ORIGINAL PAPER

A mathematical model for lactate transport to red blood cells

Patrick Wahl · Zengyuan Yue · Christoph Zinner ·
Wilhelm Bloch · Joachim Mester

Abstract A simple mathematical model for the transport of lactate from plasma to red blood cells (RBCs) during and after exercise is proposed based on our experimental studies for the lactate concentrations in RBCs and in plasma. In addition to the influx associated with the plasma-to-RBC lactate concentration gradient, it is argued that an efflux must exist. The efflux rate is assumed to be proportional to the lactate concentration in RBCs. This simple model is justified by the comparison between the model-predicted results and observations: For all 33 cases (11 subjects and 3 different warm-up conditions), the model-predicted time courses of lactate concentrations in RBC are generally in good agreement with observations, and the model-predicted ratios between lactate concentrations in RBCs and in plasma at the peak of lactate concentration in RBCs are very close to the observed values. Two constants, the influx rate coefficient C_1 and the efflux rate coefficient C_2, are involved in the present model. They are determined by the best fit to observations. Although the exact electro-chemical mechanism for the efflux remains to be figured out in the future research, the good agreement of the present model with observations suggests that the efflux must get stronger as the lactate concentration in RBCs increases. The physiological meanings of C_1 and C_2 as well as their potential applications are discussed.

Keywords Erythrocytes · Lactate transport · Mathematical model · Blood compartments

Introduction

Blood lactate is a well-known important parameter for exercise physiology because the individual blood lactate threshold offers a simple and convenient way to distinguish trained from untrained subjects [1] and to see the improvement of the endurance level after certain training for a given subject. Although lactate determination in whole blood is a standard method in performance diagnostics, there is only limited knowledge about the dynamic distribution of lactate among different cells, tissues and compartments [e.g., plasma and red blood cells (RBCs)] during exercise. In this context blood represents the first great distribution space for lactate leaving the muscle. However, lactate is not homogeneously distributed in blood. The lactate concentration in plasma, denoted by $[LA]_{plasma}$ hereafter, could be much higher than the lactate concentration in RBC, denoted by $[LA]_{RBC}$ hereafter, especially during and after intensive exercises. For this reason, many researchers have studied $[LA]_{plasma}$ and $[LA]_{RBC}$ separately before, during and after certain exercises and lactate infusion. In one group of studies [2–9], $[LA]_{RBC}$ was calculated from the lactate concentrations in plasma and in whole blood. In the other group of studies [10, 11], $[LA]_{RBC}$ was measured. Although the exercise protocols used in these studies were different, some common features found in these studies could be

P. Wahl and Z. Yue contributed equally to this study.

P. Wahl (✉) · Z. Yue · C. Zinner · J. Mester
Institute of Training Science and Sport Informatics,
German Sport University Cologne,
Am Sportpark Müngersdorf 6, 50933 Cologne, Germany
e-mail: Wahl@dshs-koeln.de

P. Wahl · W. Bloch
Institute of Cardiovascular Research and Sport Medicine,
German Sport University Cologne, Cologne, Germany

P. Wahl · W. Bloch · J. Mester
German Research Centre of Elite Sport,
German Sport University Cologne, Cologne, Germany

summarized as follows: (1) In most of the studies, $[LA]_{RBC}$ was found to be significantly lower than $[LA]_{plasma}$ even in the resting state, resulting in a ratio $[LA]_{RBC} \cdot [LA]_{plasma}^{-1}$ of about 0.5. (2) In the cases of a high increase rate of $[LA]_{plasma}$ caused by intensive exercise, the increase of $[LA]_{RBC}$ had a time delay compared to the increase of $[LA]_{plasma}$, and therefore $[LA]_{RBC}$ reached the peak after $[LA]_{plasma}$ reached its peak. (3) After $[LA]_{RBC}$ reached its peak, $[LA]_{RBC}$ decreased when $[LA]_{plasma}$ was still higher than $[LA]_{RBC}$, as shown by our own studies (cf. "Experimental studies").

In order to explain the above findings, some kind of resistance against the influx associated with the plasma-to-RBC lactate gradient must be involved. Actually, some suggestions for the mechanism of the resistance/the delayed influx have been made in the literature, e.g., Donnan equilibrium [12] {the Donnan ratio ($[An^-]_{RBC} \cdot [An^-]_{plasma}^{-1}$) under resting conditions is ~ -10 mV (calculable from Cl^- concentrations) at the inside of the red blood cell membrane, forcing diffusible anions out}, a barrier provided by the membranes of red blood cells [2] and the saturation of transporters [2, 3, 7, 11, 13]. However, none of these suggestions could give a detailed explanation and quantitative description for the entire dynamic process of lactate transport to RBCs during and after exercise.

The purpose of the present study is to develop and test a simple mathematical model that describes the dynamic processes of lactate transport into and out of RBCs. This model will be justified by the comparison between the model-predicted results and observations. The formulation of the model, as well as the check of the validity of the model, will be based on the analysis of the *raw data* of the time courses of $[LA]_{RBC}$ and $[LA]_{plasma}$ for all subjects. The previous results of the observed $[LA]_{RBC}$ and $[LA]_{plasma}$ found in the literature are not sufficient for our purpose because the raw data were usually not fully published. Therefore, the method of the present study will include two parts, our own experimental studies and mathematical model, which will be described in "Experimental studies" and "Mathematical model," respectively. The results of the mathematical model and their comparison with observations will be presented in "Results of the model and the comparison with observations." The advantages and the limitations of the present model as well as its potential applications to medicine and sports will be considered in "Discussion." Finally, some conclusions will be drawn in "Conclusions."

Methods

Experimental studies

Eleven male subjects (healthy sports students aged 24.3 ± 2.1 years) participated in these studies. All subjects

gave written informed consent to contributing to the studies. The protocols used in these studies have been granted a license from the institutional office for conducting the human research described in the studies and are in line with the Declaration of Helsinki. The purpose of these studies was to see how different warm-up conditions influenced the lactate concentrations/distribution in RBCs and in plasma before and after a 30-s short-term maximal effort exercise on a cycle ergometer. But now we will only use the results of these experimental studies to formulate the ideas of the mathematical model and to check the validity of the model. Figure 1 shows the protocols of the three experimental studies, denoted by $k = 1, 2, 3$, respectively, hereafter. They differ from each other only in warm-up conditions. In the study $k = 1$ (*moderate warm-up*), there was a constant intensity (60% of individual VO_{2peak}) of warming up for 12 min. VO_{2peak} was previously determined by an incremental step test on a cycle ergometer (100 W, +40 W every 5 min). In study $k = 2$ (*intensive warm-up*), three 10-s periods of intensive warm-up were arranged during the 12 min at an intensity of 200% of individual VO_{2peak}, as shown in Fig. 1. In the study $k = 3$, there was no warm-up at all. In all three studies, $k = 1, 2, 3$, the 30-s short-term maximal effort ("all-out") exercise, as shown in Fig. 1, was carried out in an isokinetic mode on a cycle ergometer (Schoberer Rad Messtechnik SRM GmbH, Jülich, Germany) set to a cadence of 120 rpm. The thick arrows in Fig. 1 show the blood sampling times.

For lactate determination in plasma and erythrocytes, 115 µl of blood was withdrawn from the earlobe and directly centrifuged for 1 min at 6,000 rpm with EBA20 (Hettich Zentrifugen, Tuttlingen, 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 <30 s to take the blood sample and to start centrifugation (since it needs 25 min at 25°C, which is ~ 5°C higher than the temperature in the laboratory during sampling) until equilibration [12]). Afterwards 20 µl of plasma or erythrocytes was analyzed respectively after discarding the buffy coat and the very lowest part of the sediment (however, there is always trapped plasma, as well as other cells in the cell pellet). All 20-µl samples 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) (cf. [11]).

Figure 2a gives the observed $[LA]_{RBC}$ (solid lines with solid symbols) and $[LA]_{plasma}$ (dashed lines with hollow symbols), given in mean $\pm$ SD (standard deviation), under the three different warm-up conditions ($k = 1, 2, 3$), while Fig. 2b gives the observed lactate concentration ratio $[LA]_{RBC} \cdot [LA]_{plasma}^{-1}$ as functions of time, also given in mean $\pm$ SD, for $k = 1, 2, 3$. In Fig. 2 and all the figures afterwards, the time point "1 min before the maximal exercise" is denoted by $t = 0$.
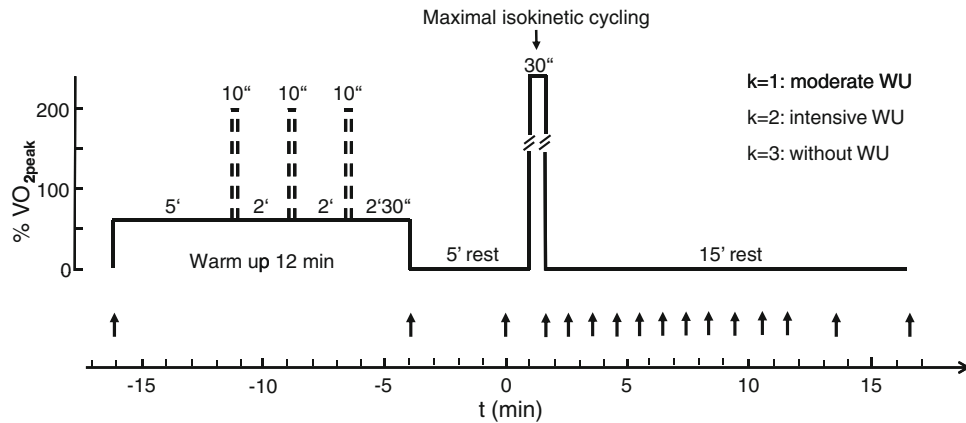
Fig. 1 Protocols of the three experimental studies, denoted by $k = 1$, 2, 3, respectively. They differ from each other by different warm-up conditions. The *thick arrows* indicate the blood sampling times; -16, -4 and 0 mean "at rest," "the end of warming up" and "1 min before the maximal exercise," respectively. During the 12 min between -16

and -4, $k = 1$ means "moderate warm-up" of constant intensity at 60% of the individual VO_{2peak}, $k = 2$ means "intensive warm-up" including three 10-s periods at 200% of the individual VO_{2peak} and $k = 3$ means without warm-up

In order to avoid too many curves, we did not show the curves of $[LA]_{RBC}$ and $[LA]_{plasma}$ for individual subjects in Fig. 2. Actually, there would be 33 curves each for $[LA]_{RBC}$ and $[LA]_{plasma}$ (11 subjects, 3 warm-up conditions). Each curve in Fig. 2a, b is the mean $\pm$ SD over 11 curves for individual subjects.

For the convenience of the mathematical model, we will denote the 16 time points for blood sampling by $t(-2), t(-1)$, $t(0), t(1),\ldots, t(13)$, where $t(0) = $ is "1 min before the maximal exercise". Thus, we have $t(-2) = -16$ min, $t(-1) = -4$ min, $t(0) = 0$, $t(1) = 1.5$ min, $t(2) = 2.5$ min,..., $t(11) = 11.5$ min, $t(12) = 13.5$ min, $t(13) = 16.5$ min.

Mathematical model

The mathematical model will describe the dynamic processes of lactate transport into and out of RBCs. Influx and efflux rate coefficients, representing lactate transfer from plasma to and from RBCs, will be calculated. The mathematical model we are going to present is based on the analysis of the results of experimental studies. Some important features of the observations shown in Fig. 2a, b can be summarized as follows: (1) Even for the resting state, $[LA]_{RBC}$ was lower than $[LA]_{plasma}$. The ratio $[LA]_{RBC} \cdot [LA]_{plasma}^{-1}$ was about 0.5 for the resting state (more precisely 0.51 as the mean ratio over all 11 subjects and all warm-up conditions for the resting state). (2) The supra-maximal exercise caused a more rapid increase for $[LA]_{plasma}$ than for $[LA]_{RBC}$ during and shortly after the exercise. (3) $[LA]_{RBC}$ reached its peak later than $[LA]_{plasma}$ reached its peak. (4) When $[LA]_{RBC}$ reached the peak, the ratio $[LA]_{RBC} \cdot [LA]_{plasma}^{-1}$ roughly recovered to its beginning value (0.54 as the mean ratio over all subjects and all warm-up conditions at the peak of $[LA]_{RBC}$). (5) After

$[LA]_{RBC}$ reached the peak, it decreased, representing an outflow of lactate from RBC, in spite of the fact that $[LA]_{plasma}$ was still much higher than $[LA]_{RBC}$. These experimental findings further confirmed the common features found by previous studies that we described in the introduction. The full raw data for all individual subjects of our own experimental studies are necessary for the development of the mathematical model and for the check of the validity of the model.

How should we explain the above-summarized features of observations found by our own and by previous studies? First, an inward force associated with the plasma-to-RBC $[LA]$ gradient must exist. The rapid increase of $[LA]_{plasma}$ during and shortly after the exercise enlarged the plasma-to-RBC $[LA]$ gradient and therefore induced the inward transport of lactate to RBC, resulting in the increase of $[LA]_{RBC}$ with a time delay. Second, the inward force alone cannot account for the ratio $[LA]_{RBC} \cdot [LA]_{plasma}^{-1}$ at rest (about 0.5), and the outflow of lactate for the late stage of recovery after $[LA]_{RBC}$ reached the peak. Thus, in addition to the inward force associated with the plasma-to-RBC $[LA]$ gradient, there must be an outward force against the inward force. Third, this outward force must not be a constant for the following reason. Let us compare the late recovery stage, say 11 min $< t < 13$ min in Fig. 2a, with the period during and shortly after the supra-maximal exercise, say $0 < t < 1.5$ min. The difference $[LA]_{plasma} - [LA]_{RBC}$ for the period 11 min $< t < 13$ min was even larger than for the period $0 < t < 1.5$ min. However, in contrast to the influx of lactate for the period $0 < t < 1.5$ min, there was an efflux of lactate for the period 11 min $< t < 13$ min. The only explanation would be that the outward force was not a constant; instead it increased as $[LA]_{RBC}$ increased. The simplest approximation would be to assume that the outward
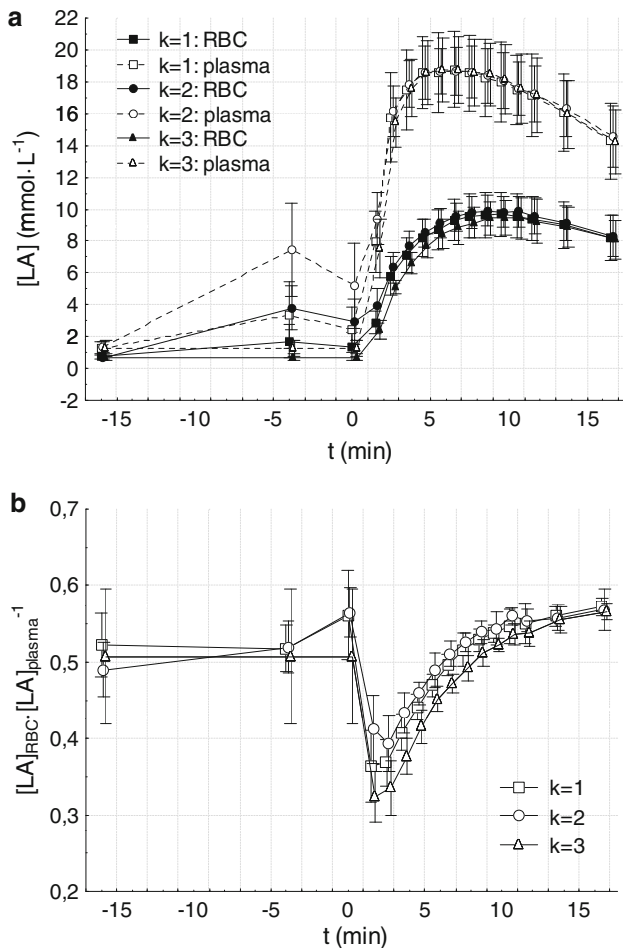
a

b

Fig. 2 **a** Observed lactate concentrations in red blood cells (*solid lines* with *solid symbols*) and in plasma (*dashed lines* with *hollow symbols*), given in mean ± standard deviation, under the three different warm-up conditions ($k = 1, 2, 3$), where the unit of lactate concentration is millimole per liter, denoted by mmol l^{-1}. **b** Observed lactate concentration ratio between red blood cells and plasma as functions of time, also given in mean ± standard deviation, for $k = 1, 2, 3$. $k = 1$, Moderate warm-up; $k = 2$, intensive warm-up; $k = 3$, without warm-up

force is proportional to [LA]$_{RBC}$. Thus, our mathematical model can be formulated as follows:

"The transport of lactate to RBCs during and after exercise is caused by an influx associated with the plasma-to-RBC [LA] gradient and an efflux that is assumed to have a rate proportional to the lactate concentration in RBC."

If we simply denote [LA]$_{RBC}$ and [LA]$_{plasma}$ by x and y, respectively, this mathematical model can be expressed by the following differential equation:

$$\frac{dx}{dt} = C_1(y - x) - C_2 x \qquad (1)$$

where C_1 and C_2 are called the *influx rate coefficient* and *efflux rate coefficient* [min^{-1}], respectively. For the convenience of notations, we will use three indices, i, j and k, to distinguish different time points for sampling ($i = -2$ to 13), different subjects ($j = 1$–11) and different warm-up

conditions ($k = 1$–3), respectively, where, e.g., $t(-2) = -16$ min is for the resting state, $t(0) = 0$ for *pre* and $t(13) = 16.5$ min for the last sampling. For given (j, k), C_1 and C_2 are constants, determined by the best fit to the observed [LA]$_{RBC}$ as a function of time for a given individual subject and a specific warm-up condition as follows.

Determinations of C_1 and C_2 as well as the model-predicted [LA]$_{RBC}$ were made as follows.

Now, let us denote the observed and the model-predicted [LA]$_{RBC}$ at $t(i)$ by $x(i)$ and $\tilde{x}(i)$, respectively. The time range from $t(0)$ to the moment for the last blood sampling $t(13)$ contains the most measurements with a blood sampling frequency nearly one sample per minute, much higher than the sampling frequency in the time period from the resting state $t(-2)$ to $t(0)$. For this reason, we will call the time range $t(0)$ to $t(13)$ the major range and seek the best fit to the observed ones only for the major range. [An alternative fit for the entire range from $t(-2)$ to $t(13)$ and its disadvantages will be discussed later in "Discussions."] Thus, the best fit means the minimization of the error

$$\Delta = \sum_{i=0}^{13} [\tilde{x}(i) - x(i)]^2 \qquad (2)$$

where

$$\tilde{x}(0) = x(0) \qquad (3)$$

$$\tilde{x}(i) = x(0) + C_1 \int_{t(0)}^{t(i)} [y(t) - x(t)]dt - C_2 \int_{t(0)}^{t(i)} x(t)dt$$

$$(i = 1, \ldots, 13) \qquad (4)$$

Equation 4 is simply the integration of Eq. 1. We denote the two integrals in Eq. 4 by $I_1(i)$ and $I_2(i)$, respectively:

$$I_1(i) = \int_{t(0)}^{t(i)} [y(t) - x(t)]dt \quad I_2(i) = \int_{t(0)}^{t(i)} x(t)dt \qquad (5)$$

Now Eq. 4 becomes

$$\tilde{x}(i) = x(0) + C_1 I_1(i) - C_2 I_2(i) \quad (i = 1, \ldots, 13) \qquad (6)$$

The error is then given by

$$\Delta = \sum_{i=1}^{13} [\tilde{x}(i) - x(i)]^2 = \sum_{i=1}^{13} [x(0) + I_1(i)C_1 - I_2(i)C_2 - x(i)]^2 \qquad (7)$$

The minimization of Δ requires that

$$\frac{\partial \Delta}{\partial C_1} = 2 \sum_{i=1}^{13} I_1(i)[x(0) + I_1(i)C_1 - I_2(i)C_2 - x(i)] = 0 \qquad (8)$$

$$\frac{\partial \Delta}{\partial C_2} = -2 \sum_{i=1}^{13} I_2(i)[x(0) + I_1(i)C_1 - I_2(i)C_2 - x(i)] = 0 \qquad (9)$$

Thus, we get two linear equations for C_1 and C_2 as follows:

$$\begin{bmatrix} a_{11} & a_{12} \\ a_{21} & a_{22} \end{bmatrix} \begin{bmatrix} C_1 \\ C_2 \end{bmatrix} = \begin{bmatrix} b_1 \\ b_2 \end{bmatrix} \quad (10)$$

where

$$\begin{bmatrix} a_{11} & a_{12} \\ a_{21} & a_{22} \end{bmatrix} = \begin{bmatrix} \sum_{i=1}^{13} I_1^2(i) & -\sum_{i=1}^{13} I_1(i)I_2(i) \\ -\sum_{i=1}^{13} I_1(i)I_2(i) & \sum_{i=1}^{13} I_2^2(i) \end{bmatrix} \quad (11)$$

and

$$\begin{bmatrix} b_1 \\ b_2 \end{bmatrix} = \begin{bmatrix} \sum_{i=1}^{13} I_1(i)[x(i) - x(0)] \\ -\sum_{i=1}^{13} I_2(i)[x(i) - x(0)] \end{bmatrix} \quad (12)$$

$I_1(i)$ and $I_2(i)$ will be calculated by the linear interpolations of $x(t)$ and $y(t)$ in any interval $[t(i), t(i + 1)]$. Thus, we have

$$I_1(i) = \sum_{j=1}^{i} \frac{1}{2}[d(j-1) + d(j)][t(j) - t(j-1)]$$
$$(i = 1, \ldots, 13) \quad (13)$$

where $d = y - x$ and

$$I_2(i) = \sum_{j=1}^{i} \frac{1}{2}[x(j-1) + x(j)][t(j) - t(j-1)]$$
$$(i = 1, \ldots, 13) \quad (14)$$

Once $I_1(i)$ and $I_2(i)$ ($i = 1,\ldots,13$) have been calculated by Eqs. 13 and 14, respectively, the coefficients a_{11}, a_{12}, a_{21}, a_{22}, b_1 and b_2 are obtained by the substitution of $I_1(i)$ and $I_2(i)$ into Eqs. 11 and 12. Thus, C_1 and C_2 as solutions of Eq. 10 are given by

$$C_1 = D_1/D \quad C_2 = D_2/D \quad (15)$$

where D_1, D_2, D_3 are the following determinants:

$$D = \begin{vmatrix} a_{11} & a_{12} \\ a_{21} & a_{22} \end{vmatrix} \quad D_1 = \begin{vmatrix} b_1 & a_{12} \\ b_2 & a_{22} \end{vmatrix} \quad D_2 = \begin{vmatrix} a_{11} & b_1 \\ a_{21} & b_2 \end{vmatrix} \quad (16)$$

Once C_1 and C_2 have been determined by Eq. 15, the model-predicted [LA]$_{RBC}$ can be determined by Eq. 6. The justification of the model will be given by the comparison of various features of the model-predicted [LA]$_{RBC}$ with those from observations as presented in the next section.

Results of the model and the comparison with observations

For all 33 cases (11 subjects $j = 1$–11 and 3 warm-up conditions $k = 1, 2, 3$), the model-predicted [LA]$_{RBC}$, as

calculated by the procedure described in "Mathematical model," turns out to be in reasonable agreement with the observed [LA]$_{RBC}$. In order to avoid too many figures, in Fig. 3, we only show the comparison between the model-predicted and the observed individual [LA]$_{RBC}$ for subject 7 as one example. Figure 4 shows the comparison of the mean $\pm$ SD of [LA]$_{RBC}$ over all subjects between model predictions and observations for (a) $k = 1$, (b) $k = 2$ and (c) $k = 3$ respectively. Again, we see reasonable agreement between model predictions and observations.

As a statistical comparison, we show the correlation between the observed and the model-predicted values of [LA]$_{RBC}$ for all the subjects, under all three warm-up conditions at all time points in the major range in Fig. 5. It shows very good and very significant correlation ($r = 0.9773$, $p = 0.0000$), and the best linear fit (the thin solid line) is very close to the thick solid line, which means the equality of the model predictions and the observations.

The influx rate coefficients C_1 and the efflux rate coefficients C_2 for all subjects ($j = 1$–11) and all warm-up conditions ($k = 1, 2, 3$) are given in Table 1.

Statistical analysis (repeated measure ANOVA) is made to compare C_1 and C_2 under different warm-up conditions, $k = 1, 2, 3$. The results are shown in Fig. 6. C_2 is significantly smaller than C_1 ($p = 0.000000$), while the differences made by different k are not significant ($p = 0.19$).

As a further check of the validity of the model, let us consider the ratio [LA]$_{RBC} \cdot$[LA]$_{plasma}^{-1}$. The observed approximate equality between the ratios at the peak of [LA]$_{RBC}$ and at rest can be easily explained by the present model. Actually, for both resting state and the peak of [LA]$_{RBC}$, the time derivative of [LA]$_{RBC}$, i.e., dx/dt on the
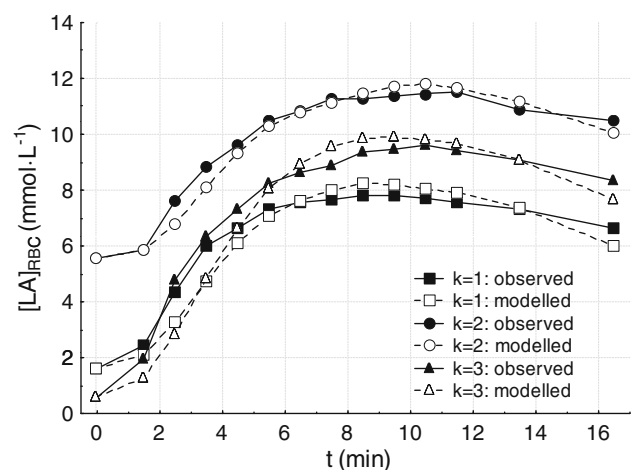

Fig. 3 Observed (*solid lines* with *solid symbols*) and model-predicted (*dashed lines* with *hollow symbols*) lactate concentrations in red blood cells under conditions $k = 1, 2, 3$ for subject 7. $k = 1$, Moderate warm-up; $k = 2$, intensive warm-up; $k = 3$, without warm-up
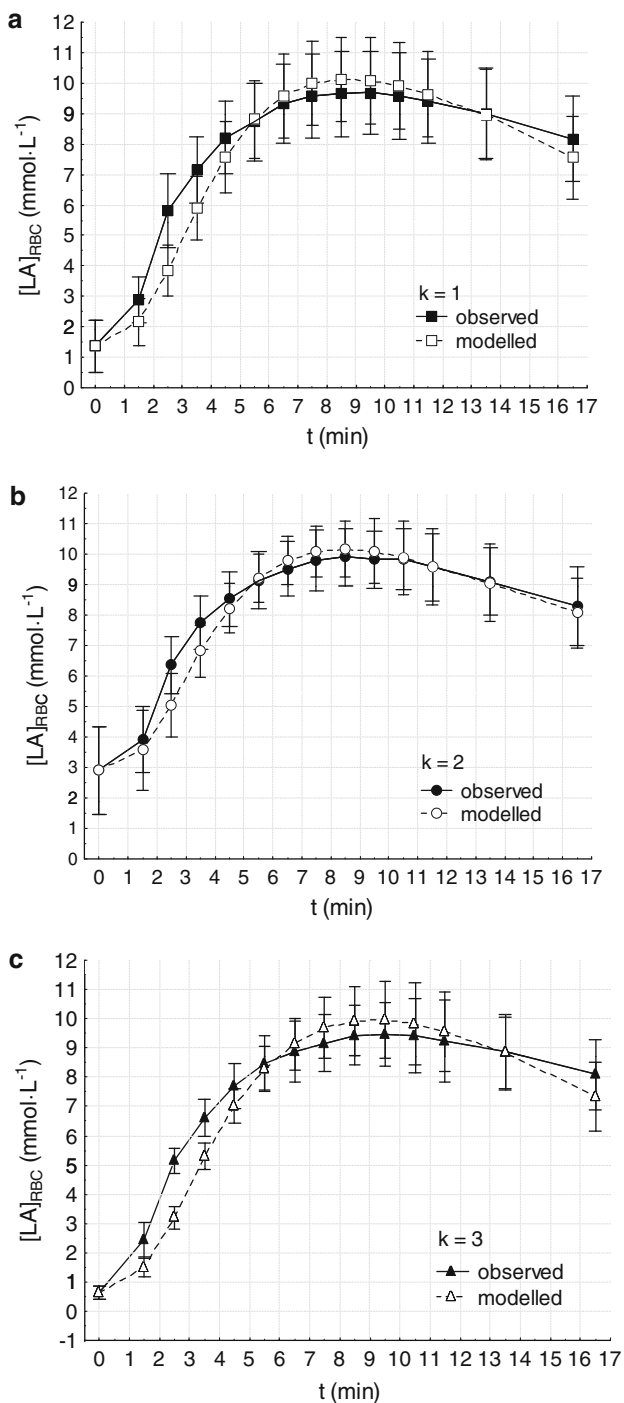
Fig. 4 Observed (*solid lines* with *solid symbols*) and model-predicted (*dashed lines* with *hollow symbols*) lactate concentrations in red blood cells, given in mean ± standard deviation, for (**a**) $k = 1$, (**b**) $k = 2$ and (**c**) $k = 3$. $k = 1$, Moderate warm-up; $k = 2$, intensive warm-up; $k = 3$, without warm-up

Fig. 5 Correlation between the observed and the model-predicted $[LA]_{RBC}$ for all 11 subjects, under all three warm-up conditions, and at all 14 time points for blood sampling [$t(0)$ to $t(13)$]. Thus, the graph includes $11 \times 3 \times 14 = 462$ *circles* where each *circle* represents the observed and the model-predicted values of $[LA]_{RBC}$ for a given subject under a given warm-up condition at a given moment for blood sampling. The *thin solid line* shows the best linear fit, which is close to the *thick solid line*, which means the equality of the observations and the model predictions. The very good and very significant correlation is shown by $r = 0.9773$ and $p = 0.0000$

Table 1 C_1 (influx rate coefficient) and C_2 (efflux rate coefficient) for different subjects ($j = 1–11$) and different warm-up conditions ($k = 1–3$)

j	C_1 (min^{-1})			C_2 (min^{-1})		
	$k = 1$	$k = 2$	$k = 3$	$k = 1$	$k = 2$	$k = 3$
1	0.6883	0.5427	0.4131	0.6397	0.462	0.4188
2	0.426	0.4136	0.3797	0.368	0.3733	0.3476
3	0.4271	0.4218	0.5321	0.3581	0.3504	0.4468
4	0.5137	0.512	0.5678	0.4405	0.4451	0.5398
5	0.4749	0.4438	0.3814	0.4128	0.3879	0.3527
6	0.4475	0.3854	0.4226	0.3707	0.3225	0.3934
7	0.4335	0.4548	0.3021	0.4057	0.3546	0.2808
8	0.3358	0.4117	0.3379	0.312	0.3974	0.3163
9	0.5132	0.5473	0.482	0.4531	0.4628	0.4476
10	0.4321	0.4673	0.3635	0.3959	0.4141	0.3344
11	0.5334	0.4484	0.4185	0.4754	0.3838	0.387
Mean ± SD	0.4508 ± 0.0778			0.4015 ± 0.0698		

$k = 1$, Moderate warm-up; $k = 2$, intensive warm-up; $k = 3$, without warm-up

left of Eq. 1, should be zero. Therefore, the right side of Eq. 1 must also be zero at these two moments. Thus, for both resting state and the peak of $[LA]_{RBC}$, the model-predicted ratio should be the same, given by
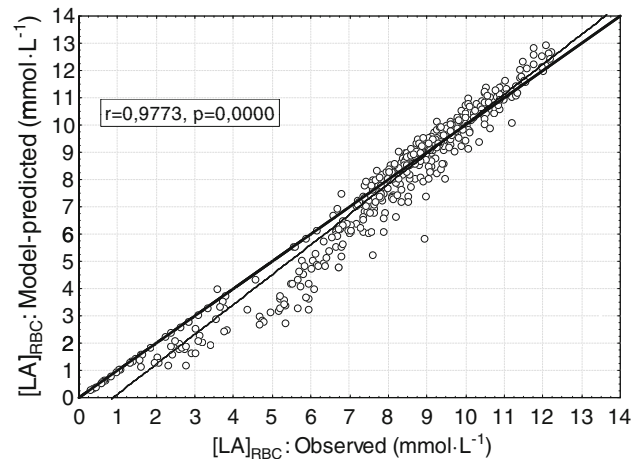
$$\frac{x}{y} = \frac{1}{1 + C_2/C_1}. \tag{17}$$

This is directly derived by letting the right side of Eq. 1 be zero. In Fig. 7, we give the comparison between the model-predicted ratios $[LA]_{RBC} \cdot [LA]_{plasma}^{-1}$ at the peak of $[LA]_{RBC}$, based on Eq. 17, and those from observations for
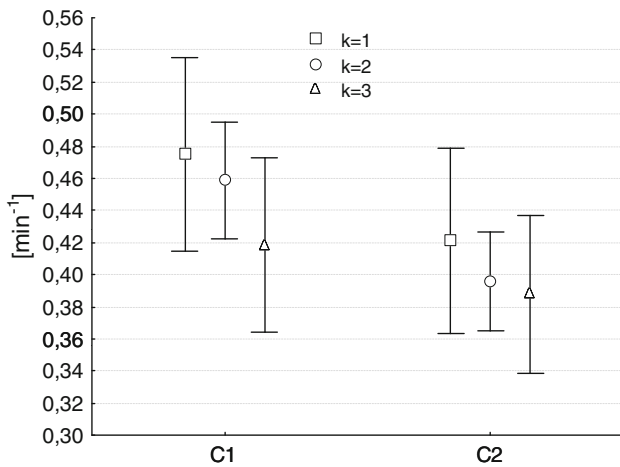
Fig. 6 Repeated measure ANOVA for C_1 (influx rate coefficient) and C_2 (efflux rate coefficient) under the three warm-up conditions, $k = 1$, 2 and 3, where the symbols stand for the means over the 11 subjects and the *vertical bars* give the 95% confidence ranges. C_2 is significantly smaller than C_1 ($p = 0.000000$), while the differences made by different warm-up conditions are not significant ($p = 0.19$). $k = 1$, Moderate warm-up; $k = 2$, intensive warm-up; $k = 3$, without warm-up

all subjects $j = 1$–11 and for (a) $k = 1$, (b) $k = 2$ and (c) $k = 3$, respectively. For all 33 cases (11 subjects and 3 warm-up conditions), the comparison shows excellent agreement. This gives further confirmation of the validity of the model.

Discussion

Now we discuss the advantages and the limitations of the present model, the physical and chemical processes involved in the influx and efflux, the physiological meanings of the influx rate coefficient C_1 and the efflux rate coefficient C_2, as well as the potential applications of the model to medicine and sports.

The advantages and the limitations of the model

Simple as it is, the present model can quantitatively reproduce the basic features of the time courses of the observed lactate concentrations in RBC. The close agreement between the model predictions and observations confirms the main influencing factors for the transport of lactate to RBCs suggested by the model. Namely, in addition to the influx associated with the plasma-to-RBC [LA] gradient, there is an efflux that has a rate approximately proportional to the lactate concentration in RBCs. Although the existence of some kind of efflux has been mentioned in previous research, the present study is the first mathematical model that has been completed for the quantitative
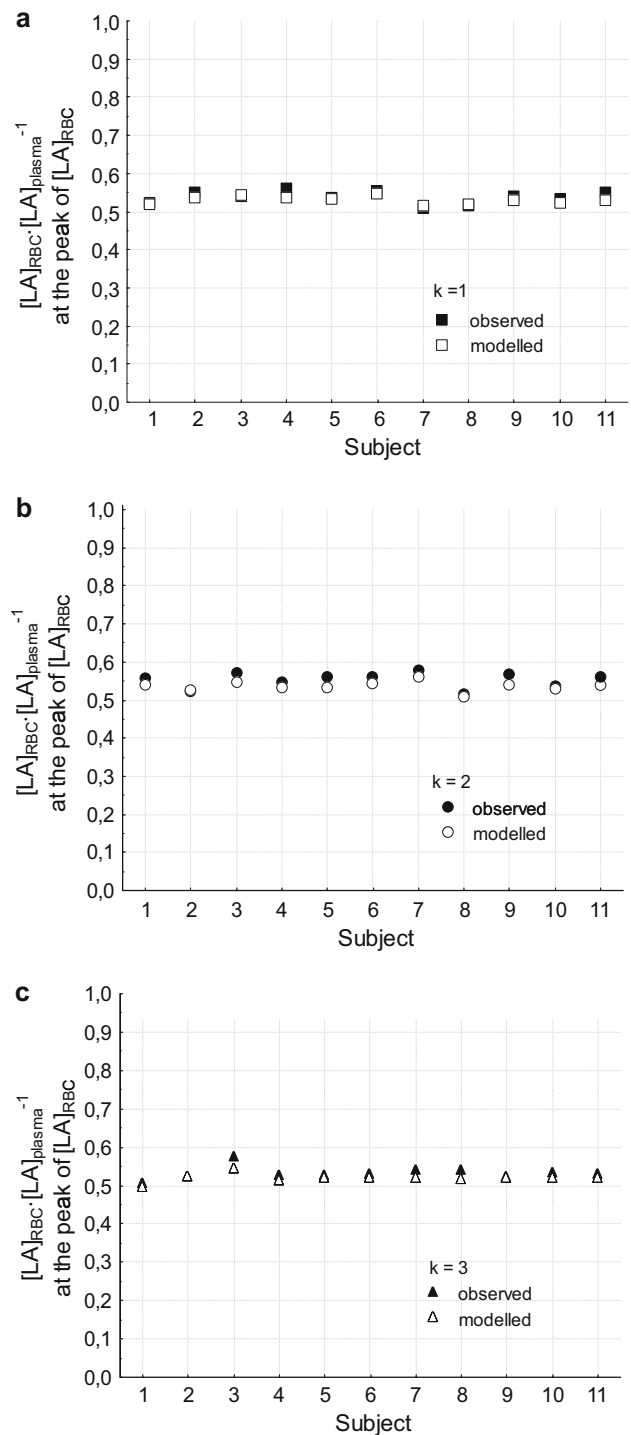

Fig. 7 Comparison between the observed and the model-predicted ratio $[LA]_{RBC} \cdot [LA]_{plasma}^{-1}$ at the peak of $[LA]_{RBC}$ for different subjects ($j = 1$–11) under different warm-up conditions, (**a**) $k = 1$, (**b**) $k = 2$ and (**c**) $k = 3$. $k = 1$, Moderate warm-up; $k = 2$, intensive warm-up; $k = 3$, without warm-up

description of both the influx and the efflux to calculate the detailed results of the model, to compare the model-predicted results with the corresponding observations in detail

and therefore to confirm the validity of the model. The validity of the model does not depend on the specific mechanism for the efflux. In other words, the present model is valid no matter what the specific mechanism for the efflux turns out to be. However, the only way to really validate the model/efflux rate coefficient would be to label a pool of intracellular lactate and directly measure efflux rates at different extracellular lactate concentrations.

Nevertheless, the present model is still a semi-empirical model because the mechanism of the efflux is still not entirely clear (cf. the next subsection) and also because the influx rate coefficient C_1 and the efflux rate coefficient C_2 are determined by the best fit to observations.

It is important to note that C_1 and C_2 are constants for a given subject during a given protocol, but C_1 and C_2 vary for a given subject between different protocols. They vary with different conditions/protocols in order to have the best fit. The present model can be applied well to all the three warm-up conditions, $k = 1$, 2 and 3. We could even think of it in another way. Namely, the data of $k = 1$ are used for the development of the model, while the data of $k = 2$ and 3 are used to further check the validity of the model. However, all three conditions used 30-s short-term maximal effort exercises. Therefore, future research needs to determine how well the present model would apply to conditions with longer term exercises. In this case, such an acute setting has been chosen because only minor changes or even no changes in the ratio $[\text{LA}]_{\text{RBC}} \cdot [\text{LA}]_{\text{plasma}}^{-1}$ occur during steady-state exercise [6] or incremental step tests (even when the work load was increased by 30 W every minute) [5, 8]. For the development of the model, major changes in the ratio are much more interesting than steady-state conditions. Our purpose was to fit the model with big changes in the lactate distribution.

On the physical and the chemical processes involved in the influx and the efflux

The terms influx and efflux used in the model are certainly just simplifications of the real physical and chemical processes involved in the lactate transport to RBCs. Actually, only a small part (roughly 4–5%) of the inward transport of lactate is caused by physical diffusion across the membrane of RBCs. The major part (80–90%) of the transport is through the transporter MCT-1 [14–16]. 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 equilibrium depends on the initial difference and on the permeability. Since MCT-1 always carries the lactate anion LA$^-$ together with the hydrogen cation H$^+$ [10, 17], not only the gradient of [LA$^-$], but also the gradient of [H$^+$] would influence the direction of transport of the couple

{LA$^-$, H$^+$}, where the bracket [] stands for the concentration. At rest, the effects of the plasma-to-RBC [LA$^-$] gradient and RBC-to-plasma [H$^+$] gradient may balance each other. During and shortly after exercise, the rapid increase of [LA$^-$]$_{\text{plasma}}$ is associated with a greater relative increase of [H$^+$] in plasma than in RBCs, resulting in the increase of the plasma-to-RBC [LA$^-$] gradient and the decrease of the RBC-to-plasma [H$^+$] gradient [3], both favoring the influx of the couple {LA$^-$, H$^+$} through MCT-1. The enlarged plasma-to-RBC [LA$^-$] gradient also favors the physical diffusion of lactate through the membrane. Since the increase of the plasma-to-RBC [LA$^-$] gradient favors both the inward physical diffusion of lactate through the membrane and the inward transport of {LA$^-$, H$^+$} through MCT-1, the influx of LA$^-$ associated with the plasma-to-RBC [LA$^-$] gradient in the mathematical model should be regarded as the combination of both.

The understanding of the efflux of LA$^-$ may involve the negative electric charge inside RBCs (~ -10 mV at the inside of the red cell membrane forcing diffusible anions out), which is caused by various anions, particularly the non-diffusible anions of hemoglobin Hb$^-$. This negative charge forces diffusible anions out and resists the inward diffusion of LA$^-$ even for the resting state (Donnan equilibrium). However, the couple (LA$^-$, H$^+$), transported through MCT-1, as a whole is electrically neutral. Therefore, there is no net electric force acting on the couple. Furthermore, it was shown that an equilibrium for other permeating anions (An$^-$) and for H$^+$ is reached within a second after a sudden rise in plasma concentration like at exhaustion, whereas such an equilibrium for LA$^-$ can only be attained after a few minutes due to the slow transport [11].

Another possibility for the forces acting on the distribution could be a preferred direction of MCT-1 transportation. Indeed, the different K_m values (Michaelis-Menten constant) for efflux and influx in RBCs may suggest asymmetric behaviors of MCT-1 [18]. This preference should also be taken into account in the balance between the gradients of [LA$^-$] and [H$^+$] for the resting state.

The above ideas for the possible mechanism of the efflux of LA$^-$ and for the reason why the efflux gets stronger as [LA]$_{\text{RBC}}$ increases are still speculative. We do not claim that we have fully understood the physical and chemical mechanism for the efflux of LA$^-$. We leave this interesting issue open to further discussions. The detailed physical and chemical mechanism of the efflux of LA$^-$ remains to be figured out in future research.

On the potential applications of the model

The present model gives a simple and convenient way to calculate C_1 and C_2, and gives a detailed explanation and

quantitative description for the entire dynamic processes of lactate transport to RBCs during and after exercises. According to Eq. 1, C_1 is the increase of $[LA]_{RBC}$ per unit time associated with a unit difference $[LA]_{plasma} - [LA]_{RBC}$, while C_2 is the decrease of $[LA]_{RBC}$ per unit time associated with a unit $[LA]_{RBC}$. The values of C_1 and C_2 could be determined by the best fit to observations as we did in "Methods" and "Results of the model and the comparison with observations." However, it is not a simple matter to determine all the properties of the blood that would influence the transport of lactate to red blood cells and the values of C_1 and C_2. *In any case, both C_1 and C_2 reflect the properties of the blood.*

The basic idea of the model was to help to understand the dynamic processes of lactate transport (kinetics) to RBC and to give a quantitative description of these processes. Of course one limitation of the model is the fact that the model is a semi-empirical model because the influx rate coefficient C_1 and the efflux rate coefficient C_2 are determined by the best fit to observations. Constants (C_1 and C_2) serve for fitting and vary between different interventions/subjects. Nevertheless, C_1 and C_2 are constants independent of sampling time points for a given subject and a given intervention. Therefore, the model might be utilizable for further studies investigating lactate kinetics in different body/tissue compartments during exercise. It might help to further understand the dynamic processes of lactate distribution.

But further investigations are needed to clarify if the model is utilizable in these areas. Of course, for the potential applications, it would be necessary to standardize the protocol of the experimental study, e.g., the warm-up condition, the duration, the intensity and the procedure of the exercise as well as the time points of blood sampling, etc. It would also be necessary to standardize the range for fitting in the mathematical model.

Conclusions

A mathematical model, as a quantitative description for the dynamic process of lactate transport to red blood cells during and after exercise, is constructed. In addition to the influx of LA associated with the plasma-to-RBC [LA] gradient, an efflux of LA with a rate proportional to the lactate concentration in red blood cells is assumed. This simple model is justified by the good agreements between the model-predicted and the observed time courses of the individual $[LA]_{RBC}$, as well as between the model-predicted and the observed ratio $[LA]_{RBC} \cdot [LA]_{plasma}^{-1}$ at the peak of $[LA]_{RBC}$ for all 33 cases (11 subjects and 3 warm-up conditions). The two constants involved in this model, i.e., the influx rate coefficient C_1 and the efflux rate coefficient C_2, reflect the corresponding blood properties favoring the influx and the efflux of lactate crossing the membrane of RBCs, respectively. These properties cannot be easily observed directly but can be calculated by the present model. Further investigations are needed to clarify if the model is utilizable for studies investigating lactate kinetics in different tissue compartments during exercise and if it helps to understand the dynamic processes of lactate distribution.

Conflict of interest The authors declare that they have no conflicts of interest.

References

1. McArdle WD, Katch FI, Katch VL (2006) Exercise physiology: energy, nutrition and, human performance. Lippincott Williams & Wilkins, Baltimore
2. Buono MJ, Yeager JE (1986) Intraerythrocyte and plasma lactate concentrations during exercise in humans. Eur J Appl Physiol Occup Physiol 55:326–329
3. Harris RT, Dudley GA (1989) Exercise alters the distribution of ammonia and lactate in blood. J Appl Physiol 66:313–317
4. McKelvie RS, Lindinger MI, Heigenhauser GJ, Jones NL (1991) Contribution of erythrocytes to the control of the electrolyte changes of exercise. Can J Physiol Pharmacol 69:984–993
5. Smith EW, Skelton MS, Kremer DE, Pascoe DD, Gladden LB (1997) Lactate distribution in the blood during progressive exercise. Med Sci Sports Exerc 29:654–660
6. Smith EW, Skelton MS, Kremer DE, Pascoe DD, Gladden LB (1998) Lactate distribution in the blood during steady-state exercise. Med Sci Sports Exerc 30:1424–1429
7. Hildebrand A, Lormes W, Emmert J, Liu Y, Lehmann M, Steinacker JM (2000) Lactate concentration in plasma and red blood cells during incremental exercise. Int J Sports Med 21:463–468
8. Sara F, Hardy-Dessources MD, Marlin L, Connes P, Hue O (2006) Lactate distribution in the blood compartments of sickle cell trait carriers during incremental exercise and recovery. Int J Sports Med 27:436–443
9. Miller BF, Lindinger MI, Fattor JA, Jacobs KA, Leblanc PJ, Duong M, Heigenhauser GJ, Brooks GA (2005) Hematological and acid-base changes in men during prolonged exercise with and without sodium-lactate infusion. J Appl Physiol 98:856–865
10. Juel C, Bangsbo J, Graham T, Saltin B (1990) Lactate and potassium fluxes from human skeletal muscle during and after intense, dynamic, knee extensor exercise. Acta Physiol Scand 140:147–159
11. Boning D, Klarholz C, Himmelsbach B, Hutler M, Maassen N (2007) Causes of differences in exercise-induced changes of base excess and blood lactate. Eur J Appl Physiol 99:163–171
12. Johnson RE, Edwards DB, Dill DB, Wilson JW (1945) Blood as a physicochemical system: the distribution of lactate. J Biol Chem 157:461–473
13. Gladden LB, Smith EW, Skelton MS (1994) Lactate distribution in blood during passive and active recovery after intense exercise. Med Sci Sports Exerc 26:35–35
14. Skelton MS, Kremer DE, Smith EW, Gladden LB (1998) Lactate influx into red blood cells from trained and untrained human subjects. Med Sci Sports Exerc 30:536–542

15. Connes P, Caillaud C, Mercier J, Bouix D, Casties JF (2004) Injections of recombinant human erythropoietin increases lactate influx into erythrocytes. J Appl Physiol 97:326–332

16. Deuticke B (1989) Monocarboxylate transport in red blood cells: kinetics and chemical modification. Methods Enzymol 173:300–329

17. Juel C, Halestrap AP (1999) Lactate transport in skeletal muscle—role and regulation of the monocarboxylate transporter. J Physiol 517(Pt 3):633–642

18. Deuticke B (1982) Monocarboxylate transport in erythrocytes. J Membr Biol 70:89–103