Warming-up affects performance and lactate distribution between plasma and red blood cells

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Abstract

Warming-up (WU) is a widely used preparation for training and competition. However, little is known about the potential mechanisms of WU on performance and on the lactate distribution in the blood compartment. The purpose of the present study was to investigate whether different WU procedures affect performance and lactate distribution between plasma and red blood cells (RBCs) after maximal exercise. At three different occasions eleven subjects performed one 30 s maximal effort exercise on a cycle ergometer. Before each exercise, subjects warmed up at different intensities: 1. no WU (NWU); 2. extensive WU (EWU); 3. intensive WU (IWU). Blood samples were taken under resting conditions, after WU, and in 1 minute intervals during recovery to determine lactate concentrations [LA] in whole blood ([LA]WB), plasma ([LA]plasma) and erythrocytes ([LA]RBC). Mean power output was +58 Watt (EWU) and +60 Watt (IWU) higher compared to NWU. For each WU condition [LA]plasma and [LA]RBC differed significantly at any time point, showing greater [LA]plasma compared to [LA]RBC. The maximal effort exercise caused a rapid decrease of the [LA]RBC/[LA]plasma ratio. [LA]RBC reached the peak 3-5 minutes later than [LA]plasma depending on the WU condition. The initial increments in [LA]RBC were 10-16% lower after IWU compared to NWU and EWU. The lower increment of [LA]RBC after IWU might be due to a “higher preloading” with lactate before exercise, causing a smaller initial [LA] gradient between plasma and RBCs. It seems that the influx decreases with increasing intracellular [LA]. Another possibility one could speculate about is, that the extracellular increase in [LA] inhibits the outflux of lactate produced by the RBC itself. This inhibited export of lactate from RBCs may lead to an intracellular lactate accumulation. But the relatively fast increase in [LA]RBC and other investigations partly contradicts this possibility.

Key words: Lactate concentration, blood, performance enhancement, anaerobic exercise, cycling, competition preparation.

Introduction

Despite limited scientific evidence supporting their effectiveness, warm-up (WU) routines prior to exercise are well-accepted and widely used. As a result, WU procedures are usually based on the trial and error experience of the athlete or coach, rather than on scientific study.

Several previous studies have demonstrated a number of physiological changes that occur with active WU, some of which are potentially capable of improving performance (Burnley et al., 2000; 2002; DeLorey et al., 2004; Gray and Nimmo 2001; Ingjer and Stromme, 1979). The majority of the effects of WU have been attributed to temperature-related mechanisms for instance decreased resistance of muscles and joints, greater release of oxygen from haemoglobin and myoglobin, speeding of metabolic reactions, increased nerve conduction rate, increased thermoregulatory strain (Bishop, 2003a; 2003b). But also non-temperature-related mechanisms have been proposed. These include increases in heart rate (Andzel 1978), elevation of baseline oxygen consumption (VO2), acceleration of VO2 kinetics (Burnley et al., 2002; DeLorey et al., 2004; Ingjer and Stromme, 1979) decreases in lactate accumulation (Martin et al., 1975) and increased blood flow to muscles (Bishop 2003 a). Potential mechanisms and the effects of warm-up have recently been reviewed by Bishop (2003a; 2003b).

It is well known that glucose transport is increased during exercise, and transport proteins (e. g. GLUT4) are translocated from intracellular stores to the cell surface when they are needed (Röckl et al., 2008). It can be speculated, that this translocation of transport proteins to the cell surface might be induced by WU procedures before the actual exercise which might lead to improved performance. But whether lactate transport is also altered during or immediately after exercise remains unknown. A recent study from our group has shown that monocarboxylate-transporter-1 (MCT-1) in red blood cells (RBCs) might be translocated from cytoplasma to the membrane in response to exercise, which might affect La flux due to a higher availability of MCT-1 in the membrane (unpublished data). As RBCs represent 40-45% of the whole blood volume, it has been suggested that they may act as an important dilution space for lactate and H+ ions leaving the muscle and thereby increasing the gradient from muscle to blood which potentially improves the rate of release from muscle (Juel et al., 2003). Previous studies also have shown that RBCs play a quantitative important role in the movement of lactate within the vascular compartment (Lindinger et al., 1995). In studies using repeated high intensity exercise, RBCs accounted for 1/3 of the total lactate release to the vascular compartment (Lindinger et al., 1995). Therefore the purpose of the present study was to investigate if different WU protocols can influence the increments in RBC lactate concentrations ([LA]RBC) the lactate distribution between plasma and RBCs and performance. A faster increase in [LA]RBC and/or an altered distribution might be a hint for a previously triggered lactate transport system in terms of the...
mentioned translocation of MCTs. This mechanism may increase the capacity of RBCs to transport lactate and H⁺ into RBCs during high intensity exercise and therefore affect performance. Lactate/H⁺ co-transport across the plasma membrane is fundamental for the metabolism and pH regulation of all cells, removing lactic acid produced by glycolysis and allowing uptake by those cells utilizing it for gluconeogenesis (liver and kidney) or as a respiratory fuel (heart and red muscle) (Chen and Chen, 1990; Halestrap and Meredith, 2004). As suggested by Lindinger et al. (1992) the uptake of lactate by RBCs plays an important role in regulating ion homeostasis within plasma and the intracellular and intracellular compartments of contracting muscle (Lindinger et al., 1992). An improved uptake capacity might decrease plasma lactate/H⁺ concentrations and thus allowing more lactate/H⁺ to leave the muscle. This regulatory process may help to maintain the function of active muscles by delaying the onset of fatigue (Lindinger et al., 1992; 1995), but it may also help to improve the cell-to-cell lactate shuttle as more lactate can be transported out of the working muscle to lactate-oxidizing tissues such as the heart or passive musculature (Brooks 2009).

Methods

Subjects

11 healthy male subjects (Mean ± SD; age: 24.3 ± 2.1 yrs; height: 1.83 ± 0.61 m; body weight: 79.9 ± 7.9 kg; VO₂peak of 54.7 ± 4.1 mL·kg⁻¹·min⁻¹) participated in this study. All subjects represented healthy male sport students from the German Sport University Cologne. All subjects gave written informed consent to the contribution to the study. The protocols used in these studies were approved by University Ethics Committee and are in line with the Declaration of Helsinki. All subjects abstained from alcohol consumption for 24 h before and during the training intervention and were non-smokers.

Exercise study protocol

Subjects visited the laboratory for four separate sessions. During the first visit, peak pulmonary oxygen uptake (VO₂peak) (Zan 600, Zan Messgeräte, Oberthulba, Germany), and lactate concentrations [LA] (EKF Diagnostic Sales, Magdeburg, Germany) were determined during an incremental step test to volitional fatigue on a cycle ergometer (Schoberer Rad Meßtechnik SRM GmbH, Jülich, Germany) to determine the individual WU intensity for each subject. The step test protocol started at 100 Watts [W], thereafter the power was increased by 40 Watt every 5 min.

At the three subsequent visits to the laboratory, subjects performed three different warm-up (WU) protocols followed by one 30 s short-term maximal effort (“all-out”) exercise on a cycle ergometer respectively: 1. no warm-up (NWU), 2. extensive warm-up (EWU) consisting of 12min cycling at 60 % of VO₂ peak, 3. intensive warm-up (IWU) consisting of 12min cycling at 60 % of VO₂peak including three high intensity phases of 10 s at 200% of VO₂peak. The three high intensity phases were chosen to induce high lactate levels, but to avoid fatigue compared to a continuous high intensity WU procedure.

The WU was followed by a 5 min passive rest period. Afterwards subjects performed the 30 s all-out exercise. For that the cycle ergometer was adjusted to an isokinetic mode set to a cadence of 120 rpm. Subjects performed the exercise in a seated position.

After the all-out exercise subjects were sitting on a chair for 15 min to keep lactate elimination as low as possible. Blood samples were taken under resting conditions before WU (R), directly after WU (WU), after 4 min of rest after WU (pre), and in minute intervals during the recovery of the all-out exercise (0’, 1’, 2’, ..., 9’, 10’, 12’, 15’) to determine lactate concentrations in whole blood, plasma and erythrocytes (Figure 1).

All 3 tests were performed in a randomised order with at least 4 days break between each session. During the 30 s all-out exercise subjects were vocally encouraged. During each session environmental conditions (temperature, humidity) were kept constant. Always the same two investigators attended the tests.

Figure 1. Time course of the exercise protocol. Warming-up consisted of 12-min cycling at 60% VO₂peak (extensive warm-up; EWU) or included three high intensity phases of 10 s at 200% of VO₂peak (dashed lines) additionally (intensive warm-up; IWU) followed by a passive resting phase of 5 min. Afterwards subjects performed a 30 s all-out exercise. Blood samples were taken under resting conditions (R), directly after warming-up (WU), after 4 min of rest (pre), directly after exercise (0’) and in minute intervals during recovery (1’-15’). Arrows on top assign blood samples.
Measurements

The 30 s all-out exercise was analysed for mean power output (MP), peak power output (PP) and the fatigue index (FI). The FI describes the development of fatigue. The FI is expressed as the power decline and was calculated by the following formula: FI = [(PP-Lowest Power) ·100] / PP

Lactate concentrations were determined in whole blood ([LA]WB), plasma ([LA]plasma) and erythrocytes ([LA]RBC). Whole blood samples were taken with a delay of 30 s after the plasma/erythrocyte sample (blood for plasma/RBC 0” to 15” of a minute, whole blood in the following 30” to 40” of a minute). All blood samples were taken from the earlobe.

For lactate determination in whole blood, 20 µL of blood was withdrawn from the earlobe with a 20 µL capillary tube. For lactate determination in plasma and erythrocytes 115 µL of blood were withdrawn from the earlobe with a capillary tube and directly centrifuged for 1 min at 6000 rpm with EBA20 (Hettich Zentrifugen, Tuttingen, Germany) in order to interrupt the slow entrance of lactate into the erythrocytes and to obtain true in vivo [LA]plasma and [LA]RBC. It took less than 30 s to take the blood sample and to start centrifugation (since it needs 25 min at 25°C until equilibration (Johnson et al., 1945), which is ~5°C higher than the temperature in the laboratory during sampling). Afterwards 20 µL of plasma or erythrocytes were analysed respectively after discarding the buffy coat and the very lowest part of the sediment. All 20 µL samples (WB, RBC, plasma) were directly mixed with 1 mL of the EBIO plus system solution, which hemolyzes RBCs and stops further metabolism. All samples were measured with EBIO plus (EKF Diagnostic Sales, Magdeburg, Germany).

Statistical analysis

Statistical analyses of the data were performed by using a statistics software package (Statistica for Windows, 7.0, Statsoft, Tulsa, OK). Descriptive statistics of the data are presented as means ± SD. For the comparison of different points in time (R, WU, pre, and 0’-15’ post-exercise) and for the comparison of different interventions (NWU, EWU, IWU), repeated-measures ANOVA with Fisher post hoc test was used. For the comparison of the percental differences of the increments a paired t-Test was used. Statistical differences were considered to be significant for p < 0.05.

Results

Peak Power and Mean Power Output for sprint cycling test

Peak power (PP) output, as well as the mean power (MP) output for the 30 s all-out exercise were significantly higher after both warming-up conditions (EWU and IWU) compared to NWU (Table 1). MP was 58 Watt (EWU) and 60 Watt (IWU) higher, showing an improvement of performance of 8.5 % and 8.8 % respectively due to WU. EWU and IWU differed only by 0.3 %. The FI showed no differences between all three conditions.

Lactate concentration in whole blood, plasma and RBC

After WU [LA]WB of each condition differed significantly from each other. Before the all-out exercise (pre) only [LA]WB IWU were significantly higher compared to NWU and EWU at respective points of measurement. During recovery peak [LA]WB were reached in the 5th minute (NWU & EWU) and in the 6th minute for IWU respectively. Moreover, the change between pre-values and the peak lactate concentrations during the recovery in whole blood was greater in NWU (12.9 ± 1.3 mmol·L⁻¹) and EWU (12.0 ± 1.7 mmol·L⁻¹) compared to IWU (10.0 ± 1.5 mmol·L⁻¹).

[LA]plasma and [LA]RBC differed significantly from each other at any point in time, showing greater [LA]plasma (1.3 ± 0.4 mmol·L⁻¹) compared to [LA]RBC (0.7 ± 0.2 mmol·L⁻¹) (mean resting values before WU from all conditions).

After WU [LA]plasma of each condition were significantly different from each other (Table 2). Before the all-out exercise (pre) [LA]plasma were only significantly higher after IWU compared to both other conditions. EWU was not significantly different from NWU. Peak [LA]plasma after the all-out exercise were reached in the 4th minute for all interventions (Figure 2 and Table 2). If we take the individual time to peak of each subject, time points do not differ as well. (NWU: 4.18 ± 0.98 min; EWU: 4.18 ± 1.08 min; IWU: 4.18 ± 0.87 min).

After WU [LA]RBC IWU were significantly different from NWU and EWU, whereas NWU and EWU did not differ significantly (Table 2). The same results were found before the all-out exercise (pre). In contrast to [LA]plasma, peak [LA]RBC were reached at different points in time; 7th IWU, 8th EWU and 9th NWU minute (Figure 2

Table 1. Peak Power (PP) and Mean Power (MP) output during the 30 s all-out exercise in watt [W]. Data are means (±SD).

<table>
<thead>
<tr>
<th></th>
<th>NWU</th>
<th>EWU</th>
<th>IWU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Power (MP, W)</td>
<td>680 (181)</td>
<td>738 (192) *</td>
<td>740 (191) *</td>
</tr>
<tr>
<td>Peak Power (PP, W)</td>
<td>986 (104)</td>
<td>1033 (107) *</td>
<td>1051 (80) *</td>
</tr>
<tr>
<td>Time to PP (sec)</td>
<td>5.5 (1.2)</td>
<td>5.5 (1.7)</td>
<td>5.6 (9)</td>
</tr>
<tr>
<td>Fatigue Index (%)</td>
<td>38.8 (6.1)</td>
<td>57.1 (7.8)</td>
<td>57.2 (7.5)</td>
</tr>
</tbody>
</table>

* significantly different from NWU. NWU: no warm-up; EWU: extensive warm-up; IWU: intensive warm-up.

Table 2. Comparison of [LA]plasma and [LA]RBC at certain points in time. Data are means (±SD).

<table>
<thead>
<tr>
<th></th>
<th>[LA]plasma [mmol*L⁻¹]</th>
<th>[LA]RBC [mmol*L⁻¹]</th>
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<tbody>
<tr>
<td></td>
<td>WU</td>
<td>pre</td>
</tr>
<tr>
<td>NWU</td>
<td>1.3 (5) #</td>
<td>1.3 (5) *</td>
</tr>
<tr>
<td>EWU</td>
<td>3.4 (2.1) #</td>
<td>2.4 (1.5) *</td>
</tr>
<tr>
<td>IWU</td>
<td>7.4 (2.9) #</td>
<td>5.2 (2.7)</td>
</tr>
</tbody>
</table>

* significantly different from IWU. # significant difference between all conditions.
and Table 2). If we take the individual time to peak of each subject, time points do not differ significantly (NWU: 7.82 ± 1.17 min; EWU: 7.39 ± 0.67 min; IWU: 7.55 ± 1.33 min).

The all-out exercise first caused a rapid increase of \([\text{LA}]_{\text{plasma}}\). The increase of \([\text{LA}]_{\text{RBC}}\) showed a time delay compared to the increase of \([\text{LA}]_{\text{plasma}}\) and therefore reached the peak after \([\text{LA}]_{\text{plasma}}\) reached its peak (see section ratio).

After the all-out exercise \([\text{LA}]_{\text{RBC}}\) IWU tended to be higher compared to NWU, reaching statistical significance in the 0', 1st, and 2nd minute, whereas \([\text{LA}]_{\text{plasma}}\) showed no differences, which can also be seen in the ratio (see section ratio).

### Increments (\(\Delta\)) of \([\text{LA}]_{\text{plasma}}\) and \([\text{LA}]_{\text{RBC}}\) in each time step

Differences in the increments (\(\Delta = \text{post value} - \text{pre value}\)) after the all-out exercise between all three conditions for \([\text{LA}]_{\text{plasma}}\) and \([\text{LA}]_{\text{RBC}}\) were only found in the time interval (pre – 0') (Table 2, Figure 3a and Figure 3b).

The increment in \([\text{LA}]_{\text{plasma}}\) IWU was significantly lower compared to NWU (\(p < 0.003\)) and compared to EWU (\(p < 0.024\)) (Figure 3a and Table 2). The percental differences of the increments for \([\text{LA}]_{\text{RBC}}\) are shown in Table 3 (whereas the higher value was set as 100%).

Similar results were found for \([\text{LA}]_{\text{RBC}}\). The increment of \([\text{LA}]_{\text{RBC}}\) IWU differed significantly from NWU (\(p < 0.003\)) and EWU (\(p < 0.012\)) respectively (Figure 3b and Table 2). The percental differences of the increments for \([\text{LA}]_{\text{RBC}}\) are shown in Table 3 (whereas the higher value was set as 100%).

### Ratio (\(\text{[LA]}_{\text{RBC}}/\text{[LA]}_{\text{plasma}}\))

The mean RBC-to-plasma \([\text{LA}]\) ratio under resting conditions was 0.51 ± 0.07 (NWU), 0.51 ± 0.04 (EWU) and 0.50 ± 0.04 (IWU) showing a \([\text{LA}]_{\text{plasma}}\) which is approximately twice that of \([\text{LA}]_{\text{RBC}}\) (Figure 4). The 5 min rest after both WU conditions significantly increased the ratio (0.56 ± 0.06 (EWU) and 0.56 ± 0.03 (IWU); \(p < 0.011\)). The all-out exercise then significantly decreased the ratio in all three conditions (0': 0.32 ± 0.03 (NWU), 0.36 ± 0.05 (EWU), 0.41 ± 0.04 (IWU)). The lowest ratio was reached at 0' for NWU and EWU and at 1' for IWU (0.39 ± 0.04). The ratio stayed significantly lower up to the 5th (NWU), 6th (EWU) and 8th (IWU) minute compared to pre values of each condition (significance not assigned in the graph, in order to avoid too many Table 3. Differences in percentage (%) of the increments (pre - 0') between \([\text{LA}]_{\text{plasma}}\) and \([\text{LA}]_{\text{RBC}}\). The higher value was set as 100%).

<table>
<thead>
<tr>
<th></th>
<th>NWU vs. EWU</th>
<th>NWU vs. IWU</th>
<th>EWU vs. IWU</th>
</tr>
</thead>
<tbody>
<tr>
<td>([\text{LA}]_{\text{plasma}})</td>
<td>92 (34)</td>
<td>69 (25)</td>
<td>77 (27)</td>
</tr>
<tr>
<td>([\text{LA}]_{\text{RBC}})</td>
<td>92 (29)</td>
<td>53 (25) *</td>
<td>67 (31) #</td>
</tr>
<tr>
<td>Difference</td>
<td>0</td>
<td>-16</td>
<td>-10 %</td>
</tr>
</tbody>
</table>

* \(p < 0.05\) lower than percental increment of \([\text{LA}]_{\text{plasma}}\). * \(p = 0.051\); calculated with a paired student t-Test.
Figure 3. Increments (Δ) of [LA] plasma (A) and [LA] RBC (B) in each time step. Significant differences in Δ [LA] plasma and Δ [LA] RBC were only found in the time interval (pre-0'). For NWU the ‘pre’ value equates the ‘R’ value. NWU: squares, solid line; EWU: triangles, broken line; IWU: circles, dotted line. *significantly different from IWU (p < 0.05). Calculated with a paired student t-Test.

Discussion

Several possible effects of WU are discussed in literature which might have an effect on performance. The present study was designed to answer three questions. The first question was whether there would be an improvement in maximal exercise test performance with WU. The second question was whether a more intense WU would be more effective than an extensive WU. The third question was whether the increase in [LA] RBC/the lactate distribution between plasma and RBCs is influenced by different WU procedures in vivo.

In order to bring the transport process to its limit, we chose a 30 s all-out cycling exercise to induce a maximal and fastest increase/congestion of [LA] plasma concentration.

Power output

The main finding of our investigation was that WU enhances performance irrespective of WU intensity. Thereby we found improvements of the MP of ~8.5-9 % after EWU and IWU compared to NWU. The results demonstrated a positive effect of WU on performance, although our findings failed to demonstrate an advantage of a more intense WU.

Bishop et al. (2003) performed similar WU
protocols using a continuous warm-up of 15 min at approximately 65% \( V_{O_{2\max}} \) and an intermittent, high-intensity WU similar to ours, except for the last 5 min, which included five 10 s sprints at 200% \( V_{O_{2\max}} \), separated by 50 s of recovery at approximately 55% \( V_{O_{2\max}} \). Afterwards a 2 min all out kayak test was performed. In contrast to the present study significantly greater peak power (+4.7%) and mean power (+2.2%) were recorded after the intermittent WU compared to continuous WU (Bishop et al., 2003). Burnley et al. (Burnley et al., 2005) found that moderate- and high-intensity domains can improve severe-intensity cycling performance (7-min performance trial) by ~2-3%. In a study of Hajoglou et al. both “easy WU” and “hard WU” improved 3 km cycling time trial performance by 2.6-2.8% compared to no WU (Hajoglou et al., 2005).

In contrast no significant differences were observed for average power in an earlier study from Bishop et al. in which subjects warmed up for 15 min at either the aerobic threshold (W1), the anaerobic threshold (W3), or mid-way between the aerobic threshold and anaerobic threshold (W2), followed by a 2 min, all-out kayak ergometer test (Bishop et al., 2001). Similarly, Gray et al. found no difference in cycling performance until exhaustion between active, passive and control trials (Gray and Nimmo 2001).

Summing up these results, it seems that the effect of WU on performance decreases with increasing exercise time. That might be the reason why we found higher improvements compared to other studies, as they used longer exercise times. Our results show that different WU protocols can improve short term maximal exercise performance which is important for different sport disciplines (track and field, speed skating etc.) but also for performance diagnostics, when performing Wingate-tests as a common tool to investigate anaerobic power. Therefore athletes as well as scientists should carry out a standardized WU protocol.

Lactate

Our results showed that even for the resting state, \([LAC]_{\text{RBC}}\) was lower than \([LAC]_{\text{plasma}}\). The ratio \([LAC]_{\text{RBC}}/[LAC]_{\text{plasma}}\) was about 0.5 for the resting state which is in accordance with most of the studies (Böning et al., 2007; Foxdal et al., 1990; Gladden et al., 1994; Harris and Dudley 1989; Juel et al., 1990; Sara et al., 2006; Smith et al., 1997, 1998). In contrast Hildebrandt et al. (2000) and Buono and Yeager (1986) found equal lactate concentrations in plasma and RBCs. Most studies explain this gradient between plasma and RBCs as a result of a Donnan equilibrium (Böning et al. 2007; Smith et al. 1997; 1998) or according to the membrane potential (Juel et al. 1990) respectively. Under resting conditions, there is also a significant pH gradient between the RBC (7.2) and plasma (7.4), which conforms to the Donnan distribution of \(H^+\) ions caused by the negative charge of haemoglobin (Harris et al. 1989; Jensen 2004). The RBC/plasma [L] ratio of ~0.5 at rest is mirrored by a plasma/RBC [H+] ratio of 0.62 ± 0.01 and 0.47, reported by Smith et al. and Harris et al. respectively (Harris et al. 1989; Smith et al. 1997, 1998). Different transport systems and the membrane potential possibly avoid a homogenous distribution of lactate. Minor variations in resting values between different studies as well as between the subjects in our study, may be caused by measurements near the accuracy of measurement/detection.
The maximal exercise caused a more rapid increase of $[\text{LA}]_{\text{plasma}}$ than of $[\text{LA}]_{\text{RBC}}$ during and shortly after the exercise, causing a decrease in the ratio. This is in accordance with the results of Juel et al. (1990) and McKelvie et al. (1991). In contrast, studies using an incremental step test found no changes in the ratio (Sara et al., 2006; Smith et al., 1997). It seems that only in the presence of a fast and high congestion of $[\text{LA}]_{\text{plasma}}$, the transport across RBC membrane is saturated. The contradictory results in resting values as well as in recovery values might be caused by several reasons: 1. Different exercise protocols with more or less time for equilibrium between plasma and RBCs (half time for equilibration is about 50-120 s at 37 °C (Böning et al., 2007)) and with more or less fast and high increases in plasma $[\text{LA}]$. 2. Differences in the blood samples (venous, arterial, and capillary). $[\text{LA}]$ is higher in capillary/arterial blood due to lactate elimination and more time for equilibration in venous blood. The use of capillary blood might as well be an advantage for plasma lactate determination because of the shorter time to take blood samples (minimized time for lactate distribution) (Hildebrand et al., 2000). 3. Differences in blood sample treatment to interrupt lactate transport between plasma and RBCs by rapid cooling and/or rapid centrifugation. 4. Variations in the determination of $[\text{LA}]_{\text{RBC}}$ (measured or calculated).

$[\text{LA}]_{\text{RBC}}$ reached its peak later than $[\text{LA}]_{\text{plasma}}$ reached its peak. As expected, the comparison of the points in time of peak $[\text{LA}]_{\text{RBC}}$, $[\text{LA}]_{\text{plasma}}$ and $[\text{LA}]_{\text{RBC}}$ shows that the lactate transport across RBC membrane is delayed. The peak $[\text{LA}]_{\text{RBC}}$ was reached 2–4 min later than the peak $[\text{LA}]_{\text{plasma}}$. This is in accordance with the results of Böning et al. (2007) and Hildebrand et al. (2000) where $[\text{LA}]_{\text{RBC}}$ reached its peak value 3 min and 5 min later than $[\text{LA}]_{\text{plasma}}$ during recovery after an incremental step test respectively (Böning et al., 2007; Hildebrand et al., 2000). Juel et al. (1990) reported a time delay of 2 min for peak $[\text{LA}]_{\text{RBC}}$ compared to $[\text{LA}]_{\text{plasma}}$ (Juel et al., 1990).

When $[\text{LA}]_{\text{RBC}}$ reached the peak, the ratio $[\text{LA}]_{\text{RBC}} /[\text{LA}]_{\text{plasma}}$ roughly recovered to its beginning value. After $[\text{LA}]_{\text{RBC}}$ reached the peak, it decreased, representing an outflow of lactate from red blood cells, in spite of the fact that $[\text{LA}]_{\text{plasma}}$ was still much higher than $[\text{LA}]_{\text{RBC}}$. It seems that the system reached its equilibrium, dependent on the distribution of $[\text{LA}]_{\text{RBC}}$ and $[\text{LA}]_{\text{plasma}}$ (1 RBC : 2 plasma) and dependent on the fact that $[\text{LA}]_{\text{plasma}}$ now changed only slowly.

WU affected the increase of $[\text{LA}]_{\text{RBC}}$ directly after the all-out-exercise, which was shown by the different percental increments of $[\text{LA}]_{\text{RBC}}$ and $[\text{LA}]_{\text{plasma}}$. Later during recovery no differences for $[\text{LA}]_{\text{plasma}}$, $[\text{LA}]_{\text{RBC}}$ and the increments of both were found. Some possible mechanisms for a decreased influx have previously been suggested in the literature, e.g. Donnan equilibrium (Johnson et al., 1945), barrier provided by the membranes of RBCs (Buono and Yeager 1986) and the saturation of transporters (MCT) (Böning et al., 2007; Buono and Yeager 1986; Gladden et al., 1994; Harris and Dudley 1989; Hildebrand et al., 2000). In this context it has to be considered, that the lactate transport by MCTs is a facilitated but passive transport mainly driven by the concentration gradient. Therefore the velocity of reaching the equilibrium depends on the initial difference and on the permeability. However, the exact mechanisms remain unclear. The increase in $[\text{LA}]_{\text{plasma}}$ was significantly lower in the first time interval (pre-0’) for IWU. Therefore one would expect a similar or even higher increase in $[\text{LA}]_{\text{RBC}}$ (and not a lower) because of a less saturated transport system (MCT). But the lower increment of $[\text{LA}]_{\text{RBC}}$ after IWU might be due to a “higher preloading” with lactate before exercise, causing a smaller $[\text{LA}]$ gradient (initial difference) between plasma and RBCs. It seems that the influx decreases with increasing intracellular $[\text{LA}]$. It can be speculated, that RBCs may have a maximal uptake capacity for lactate, although this has never been proven. As the $[\text{LA}]$ increases inside of RBCs, the resistance against an influx increases as well. But the negative electric charge inside RBCs, which is caused by various anions, particularly the non-diffusible anions of hemoglobin (Hb) may also involve. The negative electric charge is ~ -10 mV at the inside of the red cell membrane forcing diffusible anions out. This negative charge resists the inward diffusion of LA’ even for the resting state. However, the couple $[\text{LA}’; H^+]$, transported through MCT-1, as a whole, is electrically neutral. Therefore, there is no net electric force acting on the couple. Another possibility for the forces acting on the distribution could be a preferred direction for transport of MCT-1. Indeed, the different $K_m$ values (Michaelis Menten constant) for efflux and influx in RBCs may suggest asymmetric behaviors of MCT-1 (Deuticke 1982).

Another possibility one could speculate about is, that the extracellular increase in $[\text{LA}]$ inhibits the export of lactate (produced by the RBC itself; as they rely exclusively on glycolysis to produce energy) from RBCs (Siems et al., 2000). This inhibited export of lactate from RBCs may lead to an intracellular lactate accumulation, but not due to an uptake. But the relatively fast increase of $[\text{LA}]_{\text{RBC}}$ partly contradicts the second possibility, as the lactate production in RBCs is only 2.3-2.5 mmol·L$^{-1}$ of cells per hour (Siems et al., 2000). Furthermore studies showed that, differently trained subjects reveal different influx rates into RBCs (Skelton et al., 1998). Higher total, as well as MCT-1 mediated lactate influx was also reported for persons with sickle cell trait and sickle cell disease (Patillo et al., 2005; Sara et al., 2006).

The question remains, why RBCs should take up lactate, as they cannot use it for oxidative energy production as other tissues. However RBCs might be involved in the spreading of the oxidative substrate “lactate” between tissues. A key aspect of this cell-to-cell lactate shuttle concept is the exchange of lactate between tissues of net lactate release and gluconeogenesis/oxidative tissues (Brooks 2009). RBCs may act as a “shuttle” between these tissues. Lindinger et al. (1992) showed that large and rapid increases in $[\text{LA}]_{\text{plasma}}$ result in the transport into RBCs. They suggested that the uptake of lactate by RBCs plays an important role in regulating ion homeostasis within plasma and the interstitial and intracellular compartments of contracting muscle (Lindinger et al., 1992), RBCs function to transport lactate from the working muscle and help to maintain a concentration difference between plasma and muscle facilitating diffusion of lactate.
from the interstitial space into plasma (McKelvie et al., 1991). This regulatory process may help to maintain the function of active muscles by delaying the onset of fatigue (Lindinger et al., 1992 & 1995), but it may also help to improve the cell-to-cell lactate shuttle as more lactate can by transported out of the working muscle.

**Limitations of the study**

Although our study showed an influence of WU on the lactate distribution between plasma and RBCs the experimental model used in this study is not sufficient to give clear conclusions. Differences in lactate transport in RBCs can hardly explain performance differences in the all-out exercise test. Important compartments for the distribution of lactate like the skeletal muscle and the interstitium were not taken into account. Furthermore membrane transport characteristics, the effects of blood flow or changes in plasma volume were not determined. Based on this experimental set up, we can only speculate about the underlying mechanisms for the observed differences, like the saturation of MCTs, or an altered lactate flux.

In this study free 30 sec all-out exercise was used for the determination of differences in PP and MP output induced by different WU procedures, however, for the questions regarding lactate transport the same workload in all three conditions may have been more appropriate, as differences in lactate increase may arise from the differences in PP and MP output.

**Conclusion**

In this study, the differences in lactate transport cannot explain the higher performance levels after WU. Slightly higher [LA] were found in RBCs during recovery after WU, whereas [LA]_{plasma} did not differ. Therefore the “total uptake capacity” and thus the dilution space RBC seems to be affected. Juel et al. reported a 5-fold increase in the density of MCT-1 in RBCs after 8 weeks of chronic hypoxia (Juel et al., 2003). These tremendous changes suggested improved performance after high altitude training. However, the advantageous of the increase of the erythrocyte MCT-1 density are still unknown. A previous study showed that the recovery of pH of an erythrocyte suspension after lactic acid addition to the medium was significantly faster after a training intervention compared to the sedentary group (Aoi et al., 2004). These results underline the importance of RBCs as a dilution space and for pH regulation, but the exact mechanisms and the contribution to performance of an altered lactate transport are still not known and need to be determined.

The above ideas for the possible mechanism of the flux of LA are still speculative. The physical and chemical mechanisms for the flux of LA are still not fully understood and will be subject of future research.

**References**


Key points
- Warm-up significantly improves performance during 30 s maximal effort exercise.
- No differences in performance were found between extensive and intensive warm-up.
- Warm-up and maximal effort exercise affects the lactate distribution between plasma and RBC.
- Lactate influx into RBC decreases with increasing intracellular lactate concentrations.

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