Biochem. J. (1981) **200**, 247–255
Printed in Great Britain

Energy metabolism in relation to oxygen partial pressure in human skeletal muscle during exercise

Ann-Christin BYLUND-FELLENIUS,* Paul M. WALKER, Anna ELANDER, Sten HOLM, Jan HOLM and Tore SCHERSTÉN
Surgical Metabolic Research Laboratory, Department of Surgery I, Sahlgren's Hospital, University of Göteborg, S-413 45 Göteborg, Sweden

(*Received 7 May 1981/Accepted 17 July 1981*)

1. The intramuscular oxygen partial pressure (pO_2) in human gastrocnemius muscle was monitored during exercise and compared with metabolite concentrations reflecting the energy and the redox state in the tissue. Ten normal subjects and ten patients with peripheral vascular occlusive disease were investigated. 2. In normal subjects the pO_2 at the end of exercise was related to the intensity of the exercise, expressed as effect (J/s) per contraction. 3. In both patients and normal subjects the pO_2 was related to the [ATP]/[ADP] ratio, the [lactate]/[pyruvate] ratio and the phosphocreatine concentration in the muscle tissue at rest and during exercise. 4. At each pO_2 value, a lower [lactate]/[pyruvate] ratio was found in the muscle tissue of the patients compared with that of normal subjects. This was interpreted as a beneficial effect of the higher oxidative-enzyme capacity in the muscle of the patients. 5. The results show the importance of pO_2 for the regulation of the energy and the redox state of the tissue. During exercise the changes induced in pO_2 and thus the energy state will stimulate the respiratory rate. This might be an important link in triggering the oxidative-enzyme capacity in response to physical training as well as in peripheral vascular occlusive disease.

Increased activity of oxidative enzymes in skeletal-muscle tissue is a well-documented adaptive change in response to endurance training (Bylund *et al.*, 1977; Holloszy & Booth, 1976; Kiessling *et al.*, 1974) and has also been described as a spontaneous phenomenon in peripheral vascular occlusive disease (Bylund *et al.*, 1976; Holm *et al.*, 1972). The trigger mechanism for these changes remains unknown. A direct relationship between the intensity of daily running and the new steady-state concentration of cytochrome *c* has been shown in rats (Booth, 1977). Thus a stimulus–response relationship seems to exist, and the trigger could be any metabolic event governed by the intensity of the exercise. The observation that patients who are unable to increase the blood supply to their working muscles even during mild exercise spontaneously develop the same pattern of metabolic adaptation as in normal subjects after a period of repeated exercise suggests that the trigger could be related to the degree of hypoxia induced by exercise. In the present study metabolic changes in muscle tissue during exercise in patients with peripheral vascular occlusive disease are compared with changes found in normal subjects. Special attention has been focused on changes in intramuscular pO_2 (oxygen partial pressure) and how they relate to the energy and redox state of the tissue.

* To whom reprint requests should be addressed.

Materials and methods

Chemicals

Substrates, enzymes and cofactors for metabolite and enzyme analyses were all manufactured by Sigma (St. Louis, MO, U.S.A.).

Subjects

Ten male non-diabetic patients with peripheral vascular occlusive disease, complaining only of intermittent claudication, were studied. The mean age was 58.7 ± 9.4 (S.D.) years. The mean maximal calf blood flow, determined by venous-occlusion strain-gauge plethysmography was 13.9 ± 1.8 (S.E.M.) ml/min per 100 ml of tissue. None of the patients were taking β-blockers, but all of them were smokers.

0306-3283/81/110247-09$01.50/1

The normal subjects were ten men, aged 40.0 ± 6.9 (S.D.) years. The maximum calf blood flow was in the range 40–65 ml/min per 100 ml of tissue. They were all moderately active in different sporting activities during time off work. All but one were smokers.

Informed consent was obtained from all subjects. The study was approved by the Ethical Committee of the Medical Faculty of the University of Göteborg.

Experimental procedures

The subjects performed exercise on a foot-ergometer for 2–10 min. The intramuscular pO_2 was recorded continuously at rest, during exercise and for 10 min after exercise. Muscle biopsies were taken at rest, at the end of exercise and 10 min after cessation of work for analysis of metabolites and enzyme activities.

Exercise test

The subjects performed leg exercise in the supine position on a foot-ergometer. The exercise consisted of pressing a pedal with a work load of 8 or 12 kg per leg at a rate simulating the exercise of walking. The amplitude and the frequency of the work were recorded for both legs separately. The total work for each leg was calculated from the known distance (registered on a digital meter) and the work load [work (J) = distance (m) × 9.81 (m/s) × weight (kg)]. This type of exercise mainly involves the calf muscles and only to a minor extent the thigh muscles. The aim was to mimic the exercise of walking at maximal rate, thereby provoking the typical claudication pain in the calf region that prevents the patients from further exercise. All the patients exercised at a work load of 8 kg. The normal subjects exercised until fatigue in the calf muscle made further exercise impossible at a work load of either 8 kg or 12 kg.

Measurement of intramuscular oxygen partial pressure (pO_2)

The technique for continuous monitoring of the pO_2 during exercise has been presented in detail elsewhere (Holm & Bylund-Fellenius, 1981). A flexible catheter (diameter 1.3 mm) with an oxygen transducer mounted in the tip was used (G. D. Searle and Co., High Wycombe, Bucks., U.K.). The oxygen probe was inserted as the first procedure with the subject lying with the leg in a relaxed, comfortable position. A small incision was made under local cutaneous anaesthesia (1% Carbocain; Bofors Nobel Pharma, Karlskoga, Sweden), care being taken to avoid any injection beneath the fascia. A blunt steel cannula was then introduced 3–5 cm into the medial head of the gastrocnemius muscle. The oxygen probe was inserted through the cannula and the cannula was withdrawn. The pO_2 was recorded continuously. After the pO_2 had stabilized, which usually occurred within 1–2 min, subsequent procedures were carried out. The presence of the oxygen probe did not influence the work performance either in the patients or in the controls.

Muscle biopsies

In the first three patients the needle-biopsy technique described by Bergström (1962) was used. Since this technique gave biopsies of various sizes and the experimental set-up did not allow repeated biopsies, open surgical biopsies were taken in the other patients and in the normals. Under local cutaneous anaesthesia a 2 cm incision was made 3–4 cm proximal to the incision for the oxygen probe. For determination of metabolite concentrations, biopsies weighing 20–50 mg wet wt. were taken with a pair of scissors and immediately dropped into liquid nitrogen. The time between excision of the tissue and freezing was less than 2 s. Three biopsies were taken in each patient for metabolite analysis, one at rest, one at the end of exercise and one 10 min after cessation of work. When taking the second and third biopsies care was taken to avoid previously cut fibres.

A biopsy weighing 10–20 mg was taken before exercise for determination of enzyme activities. This biopsy was stored on ice, extracted as soon as possible (< 15 min) and analysed the same day.

Seven patients and all the normal subjects had full investigation of their muscle metabolites and enzyme activities, but in three of the patients the biopsy size only allowed determination of ATP, ADP, phosphocreatine, lactate and pyruvate.

Muscle metabolite analyses

Muscle biopsies frozen in liquid nitrogen were freeze-dried at −20°C for 24 h. The dry specimens were stored in carefully sealed glass bottles at −18°C until analysed (< 1 week). The bottles were allowed to reach room temperature before opening and extraction was started as soon as possible. The extraction procedure was performed in principle as described by Harris *et al.* (1974) for human skeletal-muscle tissue. The tissue was carefully dissected free of blood and connective tissue and minced with forceps to a powder. The muscle powder was weighed on a balance to ±0.01 mg and transferred to a conical glass tube. To 10 mg of freeze-dried tissue powder 0.2 ml of 0.5 M-$HClO_4$ containing 1 mM-EDTA was added, and extraction was then performed for 20 min in an ice bath with constant agitation. The proteins were separated by centrifugation (2000 *g* for 5 min) and the supernatant was collected and neutralized by the following procedure. After addition of 2 μl of indicator

(Bromothymol Blue + Phenol Red), 2M-KOH was added until a colour change occurred (pH 6.9–7.0). To precipitate $KClO_4$ the extracts were kept on ice for 30min and finally centrifuged (2000*g* for 10min). One part of the supernatant was then frozen in liquid nitrogen for determination of pyruvate, ADP, AMP, creatine and P_i the following day. The other part was kept on ice for determination of ATP, phosphocreatine and lactate the same day.

All metabolites were analysed fluorimetrically by enzymic methods as described by Lowry & Passonneau (1972) and were expressed per kg dry wt. Samples and standard were measured spectrophotometrically once a month. The coefficients of variation of the methods, calculated as the standard deviation of the values obtained from ten different extracts made from one muscle biopsy (obtained at rest) and expressed as a percentage of the mean value, were: ATP, 3.5%; ADP, 4.6%; AMP, 10.7%; phosphocreatine, 4.4%; creatine, 4.8%: P_i, 3.6%. The coefficients of variation for lactate (5.5%) and pyruvate (6.0%) were calculated from analyses made in duplicate on extracts of ten different muscle biopsies obtained at rest. The total adenine nucleotide content was calculated as: [ATP] + [ADP] + [AMP]. The energy-charge potential was calculated as described by Atkinson (1968):

$$\frac{[ATP] + \frac{1}{2}[ADP]}{[ATP] + [ADP] + [AMP]}$$

Enzyme analyses

For determination of the activities of phosphofructokinase (EC 2.7.1.11) and citrate synthase (EC 4.1.3.7), the muscle tissue was homogenized in 10vol. (v/w) of 50mM-Tris/HCl buffer, pH 8.2, containing 1mM-EDTA and 5mM-$MgSO_4$. The activity of phosphofructokinase was analysed in the supernatant after centrifugation at 130000*g* for 30min at 4°C, by the method of Opie & Newsholme (1967). The activity of citrate synthase was analysed in the supernatant after centrifugation at 600*g* for 10min at 4°C by the method of Shepherd & Garland (1969).

For determination of the activities of lactate dehydrogenase (EC 1.1.1.27) and 3-hydroxyacyl-CoA dehydrogenase (EC 1.1.1.35), the muscle tissue was homogenized in 10vol. of 100mM-sodium/potassium phosphate buffer, pH 7.2, containing 2mM-EDTA. Triton X-100 was added to the homogenate (final concn. 1.0%) and both enzymes were analysed in the supernatant after ultracentrifugation as above, by the method of Bass *et al.* (1969). For determination of cytochrome *c* oxidase activity (EC 1.9.3.1), the muscle tissue was homogenized in 40vol. of 0.25M-sucrose in 20mM-Tris/HCl buffer, pH 7.4. The activity of cytochrome *c* oxidase was measured polarographically as oxygen consumption with 0.20mM-cytochrome *c* as substrate, and 2mM-tetramethyl-*p*-phenylenediamine dihydrochloride and 4mM-ascorbate as reducing equivalents. The activity is given as the difference in oxygen consumption in the absence and presence of 0.6mM-KCN.

All enzyme activities were expressed per g of protein in the fraction analysed. Variation coefficients of the methods were as reported previously (Bylund *et al.*, 1977).

Statistics

Standard statistical procedures were used to calculate the mean, S.D. and S.E.M. in the different groups. The Mann–Whitney non-parametric ranking test was used for comparison between different groups (Siegel, 1956). The best curve fit to the experimental data was tested by means of a computer (Olivetti P652) programmed with six x/y functions: $y = a + bx$; $y = a + bx + cx^2$; $y = a + bx + cx^2 + dx^3$; $y = Ae^{bx}$; $y = Ax^b$; $y = Ax^b e^{cx}$.

Differences between regression coefficients and intercepts were tested by variance analysis. When the number of pairs was less than 20 the Spearman rank correlation coefficient (r_s) was also given (Siegel, 1956).

Results

The activities of phosphofructokinase and lactate dehydrogenase were not significantly different between the groups, whereas increased activities of the mitochondrial enzymes 3-hydroxyacyl-CoA dehydrogenase, citrate synthase and cytochrome *c* oxidase were found in the patients (Table 1).

The physical performance during the exercise test in terms of exercise time, total work, effect and effect per contraction is shown in Table 2 for patients and subjects.

The exercise time was considerably shorter for the patients than for the normals at a corresponding work load (8kg) because of the development of claudication pain. Thus the normals could perform 5 times more work than the patients at this work load. Increasing the work load from 8 to 12kg in the normals decreased the work time to less than that of the patients at 8kg. The total work performed by the normals during this time was, however, still more than twice as great.

Metabolite concentrations and pO_2 in the gastrocnemius muscle of patients and normal subjects at rest, at the end of exercise and after 10min of recovery are summarized in Table 3. In claudicating patients lower resting values of [ATP] and [phosphocreatine] were found than in the normal subjects, which was also reflected in a lower total adenine nucleotide pool and energy-change poten-

Table 1. *Activities of glycolytic and oxidative enzymes in gastrocnemius muscle tissue of claudicating patients and normal subjects*

Results are means ± S.E.M. Fresh muscle tissue was homogenized in 10–40 vol. of buffer and analysed immediately, spectrophotometrically or polarographically. See the Materials and methods section for further details. Abbreviation: ns, not significant ($P > 0.05$).

	Activity				
	(μmol of NADH/min per g of protein)			[(μmol of 5,5′-dithiobis-(2-nitrobenzoic acid)/min per g of protein)]	(μmol of O_2/min per g of protein)
	Phosphofructokinase	Lactate dehydrogenase	3-Hydroxyacyl-CoA dehydrogenase	Citrate synthase	Cytochrome *c* oxidase
Patients	612 ± 51	2196 ± 140	142 ± 13	176 ± 10	110 ± 21
Normal subjects	511 ± 91	1657 ± 233	96 ± 10	90 ± 9	55 ± 6
P	ns	ns	0.02	0.001	0.01

Table 2. *Physical performance during leg exercise in claudicating patients and in normal subjects*

Results are means ± S.E.M. Exercise was performed at a foot-ergometer until claudication pain in the patients, and fatigue in the normals, prevented further exercise. See the Materials and methods section for details.

	Work load (kg)	Work (kJ)	Exercise time (min)	Effect (J/s)	Effect per contraction (J/s per contraction)
Claudicating patients ($n = 7$)	8	0.79 ± 0.09	2.49 ± 0.28	5.5 ± 0.7	0.040 ± 0.005
Normal subjects ($n = 7$)	8	4.27 ± 0.31	8.11 ± 0.62	8.9 ± 0.6	0.015 ± 0.003
Normal subjects ($n = 3$)	12	1.74 ± 0.21	2.22 ± 0.06	13.2 ± 1.5	0.082 ± 0.006

tial. The variation in metabolite concentrations between individuals was generally higher in the patient group than in the normal group. The pO_2 at rest was the same in both groups.

In the claudicating patients the pO_2 decreased during exercise to about 30% of the resting value. The decrease in pO_2 was paralleled by a decrease in [ATP] and [phosphocreatine] and an increase in [lactate] and [pyruvate]. At the end of exercise, the [ATP]/[ADP] ratio was decreased and the [lactate]/[pyruvate] ratio was increased in the patients. A significant decrease in the total adenine nucleotide pool, but not in energy-charge potential, was found at the end of exercise. At 10 min after cessation of exercise the pO_2 and the [lactate]/[pyruvate] ratio were still significantly different from the resting values, whereas the other metabolite concentrations had returned to normal.

In the normal subjects a decrease in the pO_2 to about 70% of the resting value was found at the end of exercise. A concomitant decrease in the ATP concentration, the [ATP]/[ADP] ratio, total adenine nucleotide pool and energy-charge potential was found. Increases in [lactate] and [pyruvate] and in the [lactate]/[pyruvate] ratio were also found after exercise. After 10 min of recovery the pO_2 as well as the ATP concentration, [ATP]/[ADP] ratio, total adenine nucleotide pool and energy-charge potential were significantly decreased. The lactate and pyruvate concentrations were significantly increased, whereas the [lactate]/[pyruvate] ratio and the phosphocreatine concentration had returned to normal.

An increased work load from 8 to 12 kg in the controls caused a more pronounced decrease in the pO_2 during exercise. The depletion of [phosphocreatine] was more pronounced, and [lactate] increased about 3 times more than at the lower work load. In addition to the metabolite changes found at the lower work load, there were also significant changes in the concentrations of creatine and P_i. At 10 min after cessation of the exercise the pO_2 and [ATP] were still decreased, and [lactate] and [pyruvate] were still increased, compared with the resting values. In contrast with the findings at the lower work load, the [lactate]/[pyruvate] ratio was increased 10 min after exercise at the higher work load.

In the normal subjects there was a significant relationship between the pO_2 at the end of exercise and the work intensity, expressed as effect (J/s) per contraction (Fig. 1). In the patient group the corresponding relationship was not found.

The individual values for the pO_2 and the [ATP]/[ADP] ratios at rest and at the end of exercise are given in Fig. 2 for patients and controls. The best curve fit to these data was given by an exponential function as indicated in Fig. 2. There was no significant difference in the slopes or the *y* intercepts between the two study groups. The

Table 3. *Metabolite contents and intramuscular pO_2 in gastrocnemius muscle of claudicating patients and normal subjects at rest (I), at the end of exercise (II), and after 10 min of recovery (III)*

Seven patients (P) and seven normal subjects (N) performed foot-ergometer exercise at a work load of 8 kg and three normals (N′) at a work load of 12 kg. Muscle biopsies were immediately frozen in liquid nitrogen, freeze-dried and analysed fluorimetrically by use of enzymic methods. The pO_2 was continuously recorded by an oxygen transducer mounted at the tip of a flexible catheter introduced into the muscle and left inside during the exercise. See the Materials and methods section for further details. Results (mmol/kg dry wt.) are means ± S.E.M. $*P < 0.05$; $**P < 0.01$, compared with resting values. Abbreviations: ns, not significant ($P > 0.05$); PCr, phosphocreatine; Cr, creatine; ECP, energy charge potential; TAN, total adenine nucleotides; TCr total creatine.

		ATP	ADP	AMP	PCr	Cr	P_i	Lactate	Pyruvate	ATP/ADP	ECP	TAN	TCr	Lactate/Pyruvate	pO_2 (kPa)	pO_2 (mmHg)
Patients (P)	I	19.7 ±1.2	1.86 ±0.13	0.18 ±0.04	61.4 ±4.7	51.9 ±4.7	32.7 ±4.6	11.1 ±3.1	0.42 ±0.10	10.7 ±0.6	0.949 ±0.003	21.8 ±1.2	113 ±8	21.4 ±6.0	3.67 ±0.53	27.6 ±4.0
	II	16.8* ±1.1	2.07 ±0.15	0.20 ±0.02	33.5** ±7.8	68.5 ±7.9	42.7 ±6.7	32.6** ±7.5	0.67* ±0.17	8.3* ±1.0	0.939 ±0.006	18.7* ±1.2	103 ±6	56.1** ±17.4	1.22** ±0.40	9.2 ±3.0
	III	18.3 ±1.2	1.73 ±0.05	0.16 ±0.02	48.3 ±8.5	52.1 ±2.5	34.3 ±5.3	20.3 ±3.2	0.57 ±0.14	10.6 ±0.9	0.948 ±0.005	20.3 ±1.1	104 ±9	43.7* ±11.7	1.37** ±0.36	10.3 ±2.7
Normals (N)	I	23.5 ±0.9	1.88 ±0.03	0.21 ±0.01	75.3 ±4.9	49.0 ±2.6	25.9 ±2.4	8.5 ±2.0	0.27 ±0.04	12.5 ±0.6	0.956 ±0.002	25.5 ±0.9	124 ±5	30.1 ±5.7	3.83 ±0.45	28.9 ±3.4
	II	19.4** ±0.9	1.93 ±0.07	0.23 ±0.03	50.0** ±4.5	57.5 ±8.1	34.6 ±5.0	26.3** ±6.2	0.41** ±0.04	10.3** ±0.5	0.945** ±0.002	22.0** ±1.0	112 ±6	50.4* ±9.2	2.45* ±0.39	18.4 ±2.9
	III	19.7** ±0.8	1.82 ±0.06	0.21 ±0.01	70.2 ±1.4	48.8 ±4.0	29.3 ±4.9	18.0* ±3.7	0.49** ±0.05	10.9* ±0.4	0.948** ±0.001	21.7** ±0.8	119 ±5	35.6 ±5.3	2.34* ±0.48	17.6 ±3.6
Normals (N′)	I	23.7 ±0.8	1.90 ±0.04	0.15 ±0.01	81.4 ±2.9	51.3 ±3.0	27.6 ±2.2	3.05 ±0.78	0.13 ±0.03	12.5 ±0.05	0.956 ±0.004	25.9 ±0.8	133 ±4	23.7 ±5.9	3.94 ±0.24	29.6 ±1.8
	II	19.0* ±0.6	2.09 ±0.11	0.23 ±0.04	36.7* ±4.9	97.2* ±14.7	63.5* ±12.3	71.2* ±22.2	0.45* ±0.20	9.23* ±0.90	0.938* ±0.006	21.4 ±0.5	134 ±10	179* ±31	0.99* ±0.01	7.4 ±0.1
	III	18.9* ±1.1	1.93 ±0.03	0.20 ±0.02	74.8 ±3.0	52.8 ±2.9	30.0 ±2.8	29.5* ±10.1	0.48* ±0.15	9.70 ±0.36	0.944 ±0.002	21.1 ±1.2	128 ±7	60.7* ±2.7	2.33* ±1.0	17.5 ±7.5
P I versus N I: *P*		<0.05	ns	ns	<0.05	ns	ns	ns	ns	ns	<0.05	<0.05	ns	ns	ns	
P II versus N II: *P*		<0.05	ns	ns	<0.05	ns	ns	ns	<0.05	ns	ns	<0.05	ns	ns	<0.05	
P III versus N III: *P*		ns	ns	ns	<0.01	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	
N I versus N′ I: *P*		ns	ns	ns	ns	ns	ns	<0.05	<0.05	ns	ns	ns	ns	ns	ns	
N II versus N′ II: *P*		ns	ns	ns	<0.10	<0.05	<0.05	<0.01	ns	ns	ns	ns	ns	<0.01	<0.05	
N III versus N′ III: *P*		ns	ns	ns	ns	ns	ns	ns	ns	<0.05	ns	ns	ns	<0.05	ns	

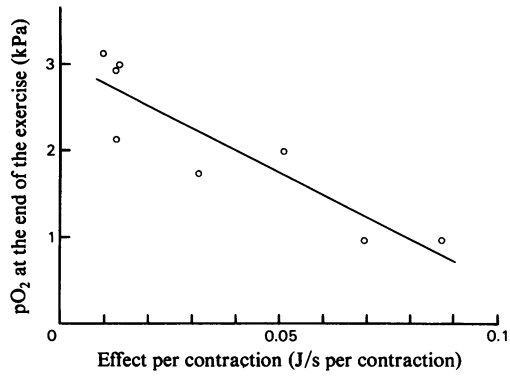

Fig. 1. *Relationship between the intensity of exercise expressed as effect (W) per contraction and the intramuscular pO_2 at the end of exercise at workloads of 8 and 12 kg in controls*

For experimental details, see the Materials and methods section. The line of best fit to the data is $y = 22.8 - 3.20x$; $r_s = 0.88$; $P < 0.005$.

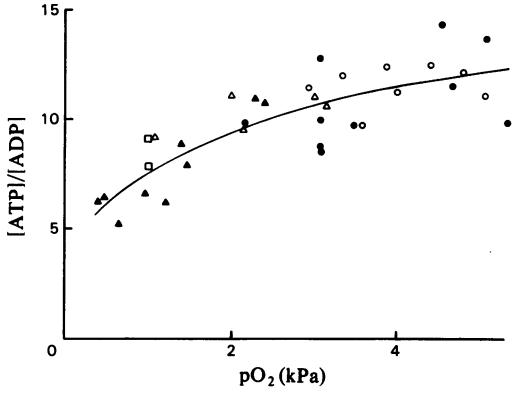

Fig. 2. *Relationship between intramuscular pO_2 and the [ATP]/[ADP] ratio in muscle tissue at rest and during exercise*

For experimental details, see the Materials and methods section. Symbols: ○, △, □, controls; ●, ▲, claudicating patients; ○, ●, rest; △, ▲, exercise with 8 kg load; □, exercise with 12 kg load. The line of best fit to the data is $y = 4.24x^{0.291}$; $r = 0.84$; $P < 0.001$.

relationship between the pO_2 and the [lactate]/[pyruvate] ratio is given in Fig. 3. The y intercepts differed significantly between the patients and the controls, as illustrated in Fig. 4. This means that the patients had a lower [lactate]/[pyruvate] ratio in the tissue at each pO_2 compared with the normal subjects.

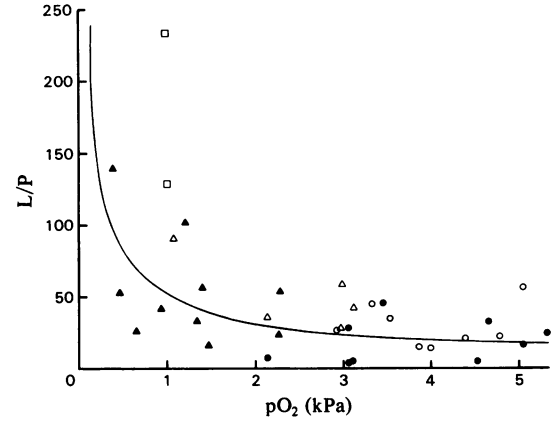

Fig. 3. *Relationship between intramuscular pO_2 and the [lactate]/[pyruvate] (L/P) ratio in muscle tissue at rest and during exercise*

For experimental details, see the Materials and methods section. Symbols are defined in Fig. 2 legend. The line of best fit to the data is $y = 205x^{-0.68}$; $r = 0.51$; $P < 0.001$.

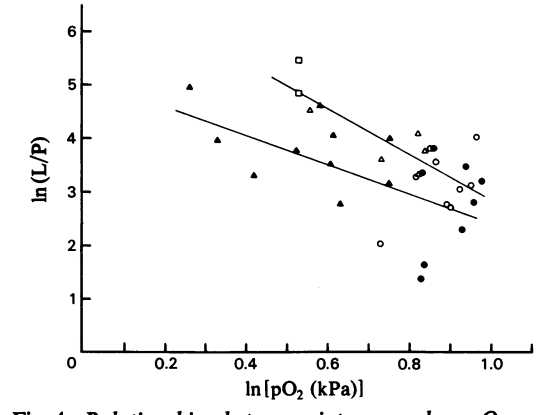

Fig. 4. *Relationship between intramuscular pO_2 and [lactate]/[pyruvate] (L/P) ratio in muscle tissue of patients and controls*

For experimental details, see the Materials and methods section. Symbols are defined in Fig. 2 legend. Lines of best fit to the data are: for controls, $y = 7.12 - 1.13x$; $r = 0.81$, $P < 0.005$; $r_s = 0.63$, $P < 0.01$; for claudicating patients, $y = 5.12 - 0.72x$; $r = 0.56$. $P < 0.025$; $r_s = 0.54$, $P < 0.01$. Difference between slopes, n.s.; difference between intercepts, $P < 0.005$.

A linear relationship was found between the pO_2 and the [phosphocreatine] as well as between the [ATP]/[ADP] ratio and the [phosphocreatine] (Figs. 5 and 6 respectively). The slopes and the y intercepts did not differ between the study groups.

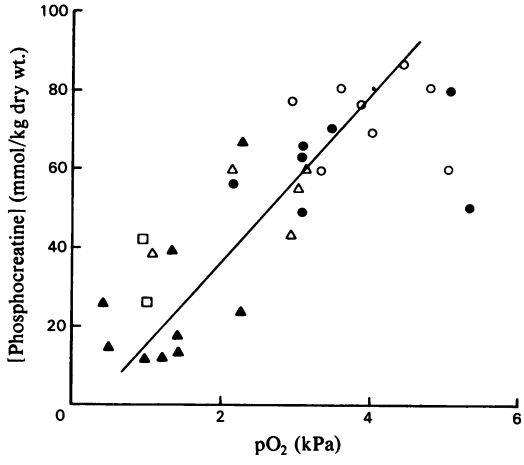

Fig. 5. *Relationship between intramuscular pO_2 and the phosphocreatine concentration in muscle tissue at rest and during exercise*

For experimental details, see the Materials and methods section. Symbols are defined in Fig. 2 legend. The line of best fit to the data is $y = 2.82x - 5.90$; $r = 0.78$; $P < 0.001$.

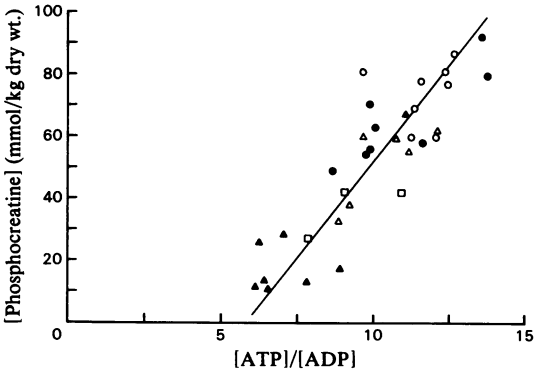

Fig. 6. *Relationship between the [ATP]/[ADP] ratio and the phosphocreatine concentration in muscle tissue at rest and during exercise*

For experimental details, see the Materials and methods section. Symbols are defined in Fig. 2 legend. The line of best fit to the data is $y = 9.92x - 46.4$; $r = 0.87$; $P < 0.005$.

Discussion

Adaptation to physical training in normal subjects is accompanied by physiological changes such as increased cardiac output, increased arterio-venous oxygen gradient and, in the muscle tissue, increased capillary density, myoglobin content and oxidative-enzyme activities (cf. Saltin & Rowell, 1980). These changes will all improve the delivery of oxygen to the muscle tissue during exercise. Associated with these adaptive changes are an increased work performance and an increased maximal oxygen uptake, which suggests that the delivery of oxygen is an important limiting factor for the metabolic function of the muscle tissue during exercise. The fact that patients with peripheral arterial insufficiency develop similar metabolic changes in the muscle tissue exposed to insufficient blood supply during exercise further suggests that a limited oxygen supply could be of importance for the development of the above-mentioned adaptive changes.

The present study was undertaken to gain information on changes in the pO_2 and the metabolic state in the muscle tissue during exercise in claudicating patients and in normal subjects.

The primary aim was to evaluate the physiological importance of the pO_2 for the energy and the redox state in the tissue. For that purpose the exercise test was designed to provoke marked changes in these respects. Thus the patients were exercised until claudication pain occurred, and the normals until fatigue in the calf muscles. An additional aim was to evaluate the physiological significance of the increased oxidative-enzyme capacity in the muscle tissue of the patients for the metabolic events during exercise.

Microelectrodes and multiwire surface electrodes have been used to measure pO_2 in human skeletal muscles at rest (Kunze, 1977; Lund *et al.*, 1980; Lübbers, 1977). These techniques are not suitable for measurements during exercise, however. In the present study we have used a flexible catheter with an oxygen electrode mounted in the tip, inserted under sterile conditions and left inside the muscle during exercise. The resting oxygen partial pressures found by us agreed well with the mean resting values in muscle reported with other techniques (Hauss *et al.*, 1978; Kunze, 1977; Lund *et al.*, 1980).

There was no difference in the resting pO_2 between claudicating patients and normal subjects. This is in line with other reports (Jussila *et al.*, 1978) and fits with the normal resting blood flow and lack of symptoms of these patients at rest.

During exercise at 8 kg a decrease in the pO_2 was observed in both patients and normal subjects. The decrease in the claudicating patients was more pronounced than in the normals, in spite of the shorter exercise time and lesser amount of exercise performed by the patients. In the normals a more pronounced decrease in the pO_2 was found when the work load was increased.

At a certain work intensity the pO_2 in the muscle tissue will be at a steady-state value determined by the balance between the oxygen delivery and the oxygen consumption. The duration of the contractions relative to that of the relaxation is of

importance for the local blood flow and hence for the delivery of oxygen to the tissue (Folkow & Halicka, 1968), which explains the significant correlation obtained in normals between the pO_2 at the end of exercise and the work, expressed as effect per contraction. In the patients a corresponding correlation was not found. The pO_2 was, however, lower in each patient at a certain work load than expected from the relationship obtained in normal subjects. Since these patients have a lower blood flow in response to exercise than do normals, this phenomenon can be explained by the decreased oxygen delivery. Thus, in the individual patient the pO_2 in the muscle tissue at a certain work intensity will be dependent on the magnitude of blood-flow impairment.

Relationships between the pO_2 and various metabolite concentrations, reflecting the energy and the redox state in the tissue, were found. This suggests that the pO_2 in the extracellular compartment is of importance for the metabolic state in the intracellular compartment in muscle tissue. This of course also strengthens the validity of the intramuscular-pO_2 measurements obtained with the technique used in this study.

The relationship obtained between pO_2 and the [ATP]/[ADP] ratio was in close agreement with that reported by Wilson *et al.* (1979), for cultured cells exposed to different oxygen partial pressures *in vitro*. This supports the causal relationship between the pO_2 and the energy state in intact human skeletal muscle. According to Wilson *et al.* (1979), the decrease in the [ATP]/[ADP] ratio can be considered as a regulatory mechanism which maintains the respiratory rate when the oxygen supply becomes limited.

According to Ferenczi *et al.* (1978), about 50% of the ADP in the muscle cell is covalently bound to actin, which makes it unavailable to metabolic reactions. When the total amount of ADP is extracted, this will reflect both the free and the bound ADP in the cell. In the present study 0.5 M-$HClO_4$ was used for the extraction of metabolites. The ADP values obtained by this procedure were lower than those reported by others (Harris *et al.*, 1974). Comparison of ADP values after extraction with 0.5 M- and 2 M-$HClO_4$ reveals about 40% lower values by the former procedure (A.-C. Bylund-Fellenius, A. Elander & T. Scherstén, unpublished work). It seems reasonable therefore to assume that the ADP extracted by the low $HClO_4$ concentration is equivalent to the ADP that is not bound to proteins, thus representing the freely available pool of ADP in the cell.

Equilibrium between the phosphorylation state of the adenine dinucleotide system and the redox state of the cytoplasmic NAD couple has been demonstrated in liver tissue (Stubbs *et al.*, 1972). In this equilibrium the oxidative phosphorylation is considered as the master process controlling the redox state. Assuming that these findings are also relevant for muscle tissue, the relationship found between the pO_2 and the [lactate]/[pyruvate] ratio can be considered as mediated by the changed [ATP]/[ADP] ratio. A direct relationship was also found between the [ATP]/[ADP] ratio and the lactate content (results not shown), confirming the previous findings of Harris *et al.* (1977) and Sahlin (1978). These authors interpreted this relationship as a steady state regulated by the intracellular pH. Our results, however, suggest that the [ATP]/[ADP] ratio is determined by the pO_2 in the tissue, and that the changes in lactate content and pH are secondary events.

The correlation between the pO_2 and [phosphocreatine] can be explained by the equilibrium maintained by creatine kinase, since a direct relationship was also found between the [ATP]/[ADP] ratio and [phosphocreatine].

Previously we have reported increased activities of key enzymes in the β-oxidation pathway, citric acid cycle and electron-transport chain in claudicating patients (Bylund *et al.*, 1976; Holm *et al.*, 1972). This was also found in the present series (Table 1). One aim of the present study was to evaluate the physiological significance of this increased oxidative enzyme capacity during exercise. From Fig. 4 it can be concluded that the patients were able to maintain a lower [lactate]/[pyruvate] ratio at each pO_2 as compared with the controls. This is most likely a consequence of the increased capacity for oxidative metabolism in the muscle tissue of the patients. The increased oxidative capacity will allow the patients to maintain a higher oxidative rate at each pO_2 and thereby a lower lactate production.

A decrease in the [ATP]/[ADP] ratio acts as a stimulus for the respiratory rate (Sussman *et al.*, 1980; Wilson *et al.*, 1979), which is one of the metabolic functions known to change its capacity in response to physical training in normal subjects, and spontaneously in claudicating patients. The present findings suggest that the decrease in the pO_2 induced during exercise directly affects the [ATP]/[ADP] ratio in the muscle tissue. A frequent stimulation of the respiratory rate by this mechanism might be an important link in triggering the increase in the oxidative-enzyme capacity found in response to physical training as well as in peripheral vascular occlusive disease.

This work was supported by the Swedish Medical Research Council (project no. 536). Technical assistance by Mrs. Marianne Andersson, Mrs. Anette Arvidsson, Miss Marianne Davidsson and Mrs. Margareta Jonsson is gratefully acknowledged. The excellent secretarial assistance by Miss Annika Ring and Mrs. Martha Tellstedt is also acknowledged.

References

Atkinson, D. E. (1968) *Biochemistry* **7**, 4030–4034

Bass, A., Brdiczka, D., Eyer, P., Hofer, S. & Pette, D. (1969) *Eur. J. Clin. Invest.* **10**, 198–206

Bergström, J. (1962) *Scand. J. Clin. Lab. Invest.* **14**, *Suppl.* **68**

Booth, F. (1977) *Ann. N.Y. Acad. Sci.* **301**, 431–439

Bylund, A.-C., Hammarsten, J., Holm, J. & Scherstén, T. (1976) *Eur. J. Clin. Invest.* **6**, 425–429

Bylund, A.-C., Bjurö, T., Cederblad, G., Holm, J., Lundholm, K., Sjöström, M., Ängquist, K. A. & Scherstén, T. (1977) *Eur. J. Appl. Physiol.* **36**, 151–169

Ferenczi, M. A., Homsher, E., Simmons, R. M. & Trentham, D. R. (1978) *Biochem. J.* **171**, 165–175

Folkow, B. & Halicka, H. D. (1968) *Microvasc. Res.* **1**, 1–14

Harris, R. C., Hultman, E. & Nordesjö, L.-O. (1974) *Scand. J. Clin. Lab. Invest.* **33**, 109–120

Harris, R. C., Sahlin, K. & Hultman, E. (1977) *J. Appl. Physiol.* **43**, 852–857

Hauss, J., Schönleben, K., Spiegel, U. & Kessler, M. (1978) *Adv. Exp. Med. Biol.* **94**, 401–418

Holloszy, J.-O. & Booth, F. W. (1976) *Annu. Rev. Physiol.* **38**, 273–291

Holm, J., Björntorp, P. & Scherstén, T. (1972) *Eur. J. Clin. Invest.* **2**, 321–325

Holm, S. & Bylund-Fellenius, A.-C. (1981) *Clin. Physiol.* in the press

Jussila, E., Niinikoski, J. & Inberg, M. W. (1978) *Adv. Exp. Med. Biol.* **94**, 623–628

Kiessling, K.-H., Pilström, L., Bylund, A.-C., Saltin, B. & Piehl, K. (1974) *Scand. J. Clin. Lab. Invest.* **33**, 63–69

Kunze, K. (1977) *Bibl. Anat.* **15**, 386–391

Lowry, O. H. & Passonneau, J. V. (1972) *A Flexible System of Enzymatic Analysis*, pp. 146–160, Academic Press, New York

Lübbers, D. W. (1977) in *Oxygen and Physiological Function* (Jöbsis, F. F., ed.), pp. 254–276, Professional Information Library, Dallas

Lund, N., Jorfeldt, L. & Lewis, D. H. (1980) *Acta Anaesthesiol. Scand.* **24**, 272–278

Opie, L. H. & Newsholme, E. A. (1967) *Biochem. J.* **103**, 391–399

Sahlin, K. (1978) *Acta Physiol. Scand. Suppl.* **455**, 1–56

Saltin, B. & Rowell, L. B. (1980) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **39**, 1506–1513

Shepherd, D. & Garland, P. B. (1969) *Methods Enzymol.* **13**, 11–17

Siegel, S. (1956) *Non-Parametric Statistics for the Behavioral Sciences*, pp. 116–127, McGraw-Hill, New York

Stubbs, M., Veech, R. L. & Krebs, H. A. (1972) *Biochem. J.* **126**, 59–65

Sussman, I., Erecinska, M. & Wilson, D. F. (1980) *Biochim. Biophys. Acta* **591**, 209–223

Wilson, D. F., Erecinska, M., Drown, C. & Silver, I. A. (1979) *Arch. Biochem. Biophys.* **195**, 485–493