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Postexercise red cell aggregation is negatively correlated with blood lactate rate of disappearance

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Abstract. In three separate studies, we have observed that the rise in blood lactate during exercise is correlated to blood viscosity and red cell aggregation. Whether these results were related to an effect of blood rheology on lactate production by muscles or on lactate disappearance remains unknown. The modelling of postexercise lactate kinetics allows a fair evaluation of lactate production by muscles ($\gamma 1$) and lactate disappearance ($\gamma 2$), the latter being easily measurable with simplified protocols. We thus investigated the relationships between pre- and postexercise blood rheology and $\gamma 2$. Ten subjects (2 female and 8 males; age 16–45 yr, weight 62–106.5 kg) exhibiting a wide range of $\gamma 2$ (from 2 to 7.7×10^{-2} min^{-1}) underwent a maximal exercise-test with postexercise calculation of $\gamma 2$ with the simplified formula $\gamma 2 = 0.0724 + 0.755(\text{Lac8} - \text{Lac20})/(\text{Lac8} \cdot \Delta t) - 0.00684\text{Lac20}$ where Lac8 and Lac20 are lactate concentrations 8 and 10 min after exercise stop at the level of $V_{O_{2\,max}}$, as previously reported. During exercise whole blood viscosity η_b increased (+15%, $p < 0.01$) due to a rise in hematocrit ($p < 0.05$) and plasma viscosity ($+0.08 \pm 0.03$ mPa.s, $p < 0.05$), while red cell rigidity was unchanged. Red cell aggregation (Myrenne M1) increased by 11% ($p < 0.05$). Postexercise M1 (measured at $V_{O_{2\,max}}$) was the only hemorheologic parameter correlated to $\gamma 2$ ($r = -0.697$, $p = 0.037$). We find once again a statistical relationship between lactate at exercise and red cell aggregation. Microcirculatory adaptations influenced by red cell aggregation may influence lactate disposal (as reflected by $\gamma 2$), adding its effect to that of the balance between carbohydrates and fat oxidation which is the major determinant of blood lactate concentrations at exercise in physiological conditions.

Keywords: Blood viscosity, plasma viscosity, hemorheology, erythrocyte deformability, erythrocyte aggregability, insulin sensitivity, insulin resistance, minimal model

1. Introduction

In three previous separate studies, we have reported that the rise in blood lactate during exercise is correlated to pre-exercise values of blood viscosity and red cell aggregation [1–3]. These results were surprising since blood lactate during exercise is nowadays considered rather as a mirror of the balance between oxidized carbohydrates and lipids than resulting from a "Pasteur-like" effect (so-called "anaerobiosis") [4–7].

Thus the following hypothesis was proposed [8]: beside the major influence of fuel balance at exercise, lactate kinetics might be also regulated by blood rheology. However, the mechanism of this influence remained unclear. Whether this regulation primarily affected lactate production by the muscle (defect or

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Table 1
Clinical and ergometric characteristics of the study subjects

Age (yr)	28.4±3
Weight (kg)	73.8±4.7
Height (m)	1.73±0.02
Body mass index (kg/m^2)	24.6±1.64
Blood pressure (systolic) mmHg	125±9.35
Blood pressure (diastolic) mmHg	82.5±5.95
$V_{O_2\,max}$ (ml/min)	2620±167
W_{max} (watts)	327±43
1st ventilatory threshold (%$V_{O_2\,max}$)	38.6±3.5
2nd ventilatory threshold (%$V_{O_2\,max}$)	65.3±2.6
[Lac] at 8 min recovery (mmol/l)	010±0.8
[Lac] at 20 min recovery (mmol/l)	6.8±0.8
lactate disappearance $\gamma 2$ (min^{-1})	5.4±0.6

inhomogeneity in O_2 supply to muscles resulting in some degree of anaerobiosis) or lactate removal by the vasculature from the muscular sites was unclear.

There is a classical and well validated technique for measuring from postexercise lactate samplings both lactate production and lactate removal [9–20]. We recently proposed a simplified measurement of lactate removal based upon this approach but with a drastically limited number of samples [21]. This method allows a quite correct evaluation of lactate removal although it remains necessary to analyze multiple samples for measuring muscular lactate output.

Thus, this study was undertaken in order to define whether the previously reported relationship between blood rheology and the magnitude of the increase in lactate concentrations at exercise is explained by an influence of hemorheological parameters on lactate removal rate as determined with this method.

2. Research design and methods

2.1. Subjects

Ten subjects underwent a maximal exercise-test with postexercise analysis of lactate kinetics and calculation of the lactate disappearance time constant $\gamma 2$. Subjects (2 female and 8 males, age 13–45 yr) were selected in order to display a large range of $\gamma 2$ values. Thus they included athletes, sedentary obese subjects, and patients explored for cramps or myalgia in whom no evidence for mitochondrial enzyme defect was obtained after the full exploration. Patients characteristics are given on Table 1.

2.2. Exercise testing

After medical routine examination performed by a trained physician, subjects (fasting) performed the exercise-test in a room whose temperature was between 19 and 22°C and whose hygrometry was between 30 and 50%. Wasserman's tables [8] were used for predicting the expected value of maximal oxygen consumption ($V_{O_2\,max}$). From these values the theoretical maximal power was calculated as $(V_{O_2\,max} - V_{O_2\,rest})/10.3$ where 10.3 ml/watt is the oxygen equivalent of the watt on cycloergometer. Pedal speed was set at the constant value of 50–60 rpm on a cycloergometer (Ergoline Ergometrics 800 with electromagnetic brake). After 3 min rest and 3 min warm-up at 20% of the theoretical maximal power,

the work load was increased by 8% every minute until exhaustion. Recovery started with a 3 min period of cycling at the warm-up power. Maximum was defined as the power level where O_2 consumption reached a plateau, while the respiratory quotient RQ which is the ratio V_{CO_2}/V_{O_2} was higher than 1.10 and the heart rate was close (±10%) from the theoretical maximal value assumed to be 210–0.65 × age (years) expressed in beats/min.

Oxygen consumption (V_{O_2}), CO_2 production and ventilation (VE) during the test were measured on a Cardio-O_2 CPX Medicalgraphics apparatus (St Paul, Minnesota, USA) which analyzes respiratory gases with a zirconium cell (for oxygen) and an infrared analyzer for CO_2.

Ventilatory thresholds (so-called anaerobic thresholds, see discussion) were determined according to the consensus guidelines of the French Society for Sports Medicine (SFMS) [22].

Lactate kinetics was analyzed with a simplified procedure [21] derived from the concepts developed by Freund [9–20] as indicated below in the Appendix.

2.3. *Laboratory measurements*

Blood samples for hemorheological measurements (7 ml) were drawn with potassium EDTA as the anticoagulant in a vacuum tube (Vacutainer). Hematocrit was measured by microcentrifugation. Viscometric measurements were done at high shear rate (1000 s^{-1}) with a falling ball viscometer (MT 90 Medicatest, F-86280 Saint Benoit) [23,24]. Accuracy of the measurements was regularly controlled with the Carrimed Rheometer "CS" (purchased from Rhéo, 91120 Palaiseau, France) [25]. The coefficient of variation of this method ranged between 0.6 and 0.8% [26]. With this device we measured apparent viscosity of whole blood at native hematocrit, plasma viscosity, and blood viscosity at corrected hematocrit (0.45) according to the equation of Quemada [27]

$$\eta_b = \eta_{pl}\left(1 - \frac{1}{2}kh\right)^{-2},$$

where η_b is blood viscosity, η_{pl} plasma viscosity, h the hematocrit and k a shear dependent intrinsic viscosity of the red cells according to Quemada.

Two indices of erythrocyte rigidity (Dintenfass' "*Tk*" and Quemada's "k") were calculated from blood viscosity, hematocrit and plasma viscosity measured at time 0 with equations derived from those given above:

$$k = 2\left(1 - \eta_r^{-0.5}\right)h^{-1} \quad [27]$$

and

$$Tk = \left(\eta_r^{0.4} - 1\right)\left(\eta_r^{0.4}h\right)^{-1} \quad [28],$$

where η_r is relative blood viscosity η_b/η_{pl}.

RBC aggregation was assessed with the Myrenne aggregometer [29] which gives two indices of RBC aggregation: "M" (aggregation during stasis after shearing at 600 s^{-1}) and "$M1$" (facilitated aggregation at low shear rate after shearing at 600 s^{-1}).

Table 2
Modifications (mean ± SEM) of rheologic parameters during submaximal exercise in study subjects

Time	Baseline	10
Blood viscosity (mPa.s)	3.02 ± 0.12	3.47 + 0.08***
Corrected viscosity η_{45} (mPa.s)	3.10 ± 0.18	3.40 ± 0.28
Plasma viscosity (mPa.s)	1.34 ± 0.02	1.41 ± 0.02***
"*Tk*" (RBC rigidity)	0.66 ± 0.03	0.67 + 0.03
Hematocrit (%)	41.9 ± 1.3	44.7 + 1.9***
Hematocrit/viscosity $mPa^{-1}\,s^{-1} \times 10^{-2}$	14 ± 0.3	12.8 ± 0.4*
RBC aggregation "*M*"	4.2 ± 0.4	4.1 ± 0.4
RBC aggregation "*M*1"	8.2 ± 0.7	9.4 + 0.5*

* $p < 0.05$; *** $p < 0.01$ vs baseline.

2.4. Statistics

Results are presented as mean ± the SE of the mean. Before and after training values were compared with the paired Student t-test after verification of the normality of distribution of differences between before and after values with the Kolmogorov–Smirnov test. A value of $p < 0.05$ was considered as significant. Comparison of methods for the calculation of the lactate disappearance time constant was performed with the software "Method Validator" by Ph Marquis, Metz, France and downladable as freeware at http://perso.easynet.fr/~philimar/methvalfra.htm.

3. Results

Subjects performed the test until a maximum fulfilling the classical criteria for maximal power output at an average of 95.5% (±11.6%) of their theoretical expected $V_{O_2\,max}$. Half of the subjects reached a maximum below this expected value (42 to 84%) while the others performed 107–145.5% of the expected maximal performance. Crude values of $V_{O_2\,max}$ ranged between 1383 and 4143 ml/min, i.e., between 13.2 and 57.5 ml/min/kg. The first ventilatory threshold was found between 30 and 51% of $V_{O_2\,max}$ and the second ventilatory threshold was found at 59–73% of $V_{O_2\,max}$.

The hemorheological effects of exercise are shown on Table 2. During exercise whole blood viscosity η_b increased (+15%, $p < 0.01$) due to a rise in hematocrit ($p < 0.05$) and plasma viscosity (+0.08 ± 0.03 mPa.s, $p < 0.05$), while red cell rigidity *Tk* was unchanged. The hematocrit/viscosity ratio decreased. Red cell aggregation (Myrenne $M1$) increased by 11% ($p < 0.05$).

Subjects, as expected, exhibited a wide range of $\gamma 2$ (from 2 to 7.7×10^{-2} min^{-1}). Postexercise $M1$ (measured at $V_{O_2\,max}$) was the only hemorheologic parameter correlated to $\gamma 2$ ($r = -0.697$, $p = 0.0374$) (Fig. 1).

4. Discussion

This study aimed at determining whether lactate disappearance was related to blood rheology and thus explained the previously observed relationship between blood viscosity (and red cell aggregation) and the magnitude of blood lactate increase during exercise. Our result evidence once again a statistical relationship between lactate at exercise and red cell aggregation. There is a correlation between aggregation

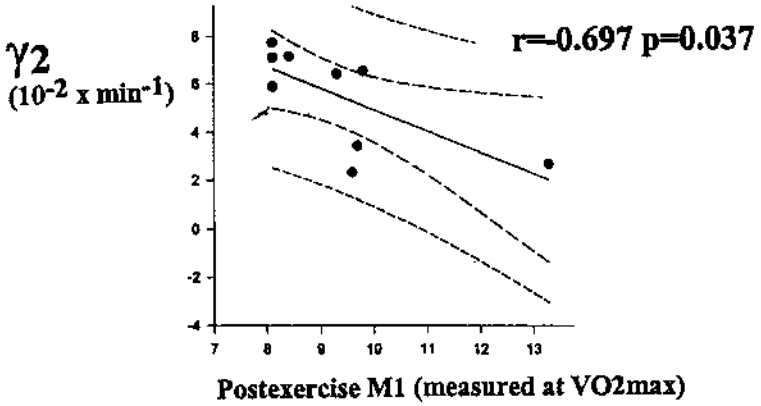

Fig. 1. Correlation between postexercise $M1$ measured at $V_{O_{2\,max}}$ and the lactate postexercise disappearance constant $\gamma 2$ ($r = -0.697$, $p = 0.037$).

and the lactate disappearance constant which suggests that microcirculatory adaptations influenced by red cell aggregation may influence lactate disposal and clearance.

Since Margaria [30] proposed in 1933 the hypothesis of an "oxygen debt" explaining via a Pasteur-like effect the rise in blood lactate during exercise, this concept has acquired a wide popularity among exercise physiologists, athletes, and sport coaches. According to this hypothesis, a relative lack of oxygen in exercising muscular cells resulted in an incomplete oxidation of carbohydrates which could not enter the Krebs cycle, as occurs in situations of anaerobiosis, so that pyruvate accumulates in the cell and is transformed into lactate which is released in the circulation. This concept provided the theoretical background of the more recent works of Wasserman [31] who proposed another very popular concept in sports medicine: that of an "anaerobic threshold". According to this author, there is a percentage of the maximal power output where oxygen supply become not sufficient to allow the full oxidation of glucose via the glycolytic pathways, resulting in a shift of pyruvate which is derived from the Krebs cycle and is transformed into lactate. The measurement of ventilation, V_{O_2} and V_{CO_2} during exercise can detect this "threshold" which is indicated by a change in the slope of the ventilatory flow rate (V_E) plotted against power [32]. This threshold is usually found between 50 and 60% of $V_{O_{2\,max}}$. Further, a second "threshold" was also described between 80 and 90% of $V_{O_{2\,max}}$, when the V_E/V_{O_2} ratio suddenly increases, and interpreted as the "onset of blood lactate-induced acidosis" [31]. In fact, while the determination of these points is now classical and widely performed in routine exercise-testing in both athletes and patients, all these concepts of oxygen debt and anaerobic thresholds are now considered to be fully obsolete [22]. Although these "thresholds" have been useful for developing in both athletes and patients personalized training programmes [22], they are no longer considered as reflecting blood lactate kinetics. Recent studies, on the contrary, give a strong evidence that the balance between carbohydrate and lipid oxidation at exercise is the major explanation of both lactate production and ventilatory "thresholds" [4–7].

Obviously, this change in our understanding of lactate metabolism during exercise does not rule out the possibility that, in some situations of "true" hypoxia, a lactate production unrelated to those mechanisms may occur as the result of a lack of O_2 in the muscular cell. Clearly, experimental hypoxia has been shown to increase muscle lactate production [33]. High lactate responses are found during exercise in altitude (or its simulation by hypobaric hypoxia [34]), and in patients with peripheral obliterative arterial disease [35]. In other terms, it remains true that muscular hypoxia promotes a higher blood lactate response to exercise.

In this respect, data indicating that increased red cell aggregation impairs oxygen distribution in muscle microcirculation [36,37] are important, since they provide the basis for an explanation of the previously reported relationships between red cell aggregation and lactate [1–3].

Theoretically, a high lactate response could be due to two mechanisms: an increased lactate production or a lower lactate removal. In this study, lactate removal, as reflected by $\gamma 2$, is negatively correlated to red cell aggregability at the end of exercise. This correlation may indicate that aggregation, via its microcirculatory effects, is one of the factors governing $\gamma 2$.

However, studies employing the full model (with parameters $A1$ and $A2$ which describe the amplitude of respectively lactate output and lactate disappearance, and the time constant $\gamma 1$ which measures muscle lactate output) will be interesting to perform in order to confirm whether $\gamma 2$ (= lactate disappearance) is the only factor of lactate disposal which is related to blood rheology or if other aspects of this kinetics are also correlated to rheological parameters.

In conclusion, this study shows that postexercise lactate removal is negatively related to red cell aggregation. Although this issue requires further research, it suggests that red cell aggregation, by adding its effect to that of the balance between carbohydrates and fat oxidation, is a regulator of blood lactate kinetics after exercise in physiological conditions.

Appendix: Calculation of lactate kinetics parameters

According to Freund and coworkers [9–20], production and disappearance of lactate can be accurately described by the modelling of postexercise lactate kinetics ($Lv(t)$) as the sum of two exponential laws:

$$Lv(t) = Lv(0) + A1\left(1 - e^{-\gamma 1 t}\right) + A2\left(1 - e^{-\gamma 2 t}\right),$$

where

$\gamma 1 =$ muscle lactate output,
$\gamma 2 =$ lactate disappearance,
$Lv(0) =$ blood lactate at $V_{O_2 max}$,
$A1$ and $A2$ amplitudes of the 2 exponentials.

While this model has been extensively investigated and its validity has been well demonstrated, it is not frequently employed since it requires a large number of experimental points (e.g., 30). We recently investigated the possibility to work with a lower number of experimental points and showed that the full model works quite well with only 15 experimental points (MAX, 1, 2, 3, 4, 5, 6, 8, 9, 12, 20, 30, 40, 70, 90) [21]. However, while working on this simplification of the sampling protocol, we noticed that the parameter which was the most easily measurable with a few points was the disappearance time constant $\gamma 2$, since it describes the second exponential which has generally a very regular shape and is displayed over a long period of time (180 min). Accordingly, we made several simulations to select a couple of experimental points which had to be the fewest and the most informative for calculating $\gamma 2$. Finally we selected times 8 and 20 min and the formula:

$$\gamma 2 = 0.0215 + 1.27(\mathrm{Lac8} - \mathrm{Lac20})/(\mathrm{Lac8} \cdot \Delta t)$$

and

$$\gamma 2 = 0.0724 + 0.755(\mathrm{Lac8} - \mathrm{Lac20})/(\mathrm{Lac8} \cdot \Delta t) - 0.00684\,\mathrm{Lac20}.$$

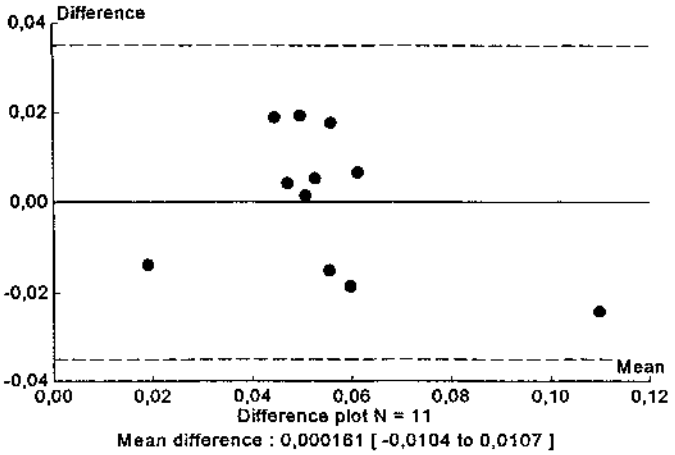

Fig. 2. Bland and Altman diagram showing the concordance of the simplified evaluation of $\gamma 2$ with the formula $\gamma 2 = 0.0724 + 0.755(\text{Lac8} - \text{Lac20})/(\text{Lac8} \cdot \Delta t) - 0.00684\text{Lac20}$ and its measurement with the full protocol procedure in 11 subjects exhibiting a wide range of $\gamma 2$.

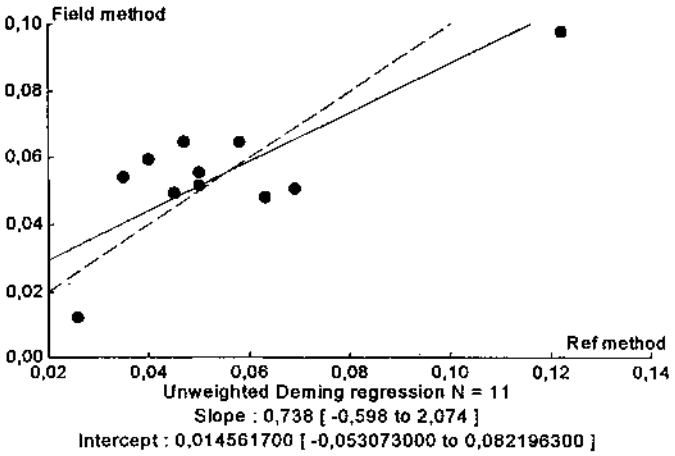

Fig. 3. Deming regression plot showing the concordance of the simplified evaluation of $\gamma 2$ with the formula $\gamma 2 = 0.0724 + 0.755(\text{Lac8} - \text{Lac20})/(\text{Lac8} \cdot \Delta t) - 0.00684\text{Lac20}$ and its measurement with the full protocol procedure in 11 subjects exhibiting a wide range of $\gamma 2$.

The accuracy of this formula was then tested on a separate sample of 11 subjects (8 non-insulin dependent diabetics and 3 patients explored for muscular soreness or cramps and thus expected to exhibit a wide range of $\gamma 2$). We give here (Figs 2 and 3) the Bland and Altman diagram and the Deming regression plot which show the concordance of the formula $\gamma 2 = 0.0724 + 0.755(\text{Lac8} - \text{Lac20})/(\text{Lac8} \cdot \Delta t) - 0.00684\text{Lac20}$ and the measurement of $\gamma 2$ with the full protocol procedure.

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