Biochem. J. (1987) 245, 551–556 (Printed in Great Britain)

Redox state and lactate accumulation in human skeletal muscle during dynamic exercise

Kent SAHLIN*, Abram KATZ*† and Jan HENRIKSSON‡
*Department of Clinical Physiology, Karolinska Institute, Huddinge Hospital, S-141 86 Huddinge, Sweden, and
‡Department of Physiology III, Karolinska Institute, S-114 33 Stockholm, Sweden

The relationship between the redox state and lactate accumulation in contracting human skeletal muscle was investigated. Ten men performed bicycle exercise for 10 min at 40 and 75% of maximal oxygen uptake [$\dot{V}O_2$(max.)], and to fatigue (4.8 ± 0.6 min; mean ± S.E.M.) at 100% $\dot{V}O_2$(max.). Biopsies from the quadriceps femoris muscle were analysed for NADH, high-energy phosphates and glycolytic intermediates. Muscle NADH was 0.20 ± 0.02 mmol/kg dry wt. of muscle at rest, and decreased to 0.12 ± 0.01 ($P < 0.01$) after exercise at 40% $\dot{V}O_2$(max.), but no change occurred in the [lactate]/[pyruvate] ratio. These data, together with previous results on isolated cyanide-poisoned soleus muscle, where NADH increased while [lactate]/[pyruvate] ratio was unchanged [Sahlin & Katz (1986) Biochem. J. **239**, 245–248], suggest that the observed changes in muscle NADH occurred within the mitochondria. After exercise at 75 and 100% $\dot{V}O_2$(max.), muscle NADH increased above the value at rest to 0.27 ± 0.03 ($P < 0.05$) and 0.32 ± 0.04 ($P < 0.001$) mmol/kg respectively. Muscle lactate was unchanged after exercise at 40% $\dot{V}O_2$(max.), but increased substantially at the higher work loads. At 40% $\dot{V}O_2$(max.), phosphocreatine decreased by 11% compared with the values at rest, and decreased further at the higher work loads. The decrease in phosphocreatine reflects increased ADP and P_i. It is concluded that muscle NADH decreases during low-intensity exercise, but increases above the value at rest during high-intensity exercise. The increase in muscle NADH is consistent with the hypothesis that the accelerated lactate production during submaximal exercise is due to a limited availability of O_2 in the contracting muscle. It is suggested that the increases in NADH, ADP and P_i are metabolic adaptations, which primarily serve to activate the aerobic ATP production, and that the increased anaerobic energy production (phosphocreatine breakdown and lactate formation) is a consequence of these changes.

INTRODUCTION

It has long been known that lactate formation in contracting muscle increases before the aerobic capacity is fully exploited. Thus increases in blood lactate occur already at exercise intensities corresponding to 50 and 60–65% of the maximal oxygen uptake, $\dot{V}O_2$(max.), in untrained and endurance-trained men respectively (Åstrand & Rodahl, 1970). The reason(s) for this early enhancement of lactate production is not known. One hypothesis is that hypoxia occurs in part of the working muscle. More recent findings in contracting dog skeletal muscle have, however, shown that the O_2 partial pressure within the muscle was far above that where cellular respiration was diminished, although lactate was produced (Connett *et al.*, 1984). In the present paper, the term 'hypoxia' is used to denote a condition where the metabolism is affected by a decreased O_2 partial pressure, but where the rate of O_2 consumption is not necessarily diminished.

Hypoxia at the cellular level would result in a reduction of the respiratory chain, including the mitochondrial NAD^+ to NADH. The redox level of mitochondria is known to be much more reduced than that of the cytosol, but apparently it is not possible to separate these compartments without affecting their redox levels (Williamson *et al.*, 1967; Jacobson & Kaplan, 1957). Furthermore, the alternative technique of using mitochondrial redox reactions to calculate the mitochondrial [NAD^+]/[NADH] ratio demands assumptions which are questionable (for a full discussion see Akerboom *et al.*, 1979). Data from cyanide-poisoned (Sahlin & Katz, 1986) and ischaemic (Sahlin, 1983) muscle, however, support the notion that changes in whole-muscle NADH primarily reflect changes within the mitochondria. Measurements of changes in total muscle NADH will therefore give information about changes in the mitochondrial redox state. When the redox state in dog skeletal muscle was monitored by surface fluorimetry, it was found that at rest the fluorescence (i.e. the NADH concentration) was close to maximal and that during contraction the fluorescence was decreased, rather than increased, although lactate was produced (Jöbsis & Stainsby, 1968). This decrease in fluorescence was considered to be due to an oxidation of mitochondrial NADH and has been used as evidence against the hypothesis that lactate formation during submaximal exercise is caused by hypoxia (Jöbsis & Stainsby, 1968).

It has been shown that, in human muscle, the chemically determined NADH concentration increased markedly after intense bicycle exercise (Sahlin, 1985) and after isometric contraction (Henriksson *et al.*, 1986). The influence of dynamic exercise at submaximal intensities on muscle NADH has not been previously studied. The

Abbreviation used: $\dot{V}O_2$(max.), maximal oxygen uptake.

† Present address: Clinical Diabetes and Nutrition Section, NIH/NIDDK, Phoenix, AZ 85016, U.S.A.

purpose of the present study was therefore to determine the muscle NADH content after submaximal exercise and to investigate whether lactate accumulation is related to the redox state of the muscle.

MATERIALS AND METHODS

Subjects

Ten healthy men participated in the study. Eight of the subjects were physical education students, of whom seven performed physical training during their leisure time in addition to the required physical activity at school. The subjects' mean (range) age, height, weight and $\dot{V}O_2$(max.) were respectively: 27 years (22–31), 182 cm (174–191), 76 kg (71–85) and 4.1 l/min (3.3–4.9). The subjects were informed about the possible risks of the study before giving their voluntary consent. The experimental protocol was approved by the Ethical Committee of the Karolinska Institute at Huddinge University Hospital.

Experimental

Before the experiment (on two separate occasions) each subject performed an exercise test (50 rev./min) in the upright position on a mechanically braked cycle ergometer (Monark) to determine $\dot{V}O_2$(max.) [for criteria of $\dot{V}O_2$(max.) see Åstrand & Rodahl, 1970]. Expired air was analysed for O_2 and CO_2 percentages in a mass spectrometer.

Subjects rested in the supine position while skin anaesthesia was applied (1–2 ml of Citanest, 10 mg/ml) and incisions at each of four muscle biopsy sites were made. The first biopsy was taken approx. 15 min later. Muscle samples were taken with the needle-biopsy technique (Bergström, 1962) from the lateral aspect of the quadriceps femoris muscle at approx. 35% of the distance from the upper margin of the patella to the anterior superior iliac spine (two in each leg, 3 cm between sites). The muscle samples were rapidly frozen in the needle in Freon maintained at its freezing point with liquid N_2.

Subjects performed incremental bicycle exercise at intensities calculated to elicit 40 and 75% of $\dot{V}O_2$(max.) for 10 min each, and at 100% $\dot{V}O_2$(max.) (292 ± 13 W; mean ± S.E.M) to fatigue (4.8 ± 0.6 min). Muscle samples were taken after each exercise bout while the subject remained on the bicycle. The time between termination of exercise and freezing the muscle sample was about 10 s, and the time between termination of exercise and start of exercise at the subsequent higher work load was 30–60 s. Capillary blood samples for lactate determination were taken from a prewarmed fingertip at rest, after 5 and 9 min of submaximal exercise and 5 min after termination of exercise to fatigue, when the peak blood lactate value was expected (Åstrand & Rodahl, 1970).

Analytical methods

The frozen muscle samples (stored in liquid N_2) were freeze-dried, dissected free from connective tissue and blood and powdered. The muscle powder was divided into two lots. One lot, weighing 1–2 mg, was extracted with a solution containing KOH (500 mM), ethanol (50%, v/v) and cysteine (5 mM), and, after neutralization with HCl, was used for the assay of NADH with a previously described bioluminescence method (Sahlin, 1983). The remaining powder (3–6 mg) was extracted with $HClO_4$ (0.5 M). After neutralization with $KHCO_3$, the extract was used for fluorimetric assays for NAD^+ (Grassl & Möllering, 1974) and creatine, phosphocreatine, ATP, ADP, AMP, pyruvate, lactate, glucose 6-phosphate and glycerol 3-phosphate (modified from Lowry & Passonneau, 1972). The muscle samples will contain, in addition to muscle, a variable amount of blood, connective tissue and fat. Although the majority of these non-muscle constituents is removed during the dissection procedure, some will remain and cause an increased intra-individual variance in the metabolite contents. To lessen this variance we have used total creatine (= phosphocreatine + creatine) as a reference base. The data were converted into mmol/kg dry wt. of muscle by multiplying by the peak total creatine value for each subject. Total creatine was not significantly changed ($P > 0.05$) during exercise, being 103 ± 3, 106 ± 3, 108 ± 3 and 108 ± 4 mmol/kg at rest and at 40%, 75% and 100% $\dot{V}O_2$(max.) respectively. Muscle glucose, lactate and pyruvate values were not adjusted, since these metabolites are also present in the extracellular compartment, and thus any adjustment would result in falsely elevated intracellular values. Blood lactate was assayed colorimetrically by the modified Barker–Summerson method (Ström, 1949).

Statistical methods

For statistical evaluation a one-way ANOVA was employed. When the ANOVA resulted in a significant F value ($P < 0.05$), the difference between means was located with the Newman–Keul test. Linear-regression analysis was performed with the method of least squares. Values are reported as means ± S.E.M.

RESULTS

NADH and glycolytic intermediates

Muscle content of NADH was 0.20 ± 0.02 mmol/kg dry wt. of muscle at rest, and decreased to 0.12 ± 0.01 after exercise at 40% $\dot{V}O_2$(max.) ($P < 0.01$, Fig. 1). Exercise at 75% $\dot{V}O_2$(max.) resulted in a significant increase in NADH above that at rest (to 0.27 ± 0.03 mmol/kg; $P < 0.05$) and to 0.32 ± 0.04 at fatigue [100% $\dot{V}O_2$(max.); $P < 0.001$ versus rest]. No significant change ($P > 0.05$) was observed in muscle NAD^+ in response to exercise (1.92 ± 0.07 at rest and 1.98 ± 0.06 mmol/kg at fatigue).

Blood [lactate] at 40% $\dot{V}O_2$(max.) was similar to the value at rest (1.4 ± 0.1 mM; $P > 0.05$), but was significantly increased after 5 and 9 min of exercise at 75% $\dot{V}O_2$(max.) (3.8 ± 0.5 and 4.3 ± 0.6 mM respectively; $P < 0.001$). The similarity between the last two values suggests that an approximate steady state was reached in the release from the exercising muscle and the uptake by resting muscle and other tissues. Blood [lactate] 5 min after exercise was 11.3 ± 0.4 mM.

Muscle [lactate] was unchanged after exercise at 40% $\dot{V}O_2$(max.), but increased substantially at the higher work loads (Table 2). Muscle content of glycerol 3-phosphate was unchanged after exercise at 40% $\dot{V}O_2$(max.), but increased in parallel with lactate at the higher work loads (Tables 1 and 2). The lactate content at fatigue was, however, more than 10 times higher than that of glycerol 3-phosphate.

Muscle pyruvate was unchanged at 40% $\dot{V}O_2$(max.),

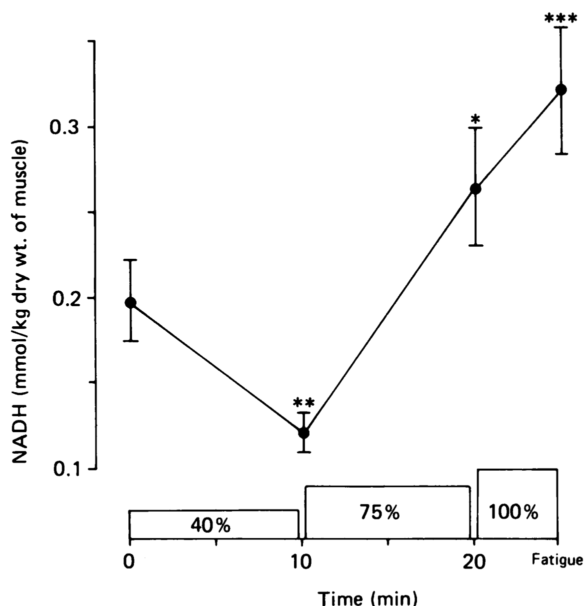

Fig. 1. Muscle content of NADH at rest and after exercise at work loads corresponding to 40, 75 and 100% $\dot{V}O_2$(max.)

Exercise at 100% $\dot{V}O_2$(max.) was continued to fatigue (4.8 ± 0.6 min). Values are means ± S.E.M. from ten subjects. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ versus rest.

but increased about 3-fold after exercise at 75% $\dot{V}O_2$(max.) (Table 2). No further increase in pyruvate occurred when exercise was performed at 100% $\dot{V}O_2$(max.) to fatigue.

The [lactate]/[pyruvate] ratio was unchanged after exercise at 40% $\dot{V}O_2$(max.), but increased at the higher work loads (Table 2). The muscle contents of lactate and glycerol 3-phosphate for each subject after 10 min of exercise at 75% $\dot{V}O_2$(max.) were both related to the corresponding [lactate]/[pyruvate] ratio (Fig. 2).

Glucose 6-phosphate increased 2-fold already at 40% $\dot{V}O_2$(max.) and increased further at the higher work loads, reaching 6.5 ± 0.3 mmol/kg dry wt. of muscle at fatigue (Table 1).

High-energy phosphates

Phosphocreatine content in muscle (79.2 ± 2.5 mmol/kg dry wt. of muscle at rest) decreased by 11% already after exercise at 40% $\dot{V}O_2$(max.) ($P < 0.01$) and decreased further at the higher exercise intensities (Table 1). The decrease in phosphocreatine was reflected by an equal increase in creatine. ATP was unchanged during submaximal exercise, but was decreased by 21% at fatigue. ADP increased significantly at 75 and 100% $\dot{V}O_2$(max.) (Table 1). The increase in inorganic phosphate (ΔP_i) has been calculated from the changes in phosphocreatine, ATP, ADP, glucose 6-phosphate and glycerol 3-phosphate, which are known to represent the major changes in phosphate-containing metabolites during exercise. ΔP_i increased progressively with increasing work load (Table 1).

DISCUSSION

It is well known from studies on liver tissue that at least two separate pools of NAD^+/NADH exist in the cell (mitochondrial and cytoplasmic) (Williamson *et al.*, 1967). Extraction of the muscle with KOH (the present study) will include NADH from all cellular compartments, whether free or bound to proteins. It is, however, likely that observed changes in muscle NADH reflect an altered mitochondrial rather than cytosolic NADH content [see the synopsis and the Introduction, as well as Sahlin & Katz (1986) and Henriksson *et al.* (1986)].

The reduction state of the nicotinamide nucleotides (i.e. NADH and NADPH) during muscle contraction has previously been measured with the surface fluorescence technique (Chance & Jöbsis, 1959; Jöbsis & Stainsby, 1968; Wendt & Chapman, 1976). The basis of this method is that light in the 366 nm region, when focused on the surface of an exposed muscle, elicits fluorescence from NADH and NADPH. Observations of the surface fluorescence from dog gastrocnemius (*in situ*)

Table 1. Phosphate compounds and glycolytic intermediates (mmol/kg dry wt. of muscle) at rest and after exercise

Values are means ± S.E.M. from ten subjects: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ versus rest.

		Exercise [(% $\dot{V}O_2$(max.)]		
	Rest	40	75	100
Phosphocreatine (PCr)	79.2 ± 2.5	70.7 ± 2.0**	33.7 ± 2.9***	16.6 ± 4.9***
Creatine	34.7 ± 2.5	43.2 ± 1.7*	80.2 ± 4.6***	97.3 ± 3.3***
ATP	25.2 ± 0.5	24.3 ± 0.5	24.2 ± 0.4	19.9 ± 0.6***
ADP	3.45 ± 0.12	3.33 ± 0.10	3.64 ± 0.14*	3.91 ± 0.10***
AMP	0.13 ± 0.01	0.14 ± 0.01	0.14 ± 0.01	0.15 ± 0.01
Glycerol 3-phosphate	0.32 ± 0.06	0.40 ± 0.09	3.30 ± 0.41***	7.57 ± 0.39***
Glucose 6-phosphate	0.81 ± 0.24	1.72 ± 0.27***	2.85 ± 0.31***	6.50 ± 0.28***
Glucose†	1.25 ± 0.29	0.35 ± 0.15	2.33 ± 0.53	9.90 ± 0.54***
ΔP_i‡	0	9.3 ± 1.1***	42.3 ± 3.9***	59.7 ± 4.0***
PCr/Cr	2.34 ± 0.15	1.66 ± 0.09***	0.45 ± 0.06***	0.18 ± 0.02***

† Glucose values are the intracellular concentration and were derived by subtracting the extracellular glucose from total muscle glucose. Extracellular glucose concentration was assumed to be 5 mM and extracellular water content to be 0.3, 0.48, 0.52 and 0.56 l/kg dry wt. of muscle at rest and at 40, 75 and 100% $\dot{V}O_2$(max.) respectively (Sjøgaard *et al.*, 1985; value at 75% was obtained by interpolation).

‡$\Delta P_i = -\Delta PCr - 2\Delta ATP - \Delta ADP - \Delta$glucose 6-phosphate $- \Delta$glycerol 3-phosphate.

Table 2. Muscle contents of lactate and pyruvate (mmol/kg dry wt. of muscle), calculated cytosolic [NADH]/[NAD$^+$] ratio and H$^+$ concentration at rest and after exercise

Values are means ± S.E.M. from ten subjects: ***$P < 0.001$ versus rest.

	Rest	Exercise [% $\dot{V}O_2$(max.)]		
		40	75	100
Lactate	1.8±0.2	2.7±0.6	33.5±5.1***	100.0±5.5***
Pyruvate	0.13±0.01	0.19±0.02	0.46±0.02***	0.46±0.04***
Lactate/pyruvate	14.9±1.7	15.5±4.6	73.2±10.4	247.2±37.5***
$10^7 \times$ [H$^+$]*	0.89	0.89	1.20	2.26
$10^4 \times$ (NADH/NAD$^+$)†	18.6	19.3	67.7	121.4

* M, calculated from the mean values of lactate + pyruvate and the relationship between muscle pH and lactate + pyruvate previously described (Sahlin *et al.*, 1976).

† Calculated from the lactate dehydrogenase equilibrium, $K_{eq.}$ = [NADH]/[NAD$^+$] × [pyruvate]/[lactate] × [H$^+$], assuming $K_{eq.} = 1.11 \times 10^{-11}$ M (Williamson *et al.*, 1967).

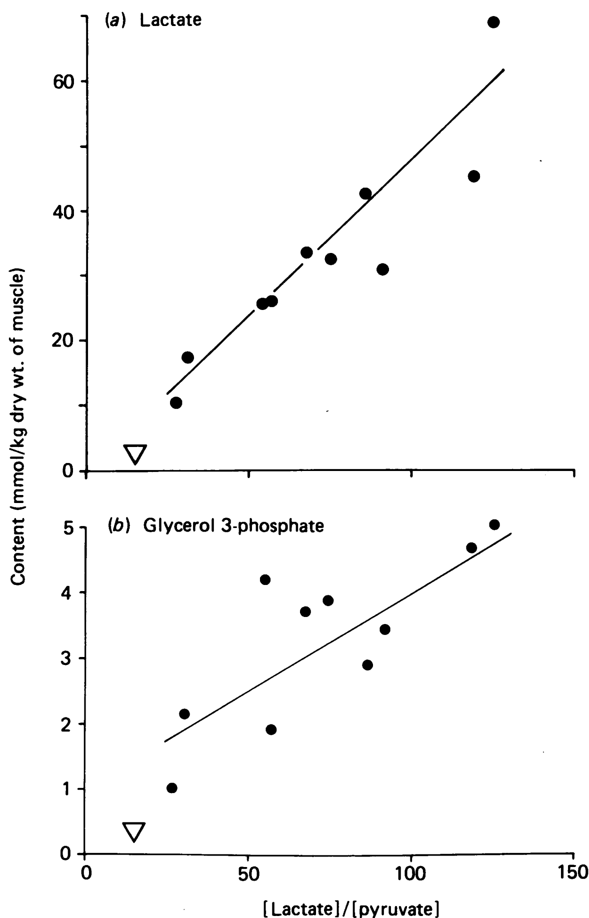

Fig. 2. Relationship between [lactate]/[pyruvate] ratio and lactate (*a*) and glycerol 3-phosphate (*b*) in muscle after exercise at 75% $\dot{V}O_2$(max.)

Mean values at rest and at 40% $\dot{V}O_2$(max.) are shown (▽). Linear-regression analysis showed:

Lactate = 0.45 [lactate]/[pyruvate] + 0.73

Glycerol 3-phosphate = 0.03 [lactate]/[pyruvate] + 0.99

$r = 0.81$, $P < 0.05$. The mean values of glycerol 3-phosphate and lactate at fatigue (7.6 and 100 mmol/kg dry wt. of muscle respectively) corresponded closely to the values expected from the linear regressions and the [lactate]/[pyruvate] ratios.

and gracilis (*in situ* and perfused) muscles have shown that, at rest, fluorescence is nearly maximal and that during supramaximal nerve stimulation at 5 Hz the fluorescence is decreased by 5–25% (Jöbsis & Stainsby, 1968). In fact, even during N_2 breathing, contraction resulted in a fluorescence that was lower than that at rest. From these results it was concluded that the oxygen supply was not limiting for the activity of the respiratory chain. These data have been used as evidence that lactate production during submaximal exercise is not caused by hypoxia (Jöbsis & Stainsby, 1968). The decrease in NADH that we observed after low-intensity exercise [40% $\dot{V}O_2$(max.)] appears to be in accordance with the decrease in surface fluorescence during muscle contraction (Chance & Jöbsis, 1959; Jöbsis & Stainsby, 1968). An increase in ADP will increase respiration and decrease the reduction level in the respiratory chain (Chance & Jöbsis, 1959). Hence the oxidation of NADH is probably explained by a contraction-induced increase in ADP (see below). The relative exercise intensity in the present study [40% $\dot{V}O_2$(max.)] was, however, low, and no lactate accumulation occurred in muscle or blood. This is in contrast with the study by Jöbsis & Stainsby (1968), where (as stated by the authors) the work load was high enough to increase the production of lactate.

In the present study, the increase in NADH at 75% $\dot{V}O_2$(max.) above the value at rest coincided with an accumulation of lactate, and is therefore consistent with the hypothesis that there is a limited availability of O_2 in at least part of the muscle. The increase in NADH above the value at rest at high exercise intensities is in contrast with the surface-fluorescence data obtained by Jöbsis & Stainsby (1968). The possible reasons why results from surface-fluorescence measurements differ from those of direct chemical NADH analysis have been discussed previously (Henriksson *et al.*, 1986), where it was concluded that reflectance fluorimetry does not provide quantitative estimates of muscle NADH.

Anaerobic energy utilization

In the present study, phosphocreatine was found to decrease progressively when the work load increased (Table 1), which is in agreement with previous findings (Hultman *et al.*, 1967). It can be calculated, however, that the amount of ATP derived from phosphocreatine

breakdown is only 1, 3 and 6% of that produced aerobically at 40 (10 min), 75 (10 min) and 100% $\dot{V}O_2$(max.) respectively. Phosphocreatine will therefore be of minor importance as an energy source during the present conditions. Furthermore, during prolonged exercise at a submaximal workload, phosphocreatine has been shown to decrease initially, but thereafter remains constant (Hultman *et al.*, 1967).

From the increased lactate in muscle and blood at 75 and 100% $\dot{V}O_2$(max.), it is clear that pyruvate formation is greatly accelerated and in excess of what is oxidized within the mitochondria. During steady-state conditions (Jorfeldt *et al.*, 1978) it can, however, be calculated (based on lactate release) that the formation of lactate contributes only about 2% of the aerobic ATP production at 71% $\dot{V}O_2$(max.). It can thus be concluded that the quantitative importance of energy production from both lactate formation and phosphocreatine breakdown at steady-state submaximal work is rather small.

During exercise the aerobic ATP production increases manyfold. The stimulus for this is likely to be increases of ADP and P_i (Chance, 1965; Wilson *et al.*, 1977). From Table 1 it is clear that a progressive increase in P_i occurs, and further, that free ADP, which is related to the decrease in phosphocreatine/creatine (Veech *et al.*, 1979), also increases progressively. Therefore we propose that the significance of phosphocreatine breakdown during dynamic steady-state exercise is more related to the activation of aerobic ATP production than to the energy release as such. This is further indicated by the observed decrease in phosphocreatine at 40% $\dot{V}O_2$(max.), which in combination with the oxidation of muscle NADH (Fig. 1) demonstrates that decreases in phosphocreatine can occur under fully aerobic conditions.

The increase in ADP will also result in increased AMP values (owing to the adenylate kinase reaction), which, together with the increase in P_i, will activate glycogenolysis (Newsholme & Start, 1974; Chasiotis *et al.*, 1982) and glycolysis (Newsholme & Start, 1974). The increased rate of pyruvate formation is, from this point of view, a consequence of the activation of aerobic ATP production. Similar ideas have been discussed by Holloszy & Booth (1976). They suggested that the lower rate of lactate production in a trained muscle was due to a lower ADP concentration necessary to attain the same rate of O_2 utilization when the mitochondrial density is increased.

At low exercise intensities [40% $\dot{V}O_2$(max.)] muscle NADH decreases, indicating that the respiratory chain is stimulated to a greater extent than the formation of NADH in the tricarboxylic acid cycle. The increase in NADH at the higher exercise intensities could be explained by a relatively low mitochondrial O_2 partial pressure, which affects the oxidation of NADH. An increase in NADH has been suggested (in addition to the increases in ADP and P_i) to be one mechanism whereby the cell adapts to a high rate of oxygen utilization when the O_2 partial pressure becomes limiting (Wilson *et al.*, 1977).

Cytosolic NADH and lactate formation

The lactate dehydrogenase reaction (pyruvate + NADH + $H^+ \rightleftharpoons$ lactate + NAD^+) is considered to be near equilibrium (Newsholme & Start, 1974), and lactate will be formed through the Law of Mass Action when pyruvate, cytosolic [NADH]/[NAD^+] or H^+ is increased. The intracellular concentration of H^+ increases during intensive exercise (Table 2), but is a consequence rather than the cause of the increased lactate concentration. Both muscle pyruvate and calculated cytosolic [NADH]/[NAD^+] are increased at 75% $\dot{V}O_2$(max.) (Table 2) and appear to be of importance for lactate formation. At 100% $\dot{V}O_2$(max.) no further increase in pyruvate occurs, whereas the calculated cytosolic [NADH]/[NAD^+] continues to increase (Table 2). These data suggest that the increase in cytosolic NADH is the major cause for the increased conversion of pyruvate into lactate in muscle during intense exercise. The increase in glycerol 3-phosphate (Table 1) and the relation between [glycerol 3-phosphate] and the [lactate]/[pyruvate] ratio (Fig. 2) are probably due to the increase in cytosolic [NADH]/[NAD^+] affecting the equilibrium of the glycerol 3-phosphate dehydrogenase reaction (dihydroxyacetone phosphate + NADH + $H^+ \rightleftharpoons$ glycerol 3-phosphate + NAD^+).

Formation of pyruvate through glycolysis is associated with an equimolar formation of NADH in the cytosol. During aerobic conditions the reducing equivalents formed are transported into the mitochondria and oxidized in the respiratory chain. The mitochondrial membrane is, however, impermeable to NADH, and the reducing equivalents are therefore transported against a concentration gradient by energy-consuming shuttle systems.

The glutamate/aspartate exchange across the mitochondrial membrane (which is essential for the function of the malate/aspartate shuttle) has been shown to be electrogenic, and thus the difference in NADH concentration between the mitochondria and the cytosol is influenced by the mitochondrial membrane potential (Davis *et al.*, 1980). An increase in mitochondrial NADH, or alternatively a decreased mitochondrial membrane potential [which could result from either a decrease in the ATP/ADP ratio (Davis *et al.*, 1980) or a decrease in the O_2 availability (Chance, 1976)] is thus expected to lead to an increase in cytosolic NADH. An increase in cytosolic NADH relative to mitochondrial NADH is also necessary to create a driving force for the increased flux of reducing equivalents into the mitochondria, occurring during high respiratory rates.

It is concluded that muscle NADH decreases during low-intensity exercise, but increases above the value at rest during high-intensity exercise. The increase in muscle NADH is consistent with the hypothesis that the accelerated lactate production during submaximal exercise is due to a limited availability of O_2 in the contracting muscle. It is suggested that the observed increases in NADH, ADP and P_i are metabolic adaptations which primarily serve to activate the aerobic ATP production, and that the increased anaerobic energy production (phosphocreatine breakdown and lactate formation) is a consequence of these changes.

This research was supported by grants from the Swedish Sports Research Council and the Swedish Medical Research Council (project no. 7670). A.K. was supported by grants from the Swedish Institute and Coca-Cola Export Corp., Sweden.

REFERENCES

Akerboom, T. M., Van Der Meer, R. & Tager, J. M. (1979) Techniques in Metabolic Research, Part 1, B205, pp. 1–33, Elsevier/North-Holland, Amsterdam

Åstrand, P.-O. & Rodahl, K. (1970) Textbook of Work Physiology, McGraw-Hill, New York

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

Chance, B. (1965) J. Gen. Physiol. **49**, 163–188

Chance, B. (1976) Circ. Res. **38**, Suppl. 1, I31–I38

Chance, B. & Jöbsis, F. F. (1959) Nature (London) **184**, 195–196

Chance, B., Williamson, J. R., Jamieson, D. & Schoener, B. (1965) Biochem. Z. **341**, 357–377

Chasiotis, D., Sahlin, K. & Hultman, E. (1982) J. Appl. Physiol. **53**, 708–715

Connett, R. J., Gayeski, T. E. J. & Honig, C. R. (1984) Am. J. Physiol. **246**, H120–H128

Davis, E. J., Bremer, J. & Åkerman, K. E. (1980) J. Biol. Chem. **255**, 2277–2280

Grassl, M. & Möllering, H. (1974) in Methods of Enzymatic Analysis (Bergmeyer, H. U., ed.), pp. 2073–2075, Academic Press, New York and London

Henriksson, J., Katz, A. & Sahlin, K. (1986) J. Physiol. (London) **380**, 441–451

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

Hultman, E., Bergström, J. & McLennan Anderson, N. (1967) Scand. J. Clin. Lab. Invest. **19**, 56–66

Jacobson, K. B. & Kaplan, N. O. (1957) J. Biol. Chem. **226**, 603–613

Jöbsis, F. F. & Stainsby, W. N. (1968) Resp. Physiol. **4**, 292–300

Jorfeldt, L., Juhlin-Dannfelt, A. & Karlsson, J. (1978) J. Appl. Physiol. **44**, 350–352

Lowry, O. H. & Passonneau, J. V. (1972) A Flexible System of Enzymatic Analysis, Academic Press, New York and London

Newsholme, E. & Start, C. (1974) Regulation in Metabolism, John Wiley and Sons, Chichester

Sahlin, K. (1983) Clin. Physiol. **3**, 477–485

Sahlin, K. (1985) Pflügers Arch. **403**, 193–196

Sahlin, K. & Katz, A. (1986) Biochem. J. **239**, 245–248

Sahlin, K., Harris, R. C., Nylind, B. & Hultman, E. (1976) Pflügers Arch. **367**, 143–149

Sjøgaard, G., Adams, R. P. & Saltin, B. (1985) Am. J. Physiol. **248**, R190–R196

Ström, G. (1949) Acta Physiol. Scand. **17**, 440–451

Veech, R. L., Lawson, J. W. R., Cornell, N. W. & Krebs, H. A. (1979) J. Biol. Chem. **254**, 6538–6547

Wendt, I. R. & Chapman, J. B. (1976) Am. J. Physiol. **230**, 1644–1649

Williamson, D. H., Lund, P. & Krebs, H. A. (1967) Biochem. J. **103**, 514–527

Wilson, D. F., Erecinska, M., Drown, C. & Silver, I. A. (1977) Am. J. Physiol. **233**, C135–C140

Received 23 December 1986/16 March 1987; accepted 6 April 1987