Original Article

Lactate distribution in red blood cells and plasma after a high intensity running exercise in aerobically trained and untrained subjects

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ABSTRACT

Introduction: To determine endurance capacity and to give specific training recommendations, blood lactate (LA) concentrations are frequently used in performance diagnostics. In blood, LA is stored in red blood cells (RBC) and in plasma. Higher LA uptake by RBC might lead to delayed muscle fatigue since RBC serve as a dilution space for LA and more LA can be taken up by plasma which is released from the working muscle. Therefore, the aim of this study was to investigate the distribution of lactate in plasma and RBC in aerobically well-trained athletes (AA) in comparison to an untrained control group (CG). Materials and Methods: 13 AA and 13 CG participated in this study and conducted a high intensive treadmill test consisting of 2x4 minutes of running at 95% of the maximal running velocity with an active break of 4 minutes. Venous blood was drawn before and after the test. LA was measured in whole blood, plasma and RBC. Further, the ratio (LA_{Ratio}) was calculated using the following formula: LA of RBC / LA of plasma. Results: AA exhibit significantly higher values in VO_2peak and maximum running velocity. After the running test, LA in whole blood, RBC and plasma is increased significantly in both groups. No interaction effect (group X time point) was observed in any parameter. Values of LA_{Ratio} did not show any significant differences. Conclusion: This study showed that the LA distribution in RBC and plasma after a high intensity running test is very similar in well trained endurance athletes and in untrained control subjects. Hence, LA uptake by RBC cannot or only in part be seen as a

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contributor to aerobic athletic performance. **Key words:** LACTATE, AEROBIC CAPACITY, RUNNING, RED BLOOD CELLS, ERYTHROCYTES, PLASMA.

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INTRODUCTION

In performance diagnostics, blood lactate (LA) concentrations are frequently used to determine endurance capacity (Broich et al., 2012; Goodwin et al., 2007), to give specific training recommendations (Stegmann et al., 1981) and to predict athletic performance in competitions (Muñoz Perez et al., 2012). Skeletal muscle produces and releases large amounts of LA during high intensity exercise. But at the same time, muscles take up LA and use it as a potential energy source for further muscle work (Gladden, 2000; Juel, 2001). The excess LA that is produced is immediately distributed in the blood and transported to LA eliminating organs and body parts (Lindinger et al., 1995).

Whole blood consists of mainly two phases, which are the erythrocytes (red blood cells, RBC) and plasma. RBC represent around 40-45% of the whole blood volume which is represented in the hematocrit (Varlet-Marie & Brun, 2004). Previous studies showed that the distribution of LA between RBC and plasma at rest is about two times higher in plasma than in RBC (Böning et al., 2007; Sara et al., 2006; Smith et al., 1997; Wahl et al., 2010). Thus, the RBC / plasma LA concentration ratio (LA_{Ratio}) is around 0.5 in these studies. In contrast, studies also show that the LA_{Ratio} is around 1 which indicates an even distribution of LA between RBC and plasma at rest (Buono & Yeager, 1986; Hildebrand et al., 2000).

LA is primarily produced when performing intensive anaerobic exercise and a disequilibrium occurs in the distribution of LA since the LA concentrations are higher in plasma than in RBC (Buono & Yeager, 1986). RBC possess different specific lactate transport systems to take up LA from plasma: (1) a non-ionic diffusion of the undissociated acid, (2) an inorganic anion-exchange system (band 3 system) and (3) a monocarboxylate-specific (MCT 1) carrier mechanism (Connes et al., 2004). In athletic performance the monocarboxylate pathways were shown to have the highest contribution to LA influx into RBC (Deuticke, 1989; Skelton et al., 1998).

It was previously shown that regular aerobic training positively alters the erythrocyte content of MCT 1 by up-regulating the content of about 90% (Opitz et al., 2015). The ability of RBC to store LA might be of particular importance for athletic performance as RBC can function as a dilution space for LA. When RBC take up LA, more LA can be taken up by the plasma. Therefore, more LA can be produced and released from the muscle into the blood and induce a delayed onset of muscle fatigue (Juel et al., 2003).

The aim of this study was to investigate the distribution of lactate in plasma and RBC in aerobically well-trained athletes in comparison to an untrained control group. Further, the importance of RBC as a possible distribution space for LA should be evaluated which might contribute to an improved athletic performance, especially in anaerobic performance modes. It is hypothesized that aerobically well-trained athletes are able to store more LA in RBC during an intensive running exercise. This might be due to an improved LA transport system from plasma into the RBC resulting from chronic endurance training.

METHODS

Participants

26 male subjects participated in this study. Of these participants were 13 aerobically well-trained athletes (AA) and 13 subjects served as an untrained control group (CG). Inclusion criteria for AA were: High activity (> 8 hours/week) in endurance sports for at least two years of training history and a VO_2peak of at least 50 ml/kg/min. Inclusion criteria for CG was a reported a physical activity of less than four hours per week and a VO_2peak less than 50 ml/kg/min. All subjects abstained from alcohol consumption 24 hours prior to the testing

and were non-smokers. Further, none of the participants did any training at the day of the testing and did not perform high-intensity training the day before the testing. Anthropometric data are presented in Table 1. Height, weight and age did not differ between groups. The study and the used protocols were approved by the ethics committee of the German Sport University Cologne. These protocols align with the declaration of Helsinki and all participants gave written informed consent to participate in this study.

Table 1. Age, height, weight and performance data of subjects.

	Aerobically well-trained athletes (AA)	Control group (CG)
Age [years]	22.92 ± 2.25	22.85 ± 2.03
Height [cm]	183.38 ± 4.80	181.31 ± 5.66
Weight [kg]	78.08 ± 7.03	83.00 ± 12.35
VO_2peak [ml/kg/min]	55.00 ± 5.03 *	45.15 ± 3.51
Max. velocity of pre-experimental test [m/s]	5.37 ± 0.28 *	4.68 ± 0.33
Applied velocity [m/s][1]	5.10 ± 0.26 *	4.44 ± 0.32

** p<0.05 vs. Control group. [1]Velocity that was applied in the main test in which lactate values were obtained (95% of max. velocity of pre-experimental test).*

Pre-experimental test

All tests were carried out in the same laboratory and all running tests were performed on the same treadmill (Woodway, Weil am Rhein, Germany). In the pre-experimental test, subjects conducted a ramp-based pre-test to determine maximal oxygen uptake (VO_2peak). The ramp-based test started with a velocity of 2 m/s for three minutes at an elevation of 1%. Then, the elevation was increased to 2.5% and the velocity was increased every 30 seconds by 0.3 m/s. Ventilatory parameters were measured breath-by-breath using a spirometer during the test (Metamax 3B, CORTEX Biophysik GmbH, Leipzig, Germany). Calibration of the oxygen and carbon dioxide analysers was conducted before each test with precision reference gases and calibration of the pneumotachograph was performed using a 3-L calibration syringe (Hans Rudolph Inc., Kansas City, USA). Heart rate was monitored using a heart rate monitor (Polar Electro GmbH, Büttelborn, Germany) and participants conducted the test until subjective exhaustion. VO_2peak was determined as the average of the highest values measured over the last 30 seconds interval. Values of VO_2peak, maximum running velocity and running velocity applied at the main test are presented in Table 1. VO_2peak, maximum running velocity, and running velocity employed in the main test were higher in AA than in CG.

Main test

At least two days after the pre test, blood was drawn from participants after five minutes of sitting rest from the vena mediana cubiti (Pre). After warming up for ten minutes at the velocity measured at the first ventilatory threshold, participants conducted an intensive test in which participants ran four minutes at the velocity of 95% of maximum running speed of the pre-experimental test. Then, participants had an active break of four minutes in which they walked. After the break, participants ran again for four minutes at the same running speed as before. Velocity was adjusted only when participants fell back on the treadmill and could not

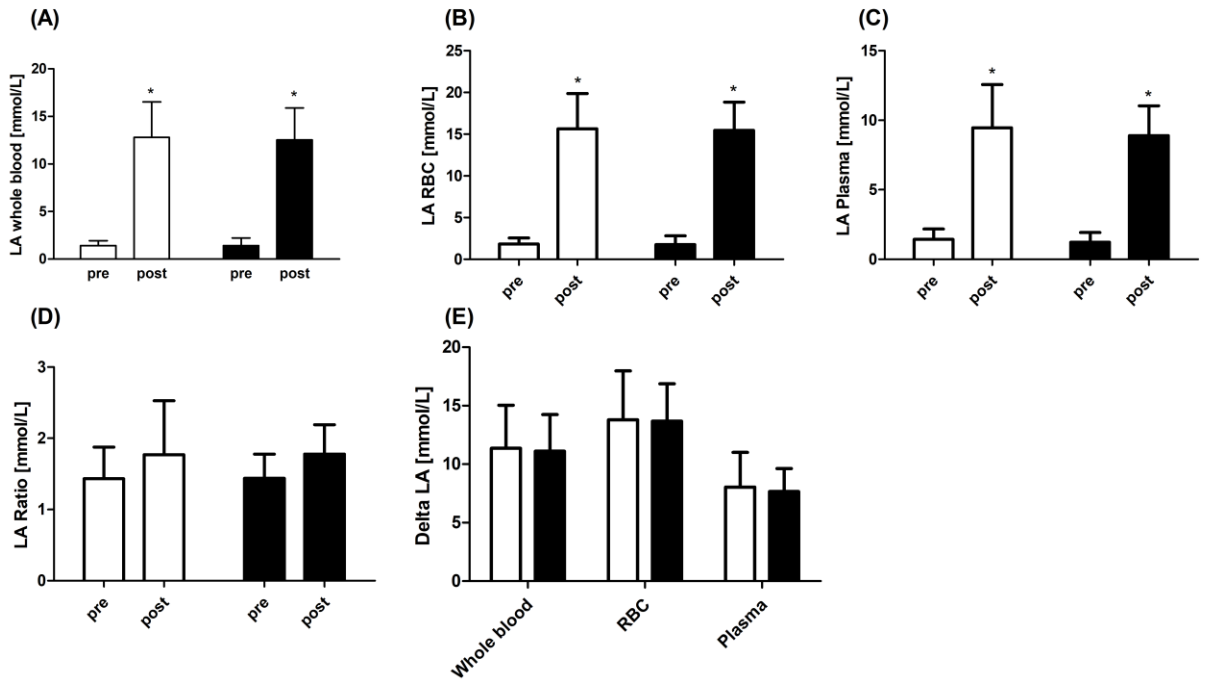
maintain the running speed, even after strong verbal motivation. Three minutes after finishing the test, venous blood was sampled again from the vena mediana cubiti (Post).

Blood sampling

LA was determined in venous whole blood, isolated RBC and in plasma. To separate RBC from plasma, the whole blood sample was centrifuged at 3000 rcf for 1 min at 4°C. 20µl of whole blood, isolated RBC and plasma, respectively, were mixed with 1ml of hemolytic liquid and then analysed using EKF-Boisen Lab+ (EKF-diagnostic GmbH, Barleben, Germany). Further, the LA_{Ratio} of LA of RBC / LA of plasma was calculated as done before (Wahl et al., 2010).

Statistics

Statistical analyses of the data were performed by using statistics software package Graph-PadPrism 6 (La Jolla, USA). All data were tested for normality of distribution using Shapiro-Wilk test which revealed no need for further transformation. To compare height, weight, age, VO_2peak, maximum running velocity, and the employed running velocity in the main test an unpaired Student's t-test was conducted. To detect effects of the two-running test, a two-way mixed ANOVA with the factors 'group' (AA, CG) and 'time point' (Pre, Post) was calculated to detect effects of the running mode and effects of the 'time point' X 'group' interaction. If differences were observed, Bonferroni post hoc analyses were conducted. To compare the specific increase of LA of each group, the delta (Δ) was calculated by subdividing the pre-value from the post value. Data of the Δ of LA measured in whole blood, RBC and plasma were compared using a Student's t-test for unpaired data to evaluate differences between the two subject groups. Differences were considered as significant with $p<0.05$. All data are expressed as means ± standard deviation.



*Note. White bars indicate aerobically trained subjects (AA) and black bars indicate subjects of the control group (CG). * p<0.05 vs. Pre of the same group. Data are presented as means ±standard deviation.*

Figure 1. LA concentration in whole blood (A), RBC (B) and plasma (C) before (Pre) and after (Post) the running exercise. (D) LA Ratio before (Pre) and after (Post) the running exercise. (E) Delta of LA in whole blood, red blood cells (RBC) and Plasma.

RESULTS

Analyses revealed a time effect of the test (pre, post) on LA measured in whole blood (Figure 1A), RBC (Figure 1B) and Plasma (Figure 1C) with significantly higher values observed after the running exercise in both groups ($p<0.05$). Whole blood LA increased from 1.43 ±0.51 to 12.79 ±3.74 in AA and from 1.42 ±0.81 to 12.52 ±3.38 in CG. RBC LA increased from 1.84 ±0.71 to 15.63 ±4.26 in AA and from 1.77 ±1.05 to 15.44 ±3.40 in CG. Plasma LA increased from 1.43 ±0.76 to 9.46 ±3.11 in AA and from 1.23 ±0.7 to 8.89 ±2.15 in CG. No interaction effect was observed for any of these three parameters. Analyses of LA_{Ratio} did not yield any significant differences. Values of LA_{Ratio} increased (not significantly) from 1.43 ±0.44 to 1.77 ±0.76 in AA and from 1.44 ±0.34 to 1.78 ±0.42 in CG (Figure 1D). Comparison of Δ of LA of whole blood, RBC and plasma showed no difference between AA and CG (Figure 1E). Δ of whole blood LA were 11.36 ±3.68 in AA and 11.10 ±3.14 in CG, Δ of RBC LA 13.79 ±4.18 in AA and 13.67 ±3.21 in CG and Δ of plasma LA 8.03 ±2.98 in AA and 7.66 ±1.97 in CG.

DISCUSSION

The study aimed to compare the rate of LA accumulation in the blood with a special focus on LA accumulation in the two main blood compartments RBC and plasma in AA and CG. Interpretation of the VO_2peak values and maximum running velocity measured in the pre-experimental test showed that the AA possessed a higher VO_2peak and thus aerobic capacity and ran longer and reached higher maximum running velocities than the CG. To reach highest possible LA concentrations we chose a very high intensity running mode in the main test which was set at a running velocity at 95% of the maximum running speed of the pre-experimental test. A higher LA uptake by RBC in athletic situations might contribute to improved performance as a high RBC LA uptake lowers the LA concentration in the plasma. The concentration difference between plasma and muscle might consequently facilitate the diffusion of LA from the interstitial space into the blood plasma (McKelvie et al., 1991) resulting in a delayed onset of muscle fatigue in anaerobic situations, such as high intensity running.

We hypothesized that the RBC of AA take up more LA due to an improved LA transport system into RBC as it was reported that the MCT 1 density of RBC increases after endurance training regimens (Juel et al., 2003; Opitz et al., 2015). But no difference in the Δ of RBC LA was observed between the groups in the present study. LA transport via the MCT system is a passive transport mechanism which is determined by the concentration gradient between LA in the plasma and RBC (Wahl et al., 2010). It is speculated that the LA influx into RBC decreases with increasing LA in RBC and in the present study high RBC LA values are observed. Further, it was shown that the direction of LA transport via MCT 1 might influence in- and efflux of LA and an asymmetric MCT 1 behaviour was suggested (Deuticke, 1982).

In the present study, venous blood samples were taken. This is important to consider as LA concentrations are higher in capillary blood compared to venous blood. Venous blood has been utilized by different organs such as passive and active musculature, the liver, the brain and the heart. Especially, when the metabolic rate of the subjects increases during exercise, LA oxidation by skeletal muscles and the heart increases (Goodwin et al., 2007). Therefore, LA concentrations are usually lower in venous blood compared to capillary blood samples (Foxdal et al., 1990; Foxdal et al., 1991).

The LA concentration in whole blood increased in both groups to LA concentrations over 12 mmol/L which was expected as the running mode was designed to exhibit very high increases in LA concentrations. Further, the Δ of whole blood LA showed no difference between both groups. This was also observed by Gmada et

al. who observed that whole blood LA peak values measured after different test modes did not differ between an aerobically trained and a untrained group (Gmada et al., 2005).

LA concentrations in RBC increased in both groups to values around 15 mmol/L and plasma LA concentrations increased to values little lower than 10 mmol/L. Neither in plasma LA, nor in RBC LA a group effect was observed, and similar Δ of plasma and RBC LA were observed in both groups. The influx of LA as an energy source is faster from plasma to utilizing muscles and organs than from RBC to those body parts (Goodwin et al., 2007; Hildebrand et al., 2000). This might explain why LA concentrations are higher in RBC compared to plasma before and after the exercise as venous blood samples were taken. This is also observed in the LA_{Ratio} which shows quotients of around 1.4 before and of 1.8 after running in both groups. These LA_{Ratio} results stand in contrast to those of studies using capillary blood for LA measurements (Buono & Yeager, 1986; Daniel et al., 1964; Devadatta, 1934). Hildebrand et al. found a LA_{Ratio} of 1 before and of 0.43 after an incremental treadmill running test to exhaustion employing venous blood (Hildebrand et al., 2000). In the present study no significant difference in the LA_{Ratio} is observed in response to the running exercise which was also reported in previous studies (Sara et al., 2006; Smith et al., 1997). A reason for the fiercer increment of the LA concentration in RBC compared to plasma LA could be the chosen running protocol. The employed protocol in the present study was very intensive and studies show that the LA accumulation in RBC increases disproportionately fast at high intensity modes (Hildebrand et al., 2000) because LA transport systems might be more activated when LA concentrations are very high in whole blood and plasma, respectively (Wahl et al., 2010). The chosen running protocol of the present study contrasts most studies that used sub-maximal training protocols which might not lead to an increased influx of LA into RBC.

Important to note is that all participants were healthy and had no diseases or any inhibition in daily activity. It can only be speculated whether more untrained subjects in the CG or more trained subjects (e.g. elite endurance athletes) might have yield different results, even though the VO_2peak data show a significant difference in aerobic performance with VO_2peak values of 55 ml/kg/min measured in AA compared to 45 ml/kg/min measured in the CG. Sara et al. reported no different LA distribution between RBC and plasma in subjects with sickle cell disease compared to healthy subjects. In this study the healthy participants showed higher VO_2peak values similar to the present study (Sara et al., 2006).

CONCLUSION

In this study we were able to show that different aerobic training status does not lead to an altered LA distribution in plasma and RBC in response to a high intensive running exercise. The discussed possible mechanisms might help to explain the absence of differences between the two groups in LA distribution, but the exact chemical and physical mechanisms and the contribution of LA storage in the blood compartments to athletic performance still need further research.

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