

Reciprocal relationships between blood lactate and hemorheology in athletes: Another hemorheologic paradox?

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Abstract. Blood lactate increases during exercise. Although this increase was classically interpreted as a “Pasteur-like effect” resulting from anaerobiosis, it is now clear that it mostly results from a shift in the balance of oxidation of substrates in the muscle, with carbohydrate becoming the predominant fuel. However, we have repeatedly observed that the rise in blood lactate during exercise is correlated to blood viscosity and red cell aggregation. More recently we investigated this issue with the modelling of postexercise lactate kinetics, that allows a fair evaluation of lactate production by muscles (γ_1) and lactate disappearance (γ_2). Postexercise red cell aggregation (Myrenne M1) appears to be correlated to γ_2 . Thus microcirculatory adaptations influenced by red cell aggregation may influence lactate disposal, adding its effect to that of the balance between carbohydrates and fat. On the other hand, the rise in blood lactate seems to induce some alterations in erythrocyte rheology at exercise. Correlations between its concentrations during exercise and erythrocyte rigidity support the concept that lactate, at least when it rises above the 4 mmol.l^{-1} threshold impairs red cell deformability. Moreover, it seems that endurance training influences erythrocyte response to lactate. While lactate did not *in vitro* affect erythrocyte aggregation, it impaired (as expected) erythrocyte deformability in sedentary subjects but it (unexpectedly) improved it in trained subjects. This difference may be due to training-induced adaptations in erythrocyte metabolism, including transmembrane transfer via monocarboxylate transporters which show marked alterations in this context. This specific training-induced pattern of response to lactate may provide an alternative explanation to the exercise-induced arterial hypoxemia that occurs in such athletes.

Keywords: Blood viscosity, plasma viscosity, hemorheology, erythrocyte deformability, erythrocyte aggregability, exercise, lactate, crossover concept, anaerobic threshold

1. Introduction. Blood lactate during exercise: still a matter of debate?

Blood lactate, which increases during exercise, is first of all a by-product of anaerobic glycolysis. It is formed in fast-twitch white skeletal muscle fibers or transiently in populations of red fibers at the start of exercise. It is classically known that, if enough oxygen is not available, lactate is produced and begins to accumulate in the muscles. That lactate is often believed to represent the cause of the “burning” sensation felt in muscles during high intensity exercise. It is also assumed that lactate accumulation prevents muscles from working their best.

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As recently reviewed by Bruce Gladden [1] the history of lactate studies in muscle is a quite old one, since as early as 1807 Berzelius found lactic acid in muscular fluid and thought that “the amount of free lactate in a muscle [was] proportional to the extent to which the muscle had previously been exercised”. Later in the XIXth century, it was established that lactate production can result from O_2 limitation which shifts glucose metabolism away from the tricarboxylic cycle and thus derives pyruvate to lactate. Based on those classic findings, Margaria [2] 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 [3,4] 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. 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”.

Accordingly, Wasserman’s anaerobic threshold paradigm assumes that elevated lactate production and concentration during muscular contractions or exercise are the result of cellular hypoxia. This “threshold paradigm” has acquired a considerable popularity among athletes, coaches and sports physicians. However, several studies during the past 30 years have severely challenged this concept. Several lines of evidence indicate that lactate is produced in muscle even in the absence of any O_2 limitation, and that O_2 is not actually the key regulator of lactate production by muscles. For example, in a recent paper using sophisticated magnetic resonance measurements, Richardson and colleagues [5] demonstrated that lactate efflux from muscle was unrelated to muscle cytoplasmic P_{O_2} during normoxia. It is now clearly demonstrated that lactate generation does not necessarily reflect O_2 limitation.

Therefore, the old concept of cellular hypoxia being the cause of lactate production and accumulation is no longer acceptable. The current understanding of the metabolic role of lactate is embodied in the “lactate shuttle hypothesis” developed by Brooks. This hypothesis assumes that “the shuttling of lactate through the interstitium and vasculature provides a significant carbon source for oxidation and gluconeogenesis during rest and exercise”. This means that, during exercise, some lactate released into the circulation reperfuses the active muscle bed within a fraction of a minute, where uptake and oxidation in red, highly oxidative fibers occurs. Some of the lactate released from an active muscle bed can also be taken up by the heart and oxidized or taken up by the liver and kidneys where lactate serves as a gluconeogenic precursor [6].

According to this hypothesis, lactate is in fact a useful metabolic intermediate that can be exchanged rapidly among tissue compartments. Once formed in muscle cells that may have high glycogenolytic and glycolytic rates, Lactate can be transported to other sites where it may serve as an energy source and a gluconeogenic precursor. Lactate oxidation can occur in nearby oxidative muscle cells, or at other sites such as the heart or other oxidative skeletal muscles that might be either at rest or engaged in light to moderate exercise, or also it can be taken up in the liver and used for glucose production or liver glycogen storage. The lactate shuttle hypothesis also indicates that there is an intracellular shuttle, due to

the fact that lactate is an inevitable product of glycolysis, and even more during rapid glycolysis. There is an obvious reason for this unavoidable lactate production, if one considers the properties of the enzyme lactate dehydrogenase (LDH), which has the highest $V_{\max}$ of any enzyme in the glycolytic pathway, and that the K_{eq} for pyruvate to lactate is far in the direction of lactate. Thus, lactate produced in the cytosol by glycolysis can be taken up directly into mitochondria and oxidized.

Despite challenges to the paradigm that lactate hails hypoxia, it nevertheless remains clear that if mitochondria are O_2 -limited, then there will be an enhanced lactate production and accumulation. For example, in the cardiac muscle this phenomenon has recently been investigated by Sutherland [7]. The findings of these authors imply that myocardial ischemia causes myocardial O_2 limitation, resulting in accelerated lactate production and accumulation. Lactate then moves across the sarcolemma via facilitated diffusion into the extracellular fluid where the decreased pH is detected by ASIC3 channels, resulting in signals along cardiac sympathetic afferents which cause the sensation of chest pain. This is a striking example of the pathophysiological relevance of hypoxia-induced lactate production. Clearly, this phenomenon is able to trigger local pain in muscular tissues such as the cardiac one.

Therefore, the older view of lactate and hypoxia remains correct in the sense that anaerobic glycolysis, lactate production, and lactate accumulation are increased under conditions that engender O_2 -limited oxidative phosphorylation in mitochondria. In other terms, it is still true that tissue hypoxia leads to increased lactate concentration. However, the induction that elevated lactate production and accumulation necessarily indicate the presence of hypoxia is not correct. Bruce Gladden, in his recent review on this topic, defines this as “the dichotomy of lactate in metabolism: on the one hand, an end product of anaerobic glycolysis, but on the other hand, an important metabolic intermediate of aerobic glycolysis” [1].

It can be concluded from the points discussed above that hypoxia may be in some extreme or pathologic conditions a mechanism inducing excess lactate production in muscular tissues, but that the main mechanism which governs this production is not oxygen but the balance of substrates used as fuels by the muscle. Since pyruvate and lactate are products of carbohydrate breakdown but not lipid breakdown, the amount of lactate produced during exercise is largely explained by the balance between carbohydrate and fat oxidation. The more you oxidize carbohydrates, the more you generate pyruvate and, thus, lactate. By contrast, the more you oxidize lipids (a metabolic pathway increased by endurance training) the less you generate lactate. The onset of lactate accumulation measured with the classical “thresholds” described above reflects in fact the “point of crossover” of substrates where carbohydrates become predominant fuel [8,9].

2. Lactate as a modulator of erythrocyte rheology during exercise

In most (but not all) exercise protocols there are also changes in the rheological properties of erythrocytes [10]. The most classical is a decrease in erythrocyte deformability which is not a specific finding since it is also observed in most stressful events like labor [11,12], videofilm-induced emotional stress [13], and endogenous depression [14]. These effects are generally not found at exercise when red cell rheology is investigated after resuspension of cells on a buffer, indicating that they are mostly due to plasma factors rather than to intrinsic red cell properties [10].

Blood lactate, which experimentally shrinks the red cells and decreases their flexibility, is likely to explain in part this exercise-induced rigidification of erythrocytes, as supported by correlations between lactate concentrations and red cell rigidity at exercise [15]. In one study, we interestingly found a threshold value for this effect which became apparent only when blood lactate increased above 4 mmol.l^{-1} ,

i.e. the value which has been proposed to represent approximately the point where lactate induces acidosis [16]. However, in other experimental conditions, it was shown that even a moderate lactate increase during low intensity exercise results in a transient increase in erythrocyte rigidity [17]. Recently, *in vitro* experiments were conducted in order to confirm the direct role of lactate on red cell rigidity in athletes [18]. At physiological concentration (2 mM, 4 mM and 10 mM) red cell deformability was decreased in blood from sedentary subjects, confirming the more indirect previous studies.

3. Paradoxical increase of red cell deformability during exercise in athletes

While red cell rigidity was generally found to be either increased or unchanged during exercise, there was a surprising report of a decrease of this parameter, when assessed after exercise with the LORCA [19]. This paradox has recently been explained by a study on 20 highly trained athletes [20]. During a progressive exercise test conducted to $V_{O_{2\max}}$ red cell rigidity was found to paradoxically decrease in these athletes. This decrease was not found in a subgroup in whom hypoxemia appeared at maximal exercise, as discussed below. Besides, *in vitro* experiments [18] showed that lactate at concentrations ranging from 2 mM to 10 mM increased red cell deformability in such athletes while it classically decreased it in blood from sedentary subjects. Thus, in highly trained subjects, the exercise-induced increase in blood lactate does not rigidify the red cell as observed in sedentary subjects or in moderately trained ones (like footballers [15,16]) but actually improves red cell deformability.

4. Exercise-induced arterial hypoxemia in aerobically highly trained athletes

About half of all highly trained endurance athletes, defined as those with maximal oxygen uptakes greater than 60 ml/kg/min, develop exercise-induced hypoxemia (EIH), i.e. the arterial pressure in O_2 decreases during intense exercise. The potential mechanism of this phenomenon is still a matter of debate. EIH is characterised by a decrease in both oxygen arterial partial pressure (PaO_2) and arterial haemoglobin saturation (SaO_2) and is primarily due to an excessive alveolar-to-arterial O_2 difference ($A-aDO_2$). This indicates inadequate oxygen equilibration between alveolar gas and pulmonary capillary blood. Such an alteration in pulmonary gas exchange may be due to ventilation-perfusion (VA/Q) inequality or diffusion limitation. The development of mild interstitial oedema has been suggested by physiological findings, such as the persistent widening of ($A-a$) DO_2 following exercise, and radiographic observations of increased lung density and areas of opacities after triathlon performance.

Connes and coworkers [21] studied a group of 20 highly trained endurance athletes, among whom 9 experienced a low hemoglobin saturation during exercise (Low- SpO_2 group) while the 11 others had normal hemoglobin saturation (High- SpO_2 group). At 50% $V_{O_{2\max}}$ and maximal exercise, whole blood viscosity measured at high shear rate was higher in Low- SpO_2 subjects, due to a lack of improvement in red cell rigidity while this rigidity decreased in the high- SpO_2 group. The authors assume that the greater increase in blood viscosity in athletes with low hemoglobin saturation may result in exercise-induced hypoxemia.

5. Exercise-induced pulmonary hemorrhage in horses (and also humans?)

Exercise-induced pulmonary hemorrhage (EIPH) occurs in most horses undergoing competitive racing activity. Recent progress in understanding the pathophysiology of EIPH indicates that stress-induced

rupture of pulmonary capillaries may be involved. In this process, blood rheology may play a role, as suggested by several reports of excess exercise-induced hyperviscosity in EIPH-prone horses. Indeed, during intense exercise, the increased cardiac output and blood viscosity combine to rise the capillary wall stress [22,23].

Episodes of pulmonary hemorrhage following ultra marathon races in humans have also been reported in the literature. Rupture of pulmonary capillaries and alteration of the blood gas barrier are suspected as well as in race horses. Although mean capillary pressure during maximal exercise does not reach the high levels observed in horses, one actually suspects a similar process resulting in high capillary tension [24].

6. Is erythrocyte aggregability a modulator of muscle oxygen delivery during exercise?

In 1990, an experiment of continuously measured oxygen uptake during constant work exercise (15 minutes at 50 W) was conducted by the team of Witte [25]. This experiment demonstrated an increased oxygen consumption in individuals with elevated blood viscosity parameters. The authors interpreted that this increased oxygen consumption resulted from a persistent contribution of anaerobic glycolysis during steady state exercise far below the level of the expected “anaerobic threshold”. Even more interestingly, an experimental improvement of viscosity parameters by prostaglandin E1, naftidrofuryl hydrogenoxalate or hemodilution with 500 ml 6% hydroxyethylamylum MW 40000 was shown to result in significant reduction of this oxygen gradient during exercise. This experiment suggested that blood rheology modulated O₂ supply to muscles during exercise and that increased blood viscosity increased the rate of glucose waste through the so-called “anaerobic pathways”.

More recently we also published several papers which are in agreement with those early findings, i.e. they strongly suggest that there is a link between erythrocyte aggregability at baseline and the rise in blood lactate during exercise.

In a first study we observed that lower values of resting blood viscosity and erythrocyte aggregation were associated with lower increases in blood lactate during submaximal exercise [26]. We further investigated this issue during the determination of the classical lactate thresholds, which appeared also to be related to blood viscosity and erythrocyte aggregation in professional football players [27]. Finally, in a follow up study of adolescent gymnasts including the measurement of viscosity parameters, we observed that changes in resting red cell aggregability and lactate response to exercise after a whole season of training were correlated, so that when resting red cell aggregability was lower, lactate response was also lower in comparison with the measurements performed at the beginning of the season [28].

All these papers suggest that red cell aggregation may influence muscular lactate metabolism. As experimentally shown by Vicaud [29], increased RBC aggregation may impair microcirculation in muscles. Although aggregation is beneficial to some extent for microvascular perfusion, its increase, even within a physiological range, might impair aerobic metabolism in muscle, resulting in higher blood lactate. If this assumption is correct, lactate accumulation, that was classically described as an “anaerobic process” [2–4], but is rather explained nowadays by a shift in the balance of fuel oxidations [8,9], could be influenced by the aggregation-related alterations in microcirculatory supply of O₂. While the microcirculatory effects of red cell aggregation are a matter of controversy, experiments by Johnson and coworkers [30], suggest that red cell aggregation represents 60% of resistance at the venous pole in cat gastrocnemius. Aggregation could be thus the major modifier of venous resistance in skeletal muscle [30]. However, Popel [31], comparing a wide series of animals, shows that red cells from athletic animals aggregate

more. This author postulates that their capacity to aggregate would regulate postcapillary hindrance, i.e. a parameter that is not likely to be modified by size changes and thus depends rather on rheology. Across mammals, these studies demonstrate a correlation between $\dot{V}_{O_{2\max}}$ and aggregability [31]. Such a zoologic observation is not in agreement with literature on red cell aggregation and fitness in humans.

Experiments of muscle hypoxia [32] show that an anemia reducing by 25% hematocrit in dogs increases blood lactate accumulation. This increase in lactate is associated with higher muscular glucose consumption, and with an increase in glucagon, norepinephrine, epinephrine and cortisol while insulin and free fatty acids are unchanged [32]. In humans suffering from peripheral obliterative arterial disease, red cell aggregation is negatively correlated with transcutaneous oxygen pressure, further supporting the concept that aggregation impairs oxygen supply to tissues [33].

More recently we investigated this issue with the modelling of postexercise lactate kinetics, that allows a fair evaluation of lactate production by muscles (γ_1) and lactate disappearance (γ_2). Postexercise red cell aggregation (as assessed by the Myrenne M1 index) appears to be correlated to γ_2 . 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 γ_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 γ_2 . Thus microcirculatory adaptations influenced by red cell aggregation may influence lactate disposal, adding its effect to that of the balance between carbohydrates and fat [34].

7. Conclusion

On the whole, beside “the dichotomy of Lactate in metabolism” described by Bruce Gladden [1] there is another domain where lactate offers two different faces, the domain of blood rheology. The body of information summarized above indicates that this metabolite is likely to influence erythrocyte rheology, with perhaps an opposite effect in trained and untrained individuals, and that erythrocyte aggregability may modulate its production by the muscle, probably via changes in lactate clearance.

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