (7)	15.4 $\pm$ 1.4 (7)	21.6 $\pm$ 1.0 (7)**
Bloodflow	46.4 $\pm$ 11.2 (4)	34.4 $\pm$ 7.6 (4)	56.8 $\pm$ 16.4 (4)	36.0 $\pm$ 8.4 (4)	135 $\pm$ 40 (4)	100 $\pm$ 32 (4)
Stimulation						
Glucose utilization	39.8 $\pm$ 8.0 (5)	55.9 $\pm$ 4.2 (5)*	41.1 $\pm$ 7.8 (5)	53.1 $\pm$ 5.1 (5)*	46.2 $\pm$ 8.8 (5)	95.9 $\pm$ 20.7 (5)**
Bloodflow	267 $\pm$ 39 (7)	158 $\pm$ 24 (7)*	259 $\pm$ 58 (7)	164 $\pm$ 32 (7)*	422 $\pm$ 56 (7)	207 $\pm$ 40 (7)**

affected limbs of animals which had undergone unilateral femoral artery ligation and section. For this reason our main concern was establishing that there was a symmetric bilateral distribution of microspheres using the kidneys and testes for this purpose (see Table 1). In experiments where quantification of blood flow is the primary objective, it is usual to obtain a reference sample from a cannula placed in the descending aorta via the renal artery (Armstrong & Laughlin, 1983). This procedure was not carried out as it would have compromised the measurements of bead distribution to the kidneys. Therefore, to allow quantification of bloodflow, values obtained as number of microspheres/g of muscle have been referenced to the bloodflow to gastrocnemius muscle at rest given by Armstrong & Laughlin (1983).

RESULTS AND DISCUSSION

Muscle bloodflow

The radioactive microsphere technique was used to assess muscle bloodflow (Armstrong & Laughlin, 1983). Bilateral microsphere distribution was assessed at rest and during muscle contraction caused by bilateral sciatic nerve stimulation at 1 Hz; it can be seen from Table 1 that there was a uniform distribution of microspheres under both conditions. The number of beads trapped in

each muscle has been converted to bloodflow (ml/min per 100 g of tissue) as described in the Materials and methods section and the results are presented in Table 2.

Resting bloodflow to the ligated limb was not significantly different in the three muscles studied from contralateral control values; however, the hyperaemic response to muscle contraction was statistically significantly decreased in the ligated limb, being 59, 63 and 49% of control responses in gastrocnemius, plantaris and soleus muscles respectively (Table 2).

Muscle glucose utilization

The rate of glucose utilization *in vivo* has been investigated by administration of a radiolabelled glucose analogue, 2-deoxy[3 H]glucose. The use of the 2-deoxyglucose method in a wide range of tissues under different pathophysiological conditions has established it as a useful tool for investigation of carbohydrate metabolism *in vivo* (Sokoloff *et al.*, 1977; Hom *et al.*, 1984; Kraegen *et al.*, 1985; Ferré *et al.*, 1985). As pointed out in the Materials and methods section, the time between administration of 2-deoxy[3 H]glucose and killing the animal was 21 min and 11 min for animals studied at rest and during isometric contraction respectively. At these times the radioactivity remaining in the plasma was 15–20% of that initially administered (Fig. 1). Selection of these short-time courses for the

Fig. 1. Plasma glucose concentration (upper panel) and plasma disappearance curves (lower panel) for 2-deoxy[2,6-³H]glucose for animals at rest (●) or during muscle stimulation at 1 Hz (■)

At 7 days after unilateral femoral artery ligation and section animals were prepared for the experiment as described in the Materials and methods section. Seven animals were studied at rest and five during muscle stimulation. The bar indicates the stimulation period.

experiments was necessary because of the stability (defined by biochemical or physiological parameters) of the stimulated, hypoperfused muscle (see Challiss *et al.*, 1986). Therefore, preliminary experiments were performed to investigate whether the time at which the experiment was terminated affected the relative difference in 2-deoxy[³H]glucose phosphorylation observed in normally perfused and hypoperfused muscle under resting conditions. There was no significant change in the difference between 2-deoxyglucose phosphorylation rates for gastrocnemius muscle samples taken 21, 40 and 60 min after 2-deoxyglucose administration (results not shown). In addition, the ratio of non-phosphorylated to phosphorylated 2-deoxyglucose in the muscle sample did not change with increasing sampling time, suggesting that interstitial deoxyglucose does not significantly affect the determination of glucose utilization rates. These initial control experiments, taken together with the fact that normally perfused and hypoperfused muscle samples were obtained from each animal studied, convinced us that the time-course used in the study was satisfactory.

The arterial plasma glucose concentrations at rest and during muscle contraction are shown in Fig. 1 (upper panel). At rest, the plasma glucose concentration did not

alter significantly during the experimental period; however, muscle contraction caused mild hyperglycaemia which reached statistical significance after 7 min of bilateral sciatic nerve stimulation at 1 Hz ($P < 0.05$). During the 15 min stimulation period the arterial plasma glucose concentration increased from 7.2 ± 0.3 to 9.2 ± 0.3 mM (Fig. 1); however, the change during the period followed deoxyglucose administration is modest ($< 15\%$). The lower panel of Fig. 1 shows the plasma 2-deoxy[2,6-³H]glucose decay curves at rest and during muscle stimulation. To overcome the fact that plasma glucose concentration was not constant throughout the protocol the mean specific radioactivities were calculated by dividing the plasma radioactivity of 2-deoxy[2,6-³H]glucose (expressed as d.p.m./ml of plasma) by the plasma glucose concentration (expressed as $\mu\text{mol/ml}$ of plasma) for each experimental time point. The rate of disappearance of 2-deoxy[2,6-³H]glucose (expressed as the decay curve of the specific radioactivity) fitted a single exponential function for resting and stimulated conditions.

Linear regression analysis of these data as a logarithmic: linear function (resting: $y = \log 4.72 - 0.027x$; $r = -0.951$; $P < 0.01$; stimulated: $y = \log 4.69 - 0.051x$; $r = -0.963$; $P < 0.01$) allowed integrals to be calculated with a pocket scientific calculator and determination of rates of glucose utilization as detailed by Ferré *et al.* (1985). Rates of glucose utilization for gastrocnemius, plantaris and soleus muscles at rest and during bilateral muscle stimulation in animals 7 days after unilateral femoral artery ligation and section are shown in Table 2.

Rates of glucose utilization by normally perfused muscles of animals at rest are in good agreement with those measured previously (James *et al.*, 1985); bilateral sciatic nerve stimulation at 1 Hz caused a 4-fold increase in the rate of glucose utilization by gastrocnemius and plantaris and a 3-fold increase in soleus muscle glucose utilization (Table 2). In the muscles of the ligated limb higher rates of glucose utilization were observed at rest compared with the contralateral control muscles. For gastrocnemius and soleus muscles of the ligated limb the increases (26 and 40% respectively) in the rates of glucose utilization by gastrocnemius, plantaris and soleus muscles of the ligated limb increased 4–5-fold, thereby accentuating the differences between the rates of glucose utilization by muscles of the ligated and contralateral control limbs (Table 2).

Insulin sensitivity of soleus muscle *in vitro*

Soleus muscle strips were prepared from donor animals which had undergone unilateral femoral artery ligation 7 days previously; in this way two pairs of strips could be prepared from each animal, allowing direct comparison of the effect of different insulin concentrations in muscle from the ligated and contralateral limb. The effect of insulin concentration on lactate production and glycogen formation is illustrated in Fig. 2. At basal insulin concentrations lactate production was similar in muscle from ligated or contralateral control limbs, whereas muscles from the ligated limb exhibited enhanced rates of glycogen synthesis; this was statistically significant at $10 \mu\text{units}$ of insulin/ml ($P < 0.05$). At high insulin concentrations, lactate production by muscles from ligated limbs was considerably higher than that seen in muscles from contralateral control limbs,

Fig. 2. Effect of 7 days unilateral femoral artery ligation on the sensitivity of lactate formation (a) and glycogen synthesis (b) to insulin in isolated soleus muscle strips

Muscle strips were prepared from the soleus muscles of the ligated (Δ) or contralateral control ($\bullet$) limb. Muscle incubations and lactate and glycogen formation determinations were carried out as referenced in the Materials and methods section.

whereas glycogen synthesis approached a similar maximal rate (Fig. 2). It is of interest to note that the concentrations of insulin which cause half-maximal effects on glycogen synthesis and lactate production were 141 ± 42 (7) and 125 ± 30 (5) $\mu\text{units/ml}$ for muscle strips from unligated limbs and 62 ± 24 (7) and 68 ± 18 (6) $\mu\text{units/ml}$ for muscle strips from ligated limbs; however, these differences were not statistically significant.

Correlation of bloodflow and glucose utilization *in vivo*

Previously, we have studied (using the same animal model as used in the present study) the ability of the muscle mass of the ligated limb to contract isometrically at 1 Hz at various times after surgical ligation and section of the femoral artery (Challiss *et al.*, 1986). There was a time-dependent improvement in the steady-state phosphocreatine concentration achieved in the gastrocnemius/plantaris muscles of the ligated limb during isometric contraction at 1 Hz; this showed a significant correlation with the time-dependent improvement in exercise-induced hyperaemia (Hayes *et al.*, 1985b). With respect to these findings, Idström *et al.* (1986) have investigated the possible relationship between glucose uptake and muscle function using the perfused hindlimb preparation; they found a significant correlation between muscle glucose uptake and the concentration of phosphocreatine maintained during isometric contraction.

In addition, several reports have presented indirect evidence that human hypoperfused skeletal muscle exhibits increased rates of carbohydrate metabolism *in vivo* (Hagenfeldt *et al.*, 1972; Holm *et al.*, 1973; O'Donnell *et al.*, 1977) and this has been linked to the finding that peripheral tissue sensitivity to insulin *in vivo* is increased in claudicants (Hammarsten *et al.*, 1977,

Fig. 3. Relationship between the rate of glucose utilization and bloodflow at rest and during isometric contraction at 1 Hz for gastrocnemius ($\circ$, $\bullet$), plantaris (Δ , $\blacktriangle$) and soleus ($\square$, $\blacksquare$) muscles from animals which had undergone unilateral femoral artery ligation and section 7 days previously

Data are taken from Table 2. Closed and open symbols are for muscles from the ligated and contralateral limbs respectively. The lines of best fit for the relationship between glucose utilization and bloodflow for skeletal muscle of control and ligated limbs are $y = 0.114x + 4.97$ ($r = 0.952$, $P < 0.01$, $n = 6$) and $y = 0.434x - 8.90$ ($r = 0.947$, $P < 0.01$, $n = 6$) respectively.

1980). In the present study we have determined bloodflow and glucose utilization *in vivo* by gastrocnemius, plantaris and soleus muscles of ligated or contralateral normally perfused hindlimbs of rats at rest or during

stimulation (see Table 2). To illustrate the relationship between the rate of glucose utilization and bloodflow, mean values for the muscles investigated at rest or during stimulation are shown graphically in Fig. 3. Glucose utilization is shown for muscles from either ligated or normal limbs as a function of bloodflow to the muscle (Fig. 3). The functions can be defined mathematically by linear regression analysis (normal perfused limb: $y = 0.114x + 4.97$; $r = 0.952$; $P < 0.01$; ligated limb: $y = 0.434x - 8.9$; $P < 0.01$). Therefore, at any given bloodflow it can be seen that muscles of the ligated limb take up and phosphorylate significantly more glucose than the contralateral normally perfused muscle mass.

Concluding remarks

The animal model of arterial insufficiency presented here and previously (Hayes *et al.*, 1985a,b; 1986; Challiss *et al.*, 1986) allows investigation of the effects of chronic ischaemic stress in skeletal muscle and the mechanisms by which the vasculature and muscle mass itself adapt to such perturbation. Clearly, this simple, reproducible animal model does not accurately mimic the complex aetiology and heterogeneity of the human disease state. However, the animal model is similar to the human disease state in a number of respects. Clinical investigations of patients with claudication by ^{31}P -n.m.r. shows no significant abnormalities in phosphorus-containing metabolites and intramuscular pH in resting muscle; however, exercise causes an intracellular acidosis and increased rate of phosphocreatine utilization compared with age-matched control subjects (Keller *et al.*, 1985; Hands *et al.*, 1986). At 7 days after unilateral femoral artery ligation and section in the rat, ^{31}P -n.m.r. spectroscopy revealed essentially normal resting muscle with respect to phosphorus-containing metabolites, except for an elevated inorganic phosphate concentration (Challiss *et al.*, 1986); upon stimulation of the spectroscopically observed muscle mass, intracellular acidosis [ligated: pH 6.75 ± 0.09 (7); control: pH 7.02 ± 0.03 (6); $P < 0.05$] and a lower steady-state phosphocreatine concentration [ligated: 23.1 ± 1.2 mM (7); control: 28.9 ± 1.4 mM (6); $P < 0.01$] were observed (Challiss *et al.*, 1986).

Conflicting findings have been reported for alterations in the maximal activities of oxidative enzymes determined in muscle biopsies from claudicants. Bylund-Fellenius and co-workers consistently report increased oxidative capacity in muscle of claudicants (Bylund *et al.*, 1976; Bylund-Fellenius *et al.*, 1981), whereas other groups have failed to observe an enhancement of oxidative capacity (Henriksson *et al.*, 1980; Clyne *et al.*, 1985); moreover, these groups have noted an enzymic adaptation consistent with an enhanced glycolytic capacity. Indeed, it is an enhancement of the glycolytic, not the oxidative, capacity which is most commonly observed in animal models of arterial insufficiency (Bass *et al.*, 1979; Hayes *et al.*, 1986), imposition of an exercise-training programme or chronic nerve stimulation being required to observe increases in oxidative capacity in the hypoperfused muscle (Janda *et al.*, 1972; Urbanová *et al.*, 1974; Elander *et al.*, 1985). Therefore, the observed adaptive response of skeletal muscle to arterial insufficiency may be dependent upon the level of muscular contractile activity.

A number of studies have suggested that glucose extraction and insulin sensitivity muscle of patients with

arterial insufficiency are enhanced (Hagenfeldt *et al.*, 1972; Holm *et al.*, 1973; Hammarsten *et al.*, 1977, 1980). In the present study, we have presented direct evidence for increased glucose utilization by the hypoperfused muscle compared with normally perfused muscle of the contralateral limb, both at rest and during isometric contraction at 1 Hz. This finding, together with the fact that glycolytic capacity from glucose and glycogen is increased under these conditions (Hayes *et al.*, 1986), suggests an increased reliance on anaerobic glycolysis in hypoperfused muscle. In addition, the stimulation-induced decrease in intramuscular pH may indicate an increased glycolytic flux to lactate or a decreased ability to maintain intramuscular H^+ concentration (Challiss *et al.*, 1986). However, despite this adaptation, skeletal muscle of the affected limb remains extremely susceptible to hypoperfusion: a 40% decrease in bloodflow to the gastrocnemius/plantaris muscles during isometric contraction at 1 Hz causing an increased rate of phosphocreatine utilization, a failure to maintain twitch-tension and prolongation of the half-relaxation time, indicating that the hypoperfused muscle mass is still compromised (Challiss *et al.*, 1986).

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