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NADH in human skeletal muscle during short-term intense exercise

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Abstract. 1. The influence of high-intensity bicycle exercise on the redox level and lactate accumulation in skeletal muscle (m. quadriceps femoris) of man has been investigated. Six subjects exercised to exhaustion at a load corresponding to 100% $\dot{V}O_{2\ max}$.

2. Muscle content of NADH, determined by the bioluminescense technique, increased from ($\bar{x} \pm$ SEM) 0.089 ± 0.007 mmol/kg dry wt. at rest to 0.190 ± 0.031 after 2 min of exercise ($P < 0.05$) and to 0.213 ± 0.021 at exhaustion ($P < 0.05$). Values after 2 min exercise and at exhaustion were not statistically different ($P > 0.05$). Muscle lactate was increased 13-fold after 2 min of exercise and 22-fold at exhaustion as compared to the resting value.

3. After 10 min recovery NADH was restored back to the pre-exercise level whereas muscle lactate was still elevated.

4. The increase of muscle NADH during exercise is in contrast to earlier studies on isolated animal muscles, where an oxidation of NADH was observed during contractions. The difference might be due to the experimental model (isolated muscle vs. in vivo) or to the analytical method (qualitative data by reflectance fluorimetri from the surface of intact muscle vs. quantitative data from muscle extracts).

5. Calculations of the cytosolic NADH concentration from the lactate dehydrogenase equilibrium show that 95% or more of the NADH is confined to the mitochondrial compartment. The observed increase of muscle NADH therefore imply that the redox potential of the mitochondria is decreased during intense exercise.

6. The results are in conformity with that local hypoxia in the muscle cell is the mechanism for lactate production during exercise but an alternative explanation could be that the capacity of the respiratory chain to oxidize the formed NADH has been exceeded.

Key words: Lactate – Mitochondria – Muscle contraction – Muscle metabolism – NADH – Redox-state

Introduction

Lactate formation during exercise has frequently been assumed to be due to a lack of oxygen in the working muscle. Lactate accumulation has also been found to be increased when the inspired oxygen content is reduced during exercise [6, 9, 10] and decreased when the oxygen content is increased [8, 10]. Hypoxia at the cellular level would result in a reduction of the respiratory chain including the mitochondrial NAD. As the major part of NADH has been estimated to be confined to the mitochondria [14] an increase of mitochondrial NADH would also result in an increase of total muscle NADH and a decrease of muscle NAD. The observed decrease of the muscle NAD after intense exercise [4] is in conformity with this hypothesis. On the other hand [7] [13] using reflectance fluorimetry observed a decrease of the fluorescence, implying a decrease of NADH, in contracting isolated animal muscles. On the basis of these results hypoxia was questioned as being the cause of lactate formation during exercise.

A new method has recently been developed for quantitative measurements of NADH in muscle samples [11]. The method is based on bioluminescence detection of NADH in muscle extracts and has a high sensitivity. Muscle NADH determined by this technique was found to increase rapidly during ischaemia and was considered to be a sensitive index of hypoxia. The aim of the present study was to use this method for determination of NADH in skeletal muscle during maximal exercise and to establish if the NADH levels are compatible with an hypoxic condition in the working muscle or not.

Material and methods

Six healthy men participated in the study. Mean age, height, and weight were 27 years (range 23–31), 182 cm (range 176–193) and 72 kg (range 65–76), respectively. The subjects were physical education students and were relatively well-trained. Their mean maximal oxygen uptake was 4.0 l/min (range 3.7–4.3). The subjects were informed about the nature and the possible risks involved in the experiment before their voluntary consent was obtained. The experimental protocol has been approved by the Ethical Committee at Huddinge Hospital.

Experimental. Subjects rested in the supine position for about 15 min before the first muscle biopsy was taken. Muscle samples were taken by the needle biopsy technique [2] from the lateral aspect of quadriceps femoris muscle and were frozen in the needle in freon maintained at its melting point (−150° C) by liquid nitrogen.

Exercise was performed on an electrically braked bicycle ergonometer keeping a pedalling rate of 60 rpm. After a warming-up period of 3 min (50 W) subjects exercised at a load corresponding to their maximal oxygen uptake (290–

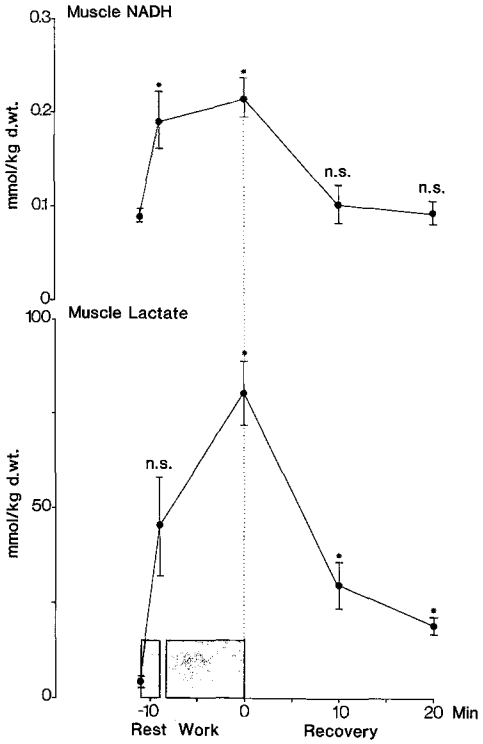

Fig. 1. Muscle content of NADH and lactate. Values are means ± SEM of 5–6 observations. Statistical significance for the difference from values at rest was tested by Student's *t*-test. * $P < 0.05$; n.s. no statistical significance $P > 0.05$

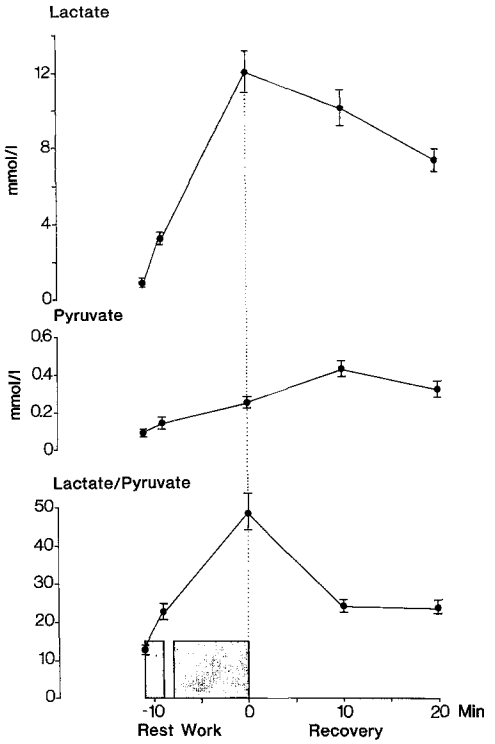

Fig. 2. Lactate, pyruvate and lactate/pyruvate in venous blood (antecubital vein). Values are means ± SEM of 6 observations

310 W, mean 300 W). Muscle biopsis were taken as fast as possible after 2 min of exercise and at exhaustion. Exhaustion was defined as the time point when the subject no longer could sustain the predetermined work-load. The time delay occurring between termination of exercise and freezing of the muscle biopsy was between 5 and 10 s. Further muscle biopsies were taken after 10 and 20 min recovery. The total work time ranged from 6 to 15 min (mean 10.5 min). The mean heart rate was 168 (range 150–180) after 2 min of exercise and 180 (165–210) at exhaustion. Blood samples were taken from an antecubital vein at the same time as the muscle biopsies.

Analytical methods. Muscle samples were freeze-dried, dissected free from blood and connective tissue and powdered [5]. The muscle powder was divided into two lots. One part (1–2 mg) was extracted with a solution containing KOH (500 mmol/l), ethanol (50%), cystein (5 mmol/l), neutralized with HCl and assayed by the bioluminescent technique for NADH [11]. Lactate and NAD were assayed enzymatically by fluorimetric methods [1] on neutralized perchloric acid extracts of the muscle powder.

Statistical methods. Values throughout this study are expressed as mean ± SEM. The differences between mean values were tested by Student's *t*-test. When repeated *t*-test was performed by using the value of NADH and lactate at rest several times the demands for statistical significance were increased, accordingly (i.e. when a significance limit of $P < 0.05$ is stated the obtained *t*-value corresponds to a significance level of $P < 0.0125$).

Results

Muscle metabolites. Muscle lactate increased rapidly during exercise and was 45.6 ± 11.9 mmol/kg dry wt. after 2 min of exercise and increased further to 80.0 ± 8.8 mmol/kg dry wt. at exhaustion (Fig. 1). The rate of lactate accumulation in muscle was significantly higher ($P < 0.05$) during the first 2 min of exercise (21 ± 6.0 mmol × kg^{-1} dry wt. × min^{-1}) than during the latter period of exercise (2 min – exhaustion; 5.6 ± 21. mmol × kg^{-1} dry wt. × min^{-1}).

The pre-exercise value of NADH (0.089 ± 0.007 mmol/kg dry wt.) was similar to that previously reported for resting muscle [11]. A two-fold increase of NADH ($P < 0.05$) was observed already after 2 min of exercise (Fig. 1). Muscle NADH increased further at exhaustion (0.213 ± 0.021 mmol/kg dry wt.) but was not statistically different ($P > 0.05$) from the value after 2 min exercise (0.190 ± 0.031 mmol/kg dry wt.). After 10 min recovery NADH was restored to the resting value while lactate was still elevated (Fig. 1). There was no relation between NADH and lactate in muscle either after 2 min of exercise or at exhaustion. No significant change ($P > 0.05$) was observed in muscle NAD in response to exercise (1.69 ± 0.09 at rest and 1.87 ± 0.11 mmol/kg dry wt. at exhaustion).

Blood metabolites. Both lactate and pyruvate increased in blood during exercise (Fig. 2). As lactate content in arterial blood after leg exercise is similar to that in blood from an antecubital vein the latter has been used to evaluate the gradient between muscle and blood. The lactate gradient increased during exercise but was higher after 2 min exercise than at exhaustion (Fig. 3). In accordance with previous

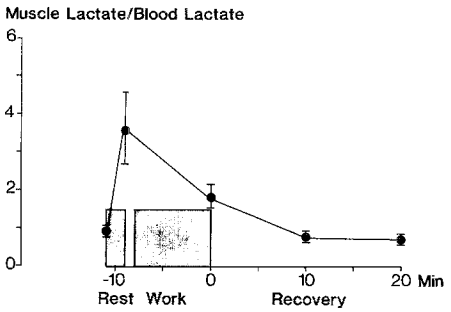

Fig. 3. Lactate gradient between muscle and blood (antecubital vein). Values are recalculated to mmol per l H_2O by assuming 77% and 85% water in muscle tissue and blood, respectively. Values are means ± SEM of 4–6 observations

studies the lactate gradient decreased during recovery below the pre-exercise value at rest [12]. The rate of lactate accumulation in blood was not significantly different ($P > 0.05$) during the first 2 min of exercise (1.04 ± 0.10 mmol × l^{-1} × min^{-1}) and during the latter period of exercise 2 min – exhaustion; 1.17 ± 0.20 mmol × l^{-1} × min^{-1}). The higher accumulation rate observed in muscle during the initial period of exercise was thus not corresponded by an equal change in the blood, probably due to a lower muscle blood flow during the initial period of exercise resulting in lower rate of lactate efflux.

Discussion

The main result of the present study is the finding that muscle NADH increases during intense exercise. If hypoxia is present in muscle and is severe enough to interfere with oxidative metabolism it would result in a reduction of the respiratory chain including NAD. The present results are thus in conformity with the hypothesis that local hypoxia occurs in the working muscle. Quantitative determinations of NADH in muscle during exercise has to our knowledge not been previously reported. Qualitative measurements of NADH by observing the fluorescence of reflecting light from the muscle surface has been performed on isolated muscle during contractions induced by electrial stimulation and during anoxia [7, 13]. These studies paradoxically showed a decrease of the fluorescence during contraction indicating an oxidation of NADH. Even when the contraction was performed in the absence of oxygen NADH remained below the initial value. The reflectance fluorometric technique might, however, be less suitable for obtaining data on NADH of the whole muscle. The technique will primarily record local changes at the surface of the muscle, which can be influenced by movement artifacts during the contraction and also by diffusion of oxygen from the surroundings. In a recent study by Connett et al. [3] the PO_2 level of contracting dog gracilis muscle was calculated from the myoglobin saturation. It was concluded that the muscle remained in a fully aerobic state (although lactate was produced) up to a work load corresponding to 70–90% of $VO_{2\ max}$. In the present study subjects were working at a higher work-load (100% $VO_{2\ max}$) which together with the difference in fibre type composition between quadriceps femoris muscle of man and gracilis muscle of dog makes a comparison difficult. Further studies are required to see if an increase of NADH occurs also at submaximal work loads.

Table 1. Calculated concentration of NADH in the cytosol compared with the total muscle concentration of NADH and NAD obtained from analyses. Cytosolic NADH was calculated from the lactate dehydrogenase equilibrium Keq (LDH) = [(pyruvate) (NADH) (H^+)]/[(lactate) (NAD)]. Conditions used in the calculations: Keq (LDH) = 1.11×10^{-11} M [14]; $NAD_{cytosol} = 0.95 \times NAD_{total}$; lactate/pyruvate = 12.8 [11], 15.3 (20 min ischaemia, [11] and 150 (bicycle exercise, [12]); muscle pH = 7.08 (rest), 6.6 (bicycle exercise, [12] and 7.08 (ischaemia). Values on NAD and NADH after 20 min ischaemia are from Sahlin [11]

	$NADH_{total}$	NAD_{total}	$NADH_{cytosol}$	$NADH_{cytosol}$
	μmol/kg dry wt.			$NADH_{total}$
Rest	89 ± 7	1,690 ± 90	2.7	3.0%
Ischaemia	266 ± 12	1,620 ± 70	3.1	1.2%
Bicycle exercise to exhaustion (10 min)	213 ± 21	1,870 ± 110	11.8	5.5%

The observed increase of muscle NADH (120 μmol/kg dry wt.) ought to be corresponded by an equal decrease of NAD. No significant change was, however, observed in the present study ($P > 0.05$). The expected change would, however, only amount to 7% of the total NAD content which appear to be lower than the variation in the analytical procedure. On the other hand Graham et al. [4] reported a decrease in NAD with 72 μmol/kg dry wt. after maximal bicycle exercise, which is of the same magnitude as the increase in NADH observed in the present study.

It is well known that during the initial period of exercise, before oxygen transport is adjusted to the increased demands, lactate is formed to a higher extent than during steady state conditions. This was also demonstrated in the present study where the rate of lactate accumulation in muscle during the first 2 min of exercise was about 4 times higher than during the last period of exercise. When the exercise is continued the cardiovascular system is adjusted to the increased oxygen demand by the working muscle resulting in increased oxygen transport and decreased utilization of the anaerobic energy sources. In the present study heart rate after 2 min of exercise (168) was only slightly lower than exhaustion (180) indicating that at this time the oxygen transport system was nearly maximally adjusted. Muscle content of NADH was similar after 2 min of exercise and at exhaustion which suggests that the redox level of the mitochondrion is similar. It is possible that muscle NADH is even higher during the first 30 s of exercise when the cardiovascular system is not fully adjusted. It should, however, be emphasized that the observed level of NADH at exhaustion is not significantly different from the value after 20 min ischaemia under which condition mitochondrial redox level would be completely reduced.

The concentration of NADH in the cytosol has been calculated from the lactate dehydrogenase (LDH) equilibrium (Table 1). The calculations show that cytosolic NADH only constitutes about 3% of total NADH in resting muscle and although it increases 4 times after exercise to exhaustion it is still only 5.5% of total NADH. From these calculations it appears as if the major part of NADH in muscle tissue is confined to the mitochondrial compartment. This is also in agreement with the data by Williamson et al. [14] where

mitochondria from liver tissue were found to be in a much more reduced state than the cytosol. Further evidence that determination of total muscle NADH is a measure of the mitochondrial NADH is the lack of correlation between change in NADH/NAD and lactate/pyruvate during ischaemia [11] and the finding that increase of NADH precedes lactate formation during ischaemia [11].

It is concluded that the observed increase in NADH during intense exercise corresponds to a decreased redox potential within the mitochondria. The results are in conformity with that local hypoxia in the muscle cell is the mechanism for lactate production during exercise. An alternative explanation could be that the capacity of the respiratory chain to oxidize the formed NADH has been exceeded.

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