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NADH content in type I and type II human muscle fibres after dynamic exercise

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The effect of dynamic exercise on the NADH content of human type I (slow-twitch) and II (fast-twitch) muscle fibres was investigated. Muscle biopsy samples were obtained from the quadriceps femoris of seven healthy subjects at rest and after bicycle exercise at 40, 75 and 100% of the maximal oxygen uptake [$\dot{V}O_2$(max.)]. At rest and after exercise at 100% $\dot{V}O_2$(max.), muscle NADH content was significantly higher ($P < 0.05$) in type I than in type II fibres. After exercise at 40% $\dot{V}O_2$(max.), muscle NADH decreased in type I fibres ($P < 0.01$), but was not significantly changed in type II fibres. After exercise at 75 and 100% $\dot{V}O_2$(max.), muscle NADH increased above the value at rest in both type I and II fibres ($P < 0.05$). Muscle lactate was unchanged at 40% $\dot{V}O_2$(max.), but increased 20- and 60-fold after exercise at 75 and 100% $\dot{V}O_2$(max.) respectively. The finding that NADH decreased only in type I fibres at 40% $\dot{V}O_2$(max.) supports the idea that type I is the fibre type predominantly recruited during low-intensity exercise. The increase of NADH in both fibre types after exercise at 75% and 100% $\dot{V}O_2$(max.) suggests that the availability of oxygen relative to the demand is decreased in both fibre types at high exercise intensities.

INTRODUCTION

In a series of studies we have demonstrated that, in response to exercise or ischaemia, muscle NADH increases either before or in parallel with increases in muscle lactate (Sahlin, 1983, 1985; Henriksson *et al.*, 1986; Katz & Sahlin, 1987; Sahlin *et al.*, 1987). It was also observed that muscle NADH decreases during low-intensity exercise, but increases above the resting value during high-intensity exercise (Sahlin *et al.*, 1987). It was suggested that the observed changes in muscle NADH occurred within the mitochondria, and the accelerated lactate production during submaximal exercise is due to a limited availability of O_2 in the contracting muscle (Sahlin, 1985; Henriksson *et al.*, 1986; Sahlin *et al.*, 1987; Katz & Sahlin, 1987).

These previous studies, however, have been performed with humans, whose skeletal muscles are composed of two fibre types, type I (slow-twitch) and type II (fast-twitch), which may exhibit different metabolic profiles during exercise. It is likely that the rate of lactate formation during exercise differs between type I and II fibres, but, as lactate can diffuse from the site of production to an adjacent fibre, the fibre lactate content may not necessarily represent the lactate formation in that fibre. In contrast with lactate, NADH cannot permeate the muscle cell membrane and will thus provide information about the redox state within each fibre. The purpose of the present study was therefore (1) to develop a method for measuring NADH in single muscle fibres, and (2) to determine the effect of exercise of different intensities on the NADH content in type I and II fibres.

MATERIALS AND METHODS

Subjects

Seven healthy men participated in the study. Six of them were physical education students and performed physical activity at school as well as during their leisure time. The subjects' mean (range) age, height, weight and maximal oxygen uptake [$\dot{V}O_2$(max.)] were respectively: 27 years (22–31), 183 cm (174–190), 77 kg (71–85) and 4.2 l/min (3.3–4.8). 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. The muscle samples used in the present study for single-fibre analysis have previously been used to measure NADH and other metabolites in mixed-muscle samples (Sahlin *et al.*, 1987).

Experimental

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.) (298 ± 14 W, mean ± S.E.M.) to fatigue (4.7 ± 0.8 min). Muscle samples were taken from the quadriceps femoris muscle at rest and after each exercise bout. For further details of the experimental procedure, see Sahlin *et al.* (1987).

Analytical methods

The frozen muscle samples (stored in liquid N_2) were freeze-dried, and divided into two lots. One lot was dissected free from connective tissue and blood, and

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

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powdered. Part of the powder (3–6 mg) was used for assays of lactate and pyruvate by fluorimetric methods (modified from Lowry & Passonneau, 1972). The blood lactate was measured by a colorimetric method (Ström, 1949). The remaining powder (1–2 mg) was extracted with a solution containing KOH (500 mM), ethanol (50%, v/v) and cysteine (0.5 mM), neutralized with HCl and analysed for NADH by a bioluminescent technique (Sahlin, 1983). Another batch was placed under a dissection microscope (magnification × 40–80) and fragments of single fibres were dissected out. The tip of the fragment was cut off and stained for myofibrillar ATPase to allow identification of type I (slow-twitch) or type II (fast-twitch) fibres as previously described (Essén *et al.*, 1975). The remaining portion of the fibre was weighed on a quartz-fibre fishpole balance that had been calibrated with quinine hydrobromide. The weights of the fibre fragments averaged 4.6 μg (range 1–11 μg).

NADH was extracted from the single muscle fibre by adding 50 μl of a precooled solution (0°C) containing KOH (500 mM) and cysteine (0.5 mM). Samples were warmed to room temperature during a period of about 10 min and thereafter agitated on a vortex mixer, which in most cases resulted in complete digestion of the fibre. The alkaline extract was cooled on ice and neutralized by addition of HCl. The supernatant was immediately assayed for NADH by a bioluminescent analytical technique using a specific monitoring kit (LKB Wallac) (see Sahlin, 1983). The assay mixture was composed of 100 μl of phosphate buffer (final concn. 0.1 M, pH 7.0), 40 μl of monitoring reagent and 60 μl of muscle extract. The sensitivity varied between different lots of monitoring kits. The average increase in luminescence after adding sample (approx. 0.5 pmol of NADH) was 1.0–3.0 units, which is about 3–9 times higher than the baseline. When the increase in luminescence was small (< 0.1 units), the volume of monitoring reagent was increased to 60 μl, which resulted in an almost doubling of the sensitivity. The average time between start of fibre dissection and extraction was about 60 min.

Statistical method

For statistical evaluation, a one-way ANOVA with repeated measures 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. Student's paired t test was used to determine differences in NADH between type I and type II fibres. Values are reported as means ± S.E.M. Coefficient of variation (C.V.) was calculated either from the difference (d) between duplicate measurements:

$$\sqrt{(\Sigma d^2/2n)} \cdot 100/\bar{x} \quad \text{or from} \quad \text{S.D.} \cdot 100/\bar{x}.$$

RESULTS

The stability of NADH in freeze-dried muscle samples during dissection and weighing was investigated in one muscle sample. After freeze-drying and dissection, fibre fragments (25–50) were pooled together and divided into six lots, which were weighed and stored in room air for different time periods (25, 60 and 120 min) before they were extracted and analysed for NADH. Two separate lots were analysed after each time interval. The NADH contents in these fibres were 0.075 (0.071, 0.078), 0.075 (0.068, 0.082) and 0.078 (0.072, 0.083) mmol/kg dry wt. of muscle respectively. The values were apparently not influenced by extending the time in room air from 25 to 120 min. The reproducibility of NADH analysis in single fibres were evaluated by splitting 12 fibre fragments into two halves and measuring NADH on each half. The C.V. was 18%.

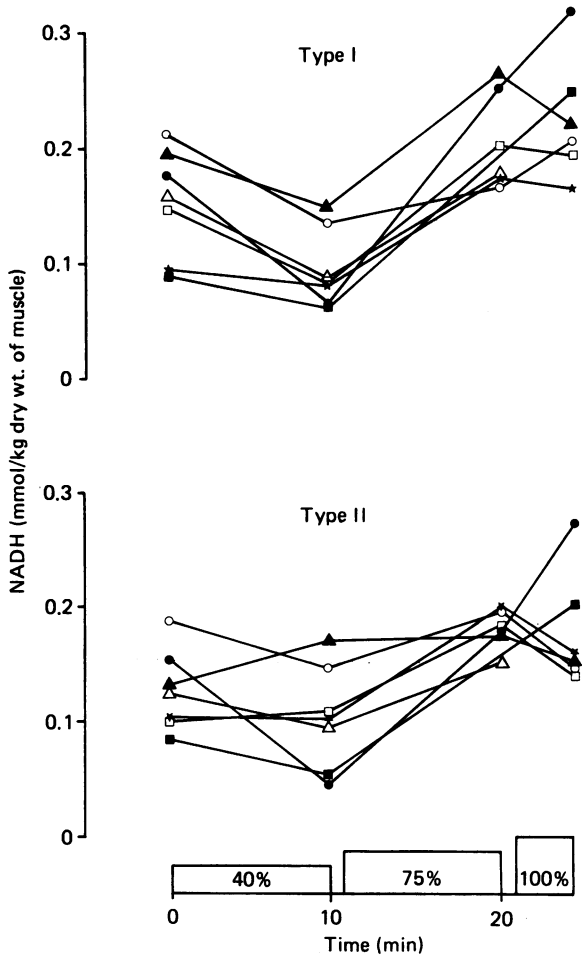

Fig. 1. NADH contents of type I and II muscle fibres at rest and after exercise

Values in boxes denote relative exercise intensities [% $\dot{V}O_2$(max.)]. Each symbol represents one subject, and each value is the mean value for several (at least three) fibres from the same subject.

NADH content in single muscle fibres

About ten fibre fragments (9–13, at least three of each type) were dissected out from each biopsy sample, and analysed for NADH. The average percentage of type I or type II fibres that was analysed in a biopsy was ~50%. In addition to an inter-individual variation in NADH and to a variation between fibre types, there was usually considerable variation between fibres of the same type from the same sample. The average C.V. was 24% (range 5–47%) for type I fibres and 22% (range 8–47%) for type II fibres.

After exercise at 40% $\dot{V}O_2$(max.), NADH content was lower than that at rest in type I fibres ($P < 0.05$), but remained unchanged ($P > 0.05$) in type II fibres (Fig. 1, Table 1). After exercise at 75% and 100% $\dot{V}O_2$(max.) to

Table 1. NADH contents of type I and II muscle fibres at rest and after exercise

Values are means ±S.E.M. for six to seven subjects and are given in mmol/kg dry wt. of muscle. * $P < 0.05$, ** $P < 0.01$, versus respective value at rest; † $P < 0.05$, †† $P < 0.01$ between types I and II.

	NADH content (mmol/kg)			
	Rest	Exercise [% $\dot{V}O_2$(max.)]		
		40	75	100
Type I	0.153±0.018 ($n = 7$) †	0.095±0.012** ($n = 7$)	0.205±0.017* ($n = 6$)	0.220±0.018** ($n = 6$) ††
Type II	0.126±0.014 ($n = 7$)	0.104±0.007 ($n = 7$)	0.183±0.007* ($n = 6$)	0.180±0.020* ($n = 6$)

Table 2. Metabolite contents (mmol/kg dry wt. of muscle) and blood lactate (mmol/l) at rest and after exercise

Values are means ±S.E.M. for seven subjects (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus respective value at rest), and are a subset of previously published data ($n = 10$; Sahlin *et al.*, 1987).

	Rest	Exercise [% $\dot{V}O_2$(max.)]		
		40	75	100
NADH	0.179±0.025	0.113±0.014**	0.226±0.029*	0.273±0.034***
Lactate	1.7±0.3	2.4±0.7	34.3±6.8***	102.4±7.4***
Pyruvate	0.12±0.02	0.21±0.02	0.46±0.03***	0.48±0.03***
Lactate/pyruvate	14.2±1.6	11.1±2.9	72.7±11.8*	220.2±27.2***
Blood lactate	1.3±0.1	1.4±0.2	4.3±0.8*	11.7±0.6*

fatigue, NADH increased above the value at rest in both fibre types (Table 1).

At rest and after exercise at 100% $\dot{V}O_2$(max.) to fatigue, the NADH content was significantly higher in type I than in type II fibres (Table 1). No significant differences between the fibre types were observed after exercise at 40% and 75% $\dot{V}O_2$(max.).

Muscle metabolites in mixed muscle samples

Mixed-muscle and blood lactate contents were unchanged after exercise at 40% $\dot{V}O_2$(max.), but increased substantially at the higher work loads (Table 2). Muscle pyruvate increased about 2- and 4-fold after exercise at 40% and 75% $\dot{V}O_2$(max.) respectively. 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.

Consistent with the single-fibre data, NADH in mixed muscle decreased significantly at 40% $\dot{V}O_2$(max.), but increased above the value at rest at the higher work loads (Table 2).

DISCUSSION

NADH content in single fibres

With the method presented in this study, quantitative biochemical determinations of NADH in individual fibres have been performed. On average the NADH content in single fibres appeared to be about 20% lower than that in mixed muscle. The reason for this is unclear, but it is probably not due to the increased time delay between dissection and extraction of single fibres (see the Results section). The muscle samples used for analysis of single-fibre NADH had been stored at −70 °C for 1 year. Before the single-fibre analysis, NADH measurements on mixed muscle were repeated on six samples. The NADH content (0.29±0.08 mmol/kg dry wt. of muscle) was similar to that (0.30±0.07 mmol/kg measured 1 year previously; and it is therefore unlikely that the lower NADH content in single fibres is due to a decrease during storage. The pattern of changes in single-fibre NADH during exercise was consistent with that in the mixed muscle, and it therefore seems reasonable to conclude that the present method provides reliable data on the NADH content in single muscle fibres.

A higher NADH content was observed in type I fibres than in type II at rest and at fatigue ($P < 0.05$ and $P < 0.01$ respectively). Previous data indicated that the changes in muscle NADH reflect the differences in the mitochondrial rather than the cytosolic NADH content (Sahlin, 1983; Sahlin & Katz, 1986; Sahlin *et al.*, 1987; Henriksson *et al.*, 1986). The higher NADH content in type I fibres could therefore be due to a higher mitochondrial content in these fibres. Consistent with this idea is the finding that the mitochondrial volume, estimated from electron micrographs, is approx. 50% larger in type I than in type II fibres (Sjöström *et al.*, 1982) and that the activity of mitochondrial enzymes is

higher in type I than in type II fibres (Essén-Gustavsson & Henriksson, 1984).

A decrease in NADH was observed in type I fibres and in mixed muscle after exercise at 40% $\dot{V}O_2$(max.). It has been suggested that the increase in ADP will stimulate cellular respiration and thereby cause an oxidation of the respiratory chain (Chance & Williams, 1955), provided that the oxygen supply is adequate. Hence, the oxidation of NADH is probably explained by an increase in ADP owing to the contractile activity. The NADH content in type II fibres was, however, not significantly changed after exercise at 40% $\dot{V}O_2$(max.), which suggests that these fibres were recruited to a lesser extent. At higher exercise intensities [75% and 100% $\dot{V}O_2$(max.)], NADH increased in both fibre types. It has been shown that selective glycogen depletion occurs in skeletal-muscle fibres of man after sustained contractions as well as dynamic exercise. At low intensity the type I fibres, and at high intensity both type I and II fibres, become glycogen-depleted (Gollnick *et al.*, 1974*a*,*b*; Vøllestad & Blom, 1985), indicating that during low-intensity exercise mostly type I fibres were activated, whereas at high intensity both type I and II fibres were recruited to a more equal extent. The observed changes in single-fibre NADH are consistent with this pattern of fibre recruitment.

NADH and lactate formation

The increase in NADH in both fibre types coincides with an accumulation of lactate, and suggests that the redox state of muscle is of importance for lactate formation. It has previously been shown that intense dynamic exercise and short-term isometric contraction resulted in a similar increase in lactate concentration in both type I and II fibres (pooled fibres), but, after isometric contraction to fatigue, lactate concentration was higher in type II than in type I fibres (Essén & Häggmark, 1975). It has also been reported that there are no major differences in lactate concentration between type I and II fibres (individual fibres) after submaximal and maximal dynamic exercise (Ivy *et al.*, 1987). Changes in lactate content within the different fibre types will, however, not give information about the site of lactate formation. It is likely that at least part of the lactate found in some fibres may have diffused from adjacent lactate-producing muscle fibres. Hence the lactate content observed in single muscle fibres does not necessarily reflect the amount produced in these fibres during exercise. In contrast, NADH cannot diffuse freely between fibres. Therefore, changes of NADH content in single fibres will give better information about the local metabolic state than do changes in the concentration of lactate.

At low exercise intensities, the increased rate of pyruvate formation is balanced by a similar increase in the rate of pyruvate oxidation, which is apparent from the absence of lactate accumulation in muscle and blood. It is interesting that the imbalance between the glycolytic and oxidative processes with increasing exercise intensities coincides with an increase in NADH above the value at rest in both fibre types. As discussed previously (Katz *et al.*, 1987; Katz & Sahlin, 1987; Sahlin *et al.*, 1987), this increase in NADH is believed to reflect an altered mitochondrial redox state, probably owing to a limited availability of O_2 in the contracting muscle in both fibre types. It appears likely that the imbalance between formation and oxidation of pyruvate is a consequence of a limited availability of O_2, as increases in ADP and P_i are activators of both oxidative phosphorylation and glycolysis (for a more detailed discussion see Sahlin *et al.*, 1987).

During exercise, the increased glycolytic rate is associated with an increased cytosolic formation of NADH. The mitochondrial membrane is, however, impermeable to NADH, and reducing equivalents are therefore transported against a concentration gradient by energy-consuming shuttle systems. Of these, the malate–aspartate shuttle is believed to be the most important in skeletal muscle (Schantz *et al.*, 1986), and it has subsequently been shown that the activity of malate–aspartate-shuttle enzymes is higher in type I than in type II fibres (Schantz & Henriksson, 1987). An increased cytosolic NADH concentration, relative to mitochondrial NADH, is expected to occur at high glycolytic rate in order to create the necessary driving force for the increased flux of reducing equivalents from cytosol to mitochondria. The lower shuttle capacity in type II fibres would necessitate a larger cytosolic NADH concentration in these fibres. This increase in cytosolic NADH, together with the high glycolytic flux, may contribute to a higher lactate production in type II fibres.

In conclusion, muscle NADH decreased during low-intensity exercise [40% $\dot{V}O_2$(max.)] in type I fibres, but was unchanged in type II fibres, suggesting that type I fibres were preferentially recruited at this work load. NADH increased above the resting value in both fibre types when the exercise intensity was increased to 75% and 100% $\dot{V}O_2$(max.), suggesting that the availability of oxygen relative to the demand was decreased in both fibre types.

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