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The 2,3-DPG Levels of Human Red Blood Cells During an Incremental Exercise Test: Relationship to the Blood Acid-Base Balance

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Summary

The aim of this study was to evaluate the influence of exercise with the intensity progressively increasing from rest until maximal oxygen uptake (VO_{2max}) on 2,3-DPG levels in red blood cells (RBC) in relation to the changes in the acid-base balance and plasma lactate concentration. Six healthy young men (age 22.5±1.5 years, VO_{2max} 3.48±0.20 l/min) participated in this study. The subjects performed an incremental exercise test on a cycloergometer until exhaustion. Blood samples were tested for acid-base balance indices (pH, HCO_3^-, BE), plasma lactate and RBC 2,3-DPG concentration. Gas exchange variables were measured continuously breath-by-breath. In this paper we present data concerning 2,3-DPG, plasma lactate, pH, HCO_3^- and BE measured at rest, at the power output corresponding to the lactate threshold (PO LT), at the power output at maximal oxygen uptake (PO VO_{2max}), as well as 5, 15 and 30 min after finishing the incremental test. Increase of power output above the lactate threshold to the PO VO_{2max} was accompanied by a significant ($p<0.01$) increase of plasma lactate from 2.58±0.78 mmol/l to 10.22±3.04 mmol/l. This was also accompanied by a significant drop ($p<0.01$) in blood pH value from 7.352± 0.025 at the PO LT to 7.294±0.041 at the PO VO_{2max}. No significant changes of the RBC 2,3-DPG level were observed at any of the analysed stages of the exercise. The RBC 2,3-DPG level expressed in relation to the changes of haematocrit showed only minor changes during the exercise period and after 15 min of recovery vs. resting value (3.21±1.19). However, after 30 min of recovery, RBC 2,3-DPG decreased to the value of 2.32±1.19 μmol/ml. We conclude that, during an incremental test, no increase in RBC 2,3-DPG concentration is required to reach the maximal oxygen uptake level. Moreover, a rapid decrease in blood pH, developing during a single bout of exercise, is not a stimulus powerful enough to cause significant changes in the RBC 2,3-DPG level during short-term exercise.

Key words
Acid-base balance – 2,3-diphosphoglycerate – Exercise – Oxygen uptake

Introduction

A progressive increase of power output requires an appropriate increase in oxygen uptake (Zoladz *et al.* 1995), which is strongly dependent upon the oxygen transport and its delivery to the working muscle. The rightward shift of the oxyhaemoglobin dissociation curve is an important factor improving oxygen delivery to tissues. It is generally accepted that such a shift can be reached by an increase in 2,3-DPG concentration in erythrocytes (Chanutin and Curnish 1967, Benesh and Benesh 1967).

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Activation of such a mechanism seems to be especially important under conditions when the need for oxygen delivery at the cellular level and its utilization are increased, e.g. during intensive physical exercise or hypoxia. This is perhaps why the erythrocyte 2,3-DPG concentration is the subject of studies in the field of exercise physiology. However, the physiological mechanisms controlling the RBC 2,3-DPG mediated increase in the red blood cell oxygen transport capacity, the subject of intense studies (Guest 1942, Bellingham *et al.* 1971, Rapoport *et al.* 1977), are not yet fully clarified.

Despite the increase in the RBC 2,3-DPG level as the result of prolonged exposure to hypoxia (Oski *et al.* 1969, MacDonald 1977, Mairbäurl 1994) or long-lasting endurance training (Remes *et al.* 1979, Brodthagen *et al.* 1985, Mairbäurl *et al.* 1986, Hespel *et al.* 1988) is well documented, but the role of the RBC 2,3-DPG during a single exercise bout is still not clear.

There is some evidence showing that even a single exercise bout causes a significant rise of RBC 2,3-DPG above the resting level (Eaton *et al.* 1969, Faulkner *et al.* 1970, Lijnen *et al.* 1986, 1988). On the other hand, in a number of studies (Thomson *et al.* 1974, Bonner *et al.* 1975, Böning *et al.* 1979, Ramsey and Pipoly 1979, Katz *et al.* 1984, Hsieh *et al.* 1986) no changes in the erythrocyte 2,3-DPG level after a single exercise bouts were observed. Thus the role of RBC 2,3-DPG in the mechanisms of oxygen transport during single exercise bouts remains unclear.

This is why in the present study, we evaluated the influence of a progressive increase in exercise intensity, during a single exercise test, starting from rest until maximal oxygen uptake (VO_{2max}) on the RBC 2,3-DPG level. Moreover, we followed the RBC 2,3-DPG concentration together with changes in acid-base balance and plasma lactate concentration induced by this kind of exercise, as well as within 30 min after terminating the effort.

Methods

Subjects

Six healthy young men (age 22.5±1.5 years, height 176.2±2.4 cm, body mass (BM) 71.2±6.6 kg, percentage of body fat 10.3±5.1 % of BM, VO_{2max} 3.48±0.20 l/min) volunteered for this study.

Procedures

The subjects performed an incremental exercise test at a pedalling rate of 70 rev./min on a cycloergometer (Ergoline 800 S, Netherlands). The power output increased by 30 W every 3 min until exhaustion. Five minutes prior to the exercise, at the end of each stage of exercise and after the 5th, 15th and 30th minute after stopping the exercise, antecubital blood samples (1 ml each) were withdrawn using 8 G/1.2 x 45 mm catethers (Int-Catether, Abbott, Ireland). Blood samples were tested for acid-base balance (pH, pCO_2, pO_2, BE, HCO_3^-), plasma lactate concentration and the RBC 2,3-DPG level. Starting from the onset of exercise until the end of cycling, gas exchange variables were continuously assessed using a breath-by-breath system (Oxycon Champion, Jaeger, Germany). Before and after finishing each test, gas analysers were calibrated with certificated gases. The blood acid-base status was determined using a blood acid-base analyser Corning 248 (England). Plasma lactate concentrations were measured on an automatic analyser (Ektachem XR 700, Kodak, USA) and the 2,3-DPG concentration in red blood cells was determined by the ultraviolet enzymatic method (kit 35-UV, Sigma, USA). The lactate threshold (LT) in this study was defined as the highest power output above which plasma lactate concentration showed a sustained increase of >0.5 mmol/l/step (see Zoladz *et al.* 1995). The percentage of body fat was assessed according to Hassager *et al.* (1986).

Statistical analysis.

Values represent means ± S.D. Statistical significance was tested by the paired t-test. The chosen levels of significance were * <0.05, ** $p<0.02$ and *** $p<0.01$.

Results

In this paper we present data concerning 2,3-DPG, plasma lactate, pH, HCO_3^- and BE measured at rest (R), at the power output corresponding to the lactate threshold (PO LT), at the power output at VO_{2max} (PO VO_{2max}), as well as 5, 15 and 30 min after finishing the incremental test.

The mean value of power output at the lactate threshold amounted to 135±31 W, whereas the power output at VO_{2max} reached 264±20 W. Mean duration of the exercise until exhaustion was 26.4±2.0 min.

The increase of power output from the lactate threshold (PO LT), to the power output at VO_{2max} (PO VO_{2max}) was accompanied by a significant ($p<0.01$) rise in plasma lactate concentration. A significant elevation in plasma lactate was observed until the 15th minute of recovery (Fig. 1, panel A). A significant decrease in the blood pH occurred at the PO VO_{2max} and until the 5th minute of the recovery period (Fig. 1, panel B). The HCO_3^- and BE were significantly ($p<0.01$) decreased after exceeding the lactate threshold. This decrease remained unchanged until the 30th minute after exercise (Fig. 1, panels C and D). Despite the significant acidosis and lactacidaemia occurring during exercise performed above the lactate threshold, as well as during the initial period of recovery (Fig. 1, panel A, B, C and D).

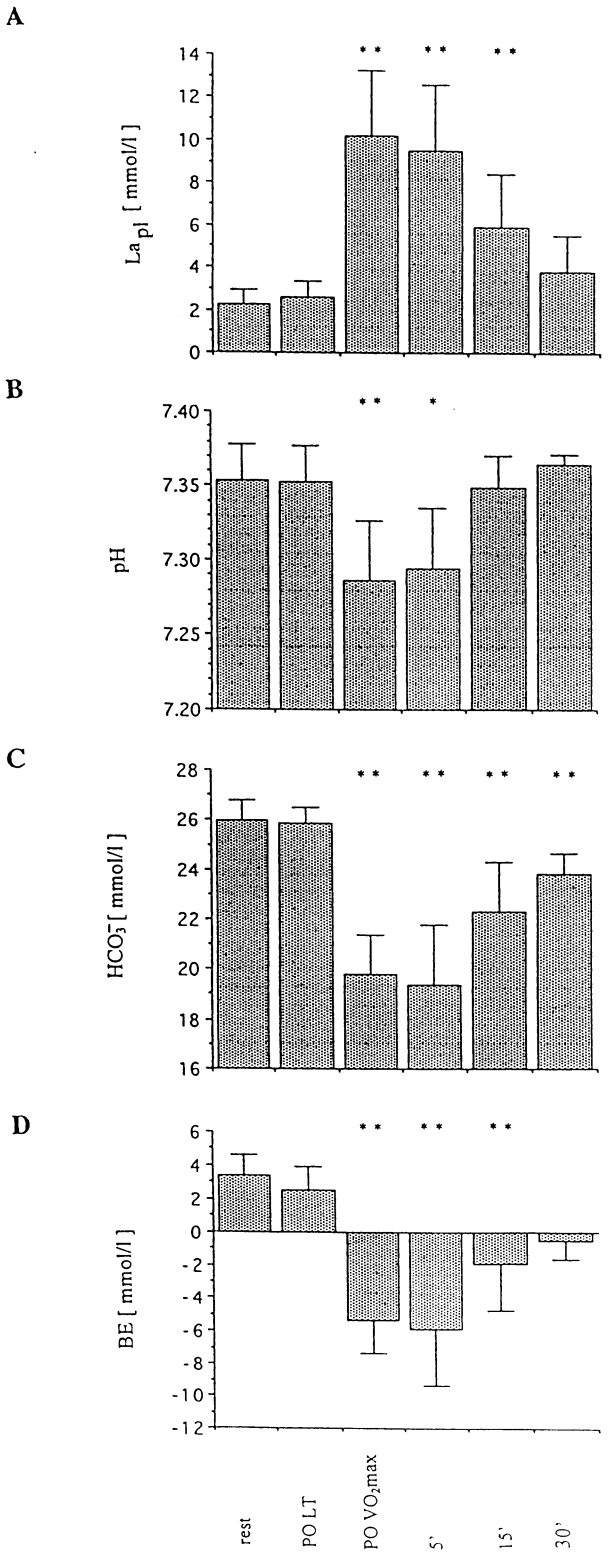

Fig. 1. *Plasma lactate concentration $[La]_{pl}$ (panel A), blood pH (panel B), HCO_3 (panel C) and BE (panel D) determined at rest, at the power output corresponding to the lactate threshold (PO LT), at the maximal oxygen uptake (PO VO_{2max}) and in the 5th, 15th and 30th minute of recovery. Significantly different from the value determined at rest (* $p<0.05$, ** $p<0.01$).*

No significant changes in the erythrocyte 2,3-DPG levels were found (Fig. 2, panel A) when compared to the resting value (1.44±0.52 μmol/ml) at any of the analysed stages of the exercise. RBC 2,3-DPG levels expressed in relation to the changes in haematocrit showed only minor changes during the exercise period and the first 15 min of recovery as compared to the resting value (3.21±1.19 μmol/ml) (Fig. 2, panel B). However, during the 30th minute of recovery after exercise, a decrease in RBC 2,3-DPG to the value 2.32±1.19 mol/ml occurred (Fig. 2, panel B).

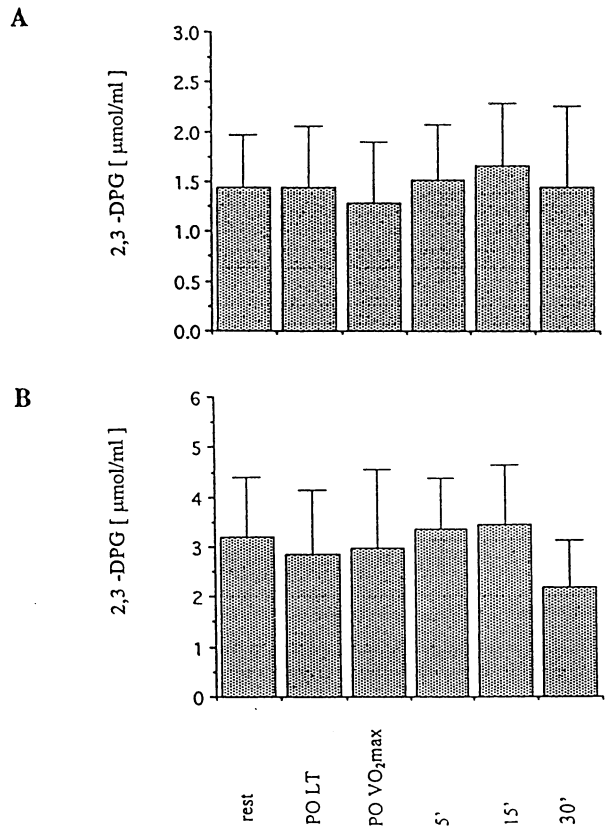

Fig. 2. *Red blood cells 2,3-DPG concentration expressed in μmol/ml (panel A) and in μmol/ml of packed cells (panel B) determined at rest, at the power output corresponding to the lactate threshold (PO LT), at the maximal oxygen uptake (PO VO_{2max}) and in the 5th, 15th and 30th minute of recovery.*

Discussion

A number of studies have shown that regular physical training causes significant rise in the erythrocyte 2,3-DPG level (Böning *et al.* 1975, Kunski and Sztobryn 1976, Remes *et al.* 1979, Shappel *et al.* 1979, Mairbäurl *et al.* 1986, Hespel *et al.* 1988). However, the physiological mechanism controlling the increase in the RBC 2,3-DPG levels following an endurance training programme is unknown. It has been

suggested that the increase in the erythrocyte 2,3-DPG level may be explained by the exercise-induced increase of red cell turnover (Brodthagen *et al.* 1985, Klein *et al.* 1980). Since young red cells have higher glycolytic activity (Bunn and Jandl 1970, Berstein 1959) than old ones, one may speculate that the increased number of reticulocytes induced by exercise may be responsible for the increase in RBC 2,3-DPG level in trained individuals. Indeed, a significant increase in the enzymatic activity of erythrocyte pyruvate-kinase and glutathione reductase was shown in rat erythrocytes following a 30 days' training period (26 training sessions) on a treadmill (Spodaryk *et al.* 1985). On the other hand, Hespel *et al.* (1988) demonstrated that the rise in erythrocyte 2,3-DPG levels developed by physical endurance training in humans was not due to the activation of red cell glycolytic enzymes or enzymes involved in the pentose-phosphate cycle.

Despite the well-documented effect of prolonged endurance training on the RBC 2,3-DPG levels, it has been reported that even single bout of exercise may induce a significant increase in the erythrocyte 2,3-DPG level (Faulkner *et al.* 1970, Meen *et al.* 1981, Lijnen *et al.* 1988). On the other hand, a number of studies (Thomson *et al.* 1974, Bonner *et al.* 1975, Böning *et al.* 1975, Ramsey and Pipoly 1979, Hsieh *et al.* 1986) have shown no effect of a single exercise bout on the erythrocyte 2,3-DPG level.

In the present experiments, we studied changes in human red blood cell 2,3-DPG level during an incremental exercise test performed until exhaustion as well as within 30 min of recovery after finishing the test. It was found that during the incremental exercise test, causing a significant decrease in the blood pH and pronounced rise in blood lactate concentration, no significant changes in the red blood cell 2,3-DPG level had occurred (see Fig. 1). Moreover, we did not observe a significant increase in the reticulocyte count during and within 30 min after the exercise (see Fig. 2). It seems to be somewhat surprising that despite pronounced decrease in plasma pH and significant plasma lactate accumulation we could not see significant changes in erythrocyte 2,3-DPG levels. Based on the studies by Guest (1942) who demonstrated that a decreased blood pH is associated with lower 2,3-DPG levels, as well as on the above mentioned studies (Eaton *et al.* 1969, Faulkner *et al.* 1970, Lijnen *et al.* 1986, 1988) in our exercise protocol one would expect a significant decrease in erythrocyte 2,3-DPG concentration during exercise accompanied by acidosis as well as during the 15-min period after the exercise during which blood pH was significantly decreased. On the other hand, as reported by Bellingham *et al.* (1971), pharmacologically induced rapid changes in the blood acid-base balance (acidosis/alkalosis) were not accompanied by significant changes in red cell 2,3-DPG levels within the first 4 hours of alkalosis or acidosis. Furthermore, maintained acidosis was accompanied by a significant decrease in RBC 2,3-DPG, whereas a significant increase in 2,3-DPG was observed in alkalosis (Bellingham *et al.* 1971). Moreover, as reported by Jones *et al.* (1977), significant *in vivo* changes in the human blood acid-base balance (alkalosis/acidosis) induced by ingestion of $NaHCO_3/NH_4Cl$ were not accompanied by significant modification of the red blood cell 2,3-DPG for the 3-hour period of observation.

On the basis of the data presented above, one may speculate that if acidosis indeed plays a significant role in the red cell 2,3-DPG concentration as reported by Bellingham *et al.* (1971), then the magnitude of the exercise-induced changes in the blood pH observed in our study was too small or the duration of exposure of erythrocytes to the low blood pH was too short to cause a significant decrease in the intraerythrocyte pH to inhibit the RBC 2,3-DPG formation. In view of our data and the evidence in the literature, exercise-induced changes in the erythrocyte 2,3-DPG concentration are not clear. Moreover, the role of the erythrocyte 2,3-DPG concentration in oxygen transport to muscles during exercise seems to be limited. According to Katz *et al.* (1984), who found a significant training-induced increase in VO_{2max} with no concomitant increase in the erythrocyte 2,3-DPG level it appears unlikely that 2,3-DPG is of any significant physiological benefit to tissue oxygenation during and following maximal exercise.

It seems to be surprising why during incremental exercise, which in the final stage requires maximal oxygen utilization, the oxygen transport supporting mechanism (2,3-DPG induced a rightward shift of the oxyhemoglobin dissociation curve) does not operate. This may be due to the rheological properties of erythrocytes. It has been shown that an increase of erythrocyte 2,3-DPG concentration decreases the deformability of erythrocytes (Suzuki *et al.* 1990). This may decrease muscle perfusion and oxygen delivery, however, further studies are required to understand the regulation of synthesis and the role of erythrocyte 2,3-DPG during exercise.

We conclude that, during an incremental test, no increase in RBC 2,3-DPG is required to reach the maximal oxygen uptake level. Moreover, a rapid decrease in the blood pH, developing during a single bout of exercise, is not a powerful enough stimulus to cause significant changes in the RBC 2,3-DPG level during short-term exercise.

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