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The Concept of Maximal Lactate Steady State

A Bridge Between Biochemistry, Physiology and Sport Science

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Abstract

The maximal lactate steady state (MLSS) is defined as the highest blood lactate concentration (MLSSc) and work load (MLSSw) that can be maintained over time without a continual blood lactate accumulation. A close relationship between endurance sport performance and MLSSw has been reported and the average velocity over a marathon is just below MLSSw. This work rate delineates the low- to high-intensity exercises at which carbohydrates contribute more than 50% of the total energy need and at which the fuel mix switches (crosses over) from predominantly fat to predominantly carbohydrate. The rate of metabolic adenosine triphosphate (ATP) turnover increases as a direct function of metabolic power

output and the blood lactate at MLSS represents the highest point in the equilibrium between lactate appearance and disappearance both being equal to the lactate turnover. However, MLSSc has been reported to demonstrate a great variability between individuals (from 2–8 mmol/L) in capillary blood and not to be related to MLSSw. The fate of enhanced lactate clearance in trained individuals has been attributed primarily to oxidation in active muscle and gluconeogenesis in liver. The transport of lactate into and out of the cells is facilitated by monocarboxylate transporters (MCTs) which are transmembrane proteins and which are significantly improved by training. Endurance training increases the expression of MCT1 with intervariable effects on MCT4. The relationship between the concentration of the two MCTs and the performance parameters (i.e. the maximal distance run in 20 minutes) in elite athletes has not yet been reported. However, lactate exchange and removal indirectly estimated with velocity constants of the individual blood lactate recovery has been reported to be related to time to exhaustion at maximal oxygen uptake.

During high-intensity exercise, lactate accumulates as the result of lactic acid production being greater than its removal. At a physiological pH, lactic acid, a strong organic acid, dissociates a proton (H^+) and almost completely dissociates to hydrogen and lactate ions; therefore, the term lactic acid and lactate are used synonymously.[1] It is the H^+ rather than the lactate ion that causes pH to decrease. Muscle and blood lactate accumulation during exercise mean that the mechanisms of lactate disposal and clearance have been exceeded. Thus, the overall system is failing with metabolic demand. Further, lactate accumulation is indicative of glycogen depletion.[2] The production of lactic acid depends on the balance of competition for pyruvate and the reduced form of nicotinamide-adenine dinucleotide (NADH) between the lactic acid dehydrogenase (LDH) on the one hand, and alanine transaminase, the NADH shuttles and the monocarboxylate transporter (MCT) on the other hand. Not all the pyruvate can be transformed into acetyl coenzyme A in the mitochondria by the activation of the enzyme pyruvate dehydrogenase (PDH). Furthermore, the phosphorylase is activated by the increase in the exercise intensity and from the increased concentration of calcium from inorganic phosphate (Pi) and from adenosine monophosphate (AMP). These metabolites (adenosine diphosphate [ADP], AMP, Pi) activate the enzyme phosphofructokinase. All these mechanisms contribute to increase the lactate appearance rate and to decrease the lactate disappearance rate.

The general scope of this review is to cross the artificial 'boundaries' between metabolic and protein biochemistry, exercise physiology and sport science through the maximal lactate steady state (MLSS) concept. The debate is no longer to assess the existence of a lactate turning point in an incremental work rate exercise but to understand the mechanisms for the stabilisation or not of blood lactate concentration at a maximal level of lactate during a constant load of exercise as this is highly discriminant of endurance performance such as the marathon (except in elite athletes).[3] Therefore, MLSS is defined as the highest blood lactate concentration (MLSSc) and work load (MLSSw) that can be maintained over time without continual blood lactate accumulation.[4-10]

MLSSw is used in the assessment of an athlete's endurance capacity.[9,11-14] MLSSw elicits a blood lactate concentration average of 4.0 mmol/L. For that reason, it has long been estimated by the onset of blood lactate accumulation which is the work load corresponding to blood lactate levels of 4.0 mmol/L determined in an incremental test.[15] However, MLSSc has been reported to have great variability between athletes (from 2–8 mmol/L in capillary blood) and not to be related to performance.[6] For an individual, MLSSw delineates the low- to high-intensity exercises at which no oxygen uptake ($\dot{V}O_2$) steady state is observed and at which the fuel mix switches (crosses over) predominantly from fat to carbohydrate and this velocity is close to the average marathon velocity.[3] Lactate represents the vehicle for shuttling chemical potential energy from one site (in contracting fast-glycolytic fibres) in the body to another (heart and oxidative fibres).[15,16] MLSSw represents the highest work rate at which

the oxidative phosphorylation is adequate for the energetic demand and is used to estimate the aerobic capacity during an exhaustive exercise performed at the lowest work rate associated with maximal oxygen uptake ($\dot{V}O_{2max}$) in an incremental test; almost 15 ± 5% of energetic demand is covered by the anaerobic metabolism whatever the level of performance.[17] MLSSw has been reported to be sensitive to endurance training. Few studies have focused on the value of MLSSc and the effect of training on its value. It is only known that the average value is 4 mmol/L and the range is large (2–7 mmol/L) and that it is linked to the power output per unit of muscle mass involved in exercise.

At very high lactate concentrations, diffusion accounts for a substantial portion of lactate movement across the plasma membrane. It has been shown that the process of protein-mediated lactate transport uses a saturable proton co-transport system that is stereo-specific for L-lactate.[18] Capillarisation[19] and carrier-mediated lactate transport[20] are the two of the determining factors for lactate release from exercising muscles. It has long been shown that the number of capillaries per muscle fibre increased with intensive endurance training.[21] Putman et al.[22] showed that 2 hours of daily training over 7 days at 60% $\dot{V}O_{2max}$ did not increase the global $\dot{V}O_{2max}$ or the local $\dot{V}O_2$ (of the inferior members), or that of the activity citrate synthase enzyme in the Krebs cycle or that of PDH (even in its active form). However, endurance training decreased the production of pyruvate by 33% and the formation of lactate from pyruvate by 59% during 15 minutes of exercise at 75% $\dot{V}O_{2max}$. This decrease, which is associated with a reduction in the oxidation of pyruvate (−24%), was partly due to a reduced pyruvate production. Furthermore, the concentrations of acetyl coenzyme A and phosphocreatine were increased. Consequently, the reduction in the post-training glycolytic flux will be more in phase with the enzymatic activity of PDH, independent of $\dot{V}O_2$, which does not vary after training.

The reduction in lactate production coincides with an improvement in the maintenance of cell phosphorylation and with an improved removal of lactate. This modification was associated with a greater potential for phosphorylation. The molecular mechanisms in the adaptation are still unknown. Pilegaard et al.[23] mentioned that sarcolemmal carrier-mediated lactate transport, which has an important role in lactate release during and after heavy exercise is higher in athletes than in less fit or untrained participants. It has been recently reported that lactate transport was mediated by a transport protein: the MCT which has several isoforms.[24] These carriers are also sensitive to endurance and intensive training and are modified after exhaustive exercise. Some data[25] suggest that among the family of MCTs, MCT1 and MCT4 are primarily responsible for lactate uptake from the circulation and lactate extrusion out of muscle, respectively. No studies have yet been published on the relationship between MLSS and lactate transporters.

This paper reviews the latest developments in the methodology for determining MLSS, its impact on performance and its physiological factors in light of the recent fundamental research on lactate transporters which contribute to a better understanding of the physiological background of MLSS and of its modification with training.

1. Physiological Mechanisms of the Maximal Lactate Steady State (MLSS)

1.1 The Process of Lactate Appearance, Removal and Turnover

The kinetics of lactate metabolism during exercise has generally been inferred from measurements of lactate concentration in blood and muscle. Production usually refers to the production rate in muscle (unless specifically referring to red cells); however, removal could occur from muscle or from the blood compartments. Donovan and Brooks[26] demonstrated that the rates of lactate appearance and disappearance are exponential functions of $\dot{V}O_2$ and are linearly related to arterial lactate concentration during graded exercise. Through the infusion of isotopically labelled lactate, the turnover and oxidation rates of lactate can be determined and have been reported to increase markedly from rest to moderate exercise with only a slight increase in circulating lactate concentration.[26] At rest and during low-intensity exercise (50% of $\dot{V}O_{2max}$), lactate is produced and removed at equal rates. This balance of production and removal is called turnover.[1] In lactate steady state, the process of lactate appearance is balanced by the process of lactate disappearance, i.e. there is an equilibrium in turnover, appearance and disappearance.

The blood lactate at MLSS represents the highest point in this equilibrium. The rate of appearance and

disappearance of lactate in the blood is measured in mg/min or normalised to body mass in mg/kg/min and is equal to about 3–4 mg/kg/min at blood lactate concentration around 4–8 mmol/L. The rate of turnover is defined for the metabolic steady state when the metabolite (lactate) is constant and lactate disappearance equals lactate appearance. The steady state arterial lactate concentration is equal to the rate of lactate turnover in the blood divided by the metabolic clearance rate (MCR) for lactate (MCR = lactate turnover/[blood lactate][27]). MCR increases during high-intensity exercise and is around 35–75 ml/kg/min for untrained and trained individuals, respectively.[28] This means that lactate disappearance is concentration-dependent as reported by turnover and is linearly related to arterial blood concentration during graded exercise.[26] MCR is a parameter that describes the volume of blood and other body fluids (mL) from which the metabolite is cleared (removed) per unit of time (minutes). MCR of the lactate is equal to the lactate distribution volume (about 500 ml/kg[29]) time the fractional turnover.[30,31] Therefore, MCR is an important parameter of lactate disappearance and provides a mean of describing the interactions between rate of removal, blood concentration, and blood flow. Blood flow is of great importance because, in the case of lactate uptake, if all other factors remain constant, an increased blood flow should increase lactate and proton delivery to the muscle, thereby maintaining more favourable extra- to intracellular lactate and proton gradients, and thus promoting net lactate uptake.[32] Blood lactate concentration will rise only if the rate of lactate entry into circulation increases relative to the rate of lactate clearance. The increase of lactate level during exercise is a result of a large increase in lactate appearance relative to a smaller rise in the MCR.[28] To our knowledge, no studies have yet focused on the relationship between lactate MCR and MLSSc. It may be possible that the wide inter-individual variation of MLSSc is because of a different MCR for the same rate of appearance.

Even though lactic acid has a rapid turnover, its concentration in the blood may not change as long as the disappearance from the blood keeps pace with the appearance in the blood. At heavier exercise intensities (50–80% of $\dot{V}O_{2max}$) blood lactate level increases compared with that at rest. If the exercise is maintained long enough, the lactate level can remain elevated but by a constant amount. If the lactate level is constant, then lactate appearance and disappearance are equal. There are several work rates at which blood lactate reaches a steady state after 10–15 minutes;[33,34] however, there is a particular work load at which the arterial lactate concentration is constant with time at its maximal level which is named maximal lactate steady state.

1.2 Mechanisms for the Balance Between Lactate Appearance and Disappearance at MLSS Work Load (MLSSw)

Both lactate appearance and disappearance and increase exponentially with $\dot{V}O_2$ during continued, graded exercise in humans.[30] Studies on the turnover of lactate in animals during exercise have revealed a direct relationship between metabolic rate ($\dot{V}O_2$) and lactate turnover.[26,27,35] Increased rates of metabolism produced by exercise result in increased rates of glycolytic carbon flux which, in turn, increases rates of lactic acid production. Approximately 50% of total glycolytic carbon flux passes through lactate which reflects the importance of lactate as a metabolic intermediary between carbohydrate storage forms (glucose and especially glycogen) and metabolic end-products (CO_2 and water), because of its rapid exchange between compartments.[36] Stanley et al.[37] demonstrated that during a moderate cycling exercise (43 ± 5% of $\dot{V}O_{2max}$) performed during 30–50 minutes, 20% of glucose utilisation went to lactate formation and 20% of the blood lactate appearance came from glucose, with the balance presumably coming from muscle glycogen. For glucose and lactate, after 20 minutes of exercise, lactate appearance equalled lactate disappearance.

Therefore, lactate appearance increases with the metabolic work rate because lactic acid is a product of glycogenolysis and glycolysis. The intracellular enzymes that process carbohydrates produce lactic acid as a function of their metabolism. It is clear that lactate is an intermediary for glycolytic metabolism and is closely dependent on exercise intensity. Furthermore, the catalytic activity of LDH greatly exceeds the combined catalytic activities of enzymes that provide alternative pathways for pyruvate metabolism. At a speed working at an exercise intensity of 65% and 90% of $\dot{V}O_{2max}$, the flux of pyruvate through LDH is 2 and 3 times greater than that through PDH, respectively.[38] Further, the Michaelis-Menten constant (K_m) for the conversion of pyruvate to lactate is about 0.08 mmol/L. Therefore, the

concentration of pyruvate found in muscle during sub-maximal exercise is sufficient to support maximal catalytic activity of LDH in the production of lactic acid.

Stanley et al.[30] reported a strong positive relationship between lactate appearance or disappearance and the arterial lactate concentration during progressive graded exercise. The increase in circulating lactate concentration during graded exercise occurred because lactate appearance increased more rapidly than lactate disappearance; it was not solely because of a sudden increase in lactate appearance. When the balance between lactate appearance and disappearance was examined during the constant load of a cycling exercise at 50% of $\dot{V}O_{2max}$, it was found that legs transiently increased the lactate release.[39] After 15 minutes, there was a switch from release to consumption and arterial level was constant, the working leg became a net lactate consumer. Significant lactate appearance and disappearance were observed in both forearms in a cycling exercise.[40] Furthermore, not only the active but also the inactive muscles and other tissues must release lactate during exercise in order to explain the maintenance of elevated arterial lactate concentration, as active muscle consumes and oxidises blood lactate, whereas lactate from active muscle falls to close to zero after 45 minutes of exercise.[41-43]

In addition, muscle tissue continuously consumes and releases lactate with the greatest consumption occurring during exercise when blood lactate level is highest.[43-45] Lactate removal through oxidation (approximately 50% at rest), increases and accounts for 75% of lactate disappearance during exercise.[39] Moreover, Brooks et al.[46] showed that the NADH could be re-oxidised in the mitochondria by the mitochondrial LDH enzymes (of the LDH-H type) by the transformation of lactate into pyruvate. Therefore, most of the blood lactate produced is not measured by lactate appearance. Thus, net release of lactate underestimates the total lactate formed in muscle.[46]

1.3 The Causes for the Increase of Lactate Appearance with Exercise Work Load

The net release of lactic acid from muscle to blood is determined by the mass of active muscle and the intensity of the activation of these muscles.[47] There will be different variations depending on fibre types, effectors of membrane lactate transport, blood flow and its distribution, especially according to thermal conditions. How high the blood lactate rises for a given mass and intensity of stimulation will also be directly related to the duration of the activity within the limits of the proposed time frame.[48]

The contribution of muscle-derived lactic acid will be significantly modified if effectors of muscle glycolysis or glycogenolysis change. For example, if the epinephrine concentration of the blood is elevated, the net output of lactic acid by the active muscles will be increased.[48] Hamann et al.[49] showed that epinephrine infusion significantly inhibited net lactate uptake. Because the effect of epinephrine infusion on net lactate uptake could be abolished by prior administration of propanolol, it appears that the effect of epinephrine on net lactate uptake is related to epinephrine/β-receptor interaction. Therefore, epinephrine has a profound effect on net lactate uptake by contracting skeletal muscle *in situ* exposed to an elevated blood lactate.

Watt et al.[50] have demonstrated that elevated plasma adrenaline levels during moderate exercise in untrained men increased skeletal muscle glycogen breakdown and PDH activation, which resulted in greater carbohydrate oxidation. The greater muscle glycogenolysis appeared to be due to increased glycogen phosphorylase transformation whilst the increased PDH activity cannot be readily explained. It has been recently demonstrated that the elevated lactacidaemia associated with hypoxia at the same absolute work load could partly be explained by the effects of hypoxia on the activities of the rate-limiting enzymes, phosphorylase and PDH.[51,52]

Glycolysis increases with the exercise intensity through β-adrenoceptor stimulation and by the recruitment of fast-twitch fibres (types IIa and IIb [fast-glycolytic]) that contain less PDH, mitochondria and oxidative enzymes from the Krebs cycle. After this feed-forward activation of glycolysis mediated by the adrenogenic activity, the increase in lactate is due to the combined effects of the increased glycolytic flow.[53] In addition, the adrenogenic activity brings about a vasoconstriction of the hepatic, renal and inactive muscle areas. This leads to a reduction in the removal of lactic acid through gluconeogenesis and hence its disappearance. The contribution of the Cori cycle to glucose production appeared to collapse.[54]

1.4 The Time to Get a Blood Lactate Steady State

The time to reach lactate steady state depends on the degree of lactate elevation and has been reported to be from 2–5 minutes depending on the work load increment in an incremental test.[55] As underlined by Freund et al.,[55] there is a range of work rate in a constant load exercise at which blood lactate is at a steady state after a first exponential increase[33] according the following equation:

$$La(t) = La\ rest + \Delta La \times [1 - e^{(K2*t)}]$$

where La (t) is the blood lactate at time t, ΔLa is the blood lactate increase (i.e. the difference between lactate appearance and disappearance and K_2 is the time constant for the lactate increase ($=1/\tau$ per minute). It may be probable that there is a relationship between K_2 and γ_1 and γ_2 the velocity constants of the bi-exponential time function fitted to the arterial lactate recovery curves obtained after muscular exercise which have been shown to supply information on the lactate exchange ability between the previously active muscles and the blood γ_1 as well as on the body's overall ability to remove lactate γ_2 during the recovery.[55,56] The relationship between the MCT concentration and the kinetics of lactate recovery is yet unknown but could be one of next challenges to validate a noninvasive tool to track and better understand the effect of training on MLSS. Indeed, γ_1 and γ_2 have been reported to be increased by 68% and 47% by endurance training, respectively,[57] and more recently have been reported to be related to the time to exhaustion at $\dot{V}O_{2max}$.[58] To date, this approach has not been applied to lactate kinetics during exercise since no studies have yet focused on the lactate kinetics during exercise as a function of exercise intensity, training state and type of training in cross-sectional or longitudinal studies. The cause of K_2 increase after training and its possible relationship with the decrease of MLSS after training remains unknown. It may be suggested that this time constant for lactate accumulation during exercise could be a key factor for the difference of the level at which blood lactate stabilises at its maximum (MLSSc).

1.5 The Lactate Shuttle Theory Between and Within Cells

The lactate steady state between rate of appearance and disappearance depends on the lactate shuttle. Systemic lactate flux and whole body oxidation data supported the concept of lactate shuttle which can help to explain the modification of MLSSw with training. The lactate shuttle theory was first formulated by Brooks at the inaugural International Congress of Comparative Physiology and Biochemistry held in Liege in August 1984:[55] "the lactate shuttle across the interstitial and vascular areas supplies an important source of carbon for the oxidation and gluconeogenesis at rest and during exercise". The original 'cell to cell' lactate shuttle theory established that the shuttling of lactate through the interstitium and vasculature provides a significant carbon source for oxidation and gluconeogenesis during rest and exercise.[59,60] Type IIb fibres are lactate producing cells, whereas type I fibres are indicated as sites of lactate oxidation. This hypothesis was developed at a time when fibre heterogeneity in skeletal muscle was recognised.[61] Furthermore, the hypothesis was based on results of rats and dogs that were infused with radioactive glucose and lactate tracers and made to exercise.[62,63] Subsequently, the same phenomenon of lactate flux and oxidation was reported in humans.[31,37]

Lactate turnover is high at rest and increases as a direct function of exercise intensity and metabolic power output. Lactate clearance through oxidation (approximately 50% at rest) increases both absolutely and relatively during exercise in such a way that oxidation accounts for 75% of lactate disposal during exercise. Skeletal as well as cardiac muscle and liver participate in lactate exchange and metabolism during exercise. Not only is lactate always present in resting muscle, but lactate release and oxidation occur simultaneously while cardiac output and blood flow to the limbs rise so that oxygen transport meets demand, and a significant amount of oxygen remains in femoral venous blood.[64]

To resolve the dilemma of tissue lactate production under fully aerobic conditions, the 'intracellular lactate shuttle' was established.[39] While there was good evidence to support activity of the 'cell-cell lactate shuttle' during exercise and at rest, the recruitment of type IIb fibres could not be invoked. A better model allowing lactate production in all fibres types during rest and exercise was needed. The 'intracellular' shuttle allows glycolysis in the cytosol to result in lactate production and lactate shuttles to mitochondria within the cell of production for oxidative removal.[46] The lactate-shuttle theory has therefore been enriched by the intracellu-

lar concept due to the discovery of intra-mitochondrial LDH and the evidence of the lactate transporters found in the internal mitochondrial membrane.[16] In effect, the lactate produced in the cytosol by glycolysis can be directly taken into the mitochondria where it is oxidised. It is still not known whether the mitochondrial respiration is linked to the mitochondrial concentration of MCT.

Recently, Chatham et al.[65] demonstrated that glycolytically-derived lactate production and oxidation of exogenous lactate operate as functionally separate metabolic pathways, consistent with the concept of a cell-cell lactate shuttle in the heart.[65] The idea of direct mitochondrial-lactate oxidation appears to be gaining wide acceptance;[16,66-68] however, this has been very recently challenged by Sahlin et al.[68] They demonstrated that mitochondria isolated from skeletal muscle readily oxidise pyruvate but cannot oxidise lactate directly. These results indicate that oxidation of lactate could only occur after extra mitochondrial conversion to pyruvate. Moreover, Sahlin et al.[68] provide theoretical arguments that direct mitochondrial lactate oxidation is energetically unlikely because, in contrast to cytosol, the redox state of the NAD^+-NADH redox couple is highly reduced in the mitochondrial matrix and the flux of the LDH^- reaction (if LDH is present) would be from pyruvate to lactate and the intracellular lactate shuttle hypothesis requires the converse flux. The system would abolish the redox gradient between mitochondria and cytosol, remove the redox drive and prevent oxidative phosphorylation. The faster lactate transport rates in oxidative fibres may reflect the role of lactate as an energy substrate for those fibres.[32]

The improvement of lactate transport between and within cells could be the key to the training effect on the increase of MLSS with training. Recent findings on the lactate transporters open new perspectives to solve this question. The study of cell membrane lactate transport proteins received a major boost when Garcia and associates[24] cloned and sequenced an MCT, which they termed MCT1.

1.6 Lactate Transport is Mediated by a Protein

Transport of lactate between muscles and blood depends on two barriers: (i) the capillary membrane; and (ii) the sarcolemma. The capillary membrane is not considered as a lactate barrier despite the fact that it is not known what fraction is diffused across the endothelial interstitium and what fraction is taken by endothelial cells before being disposed of on the other side of the capillary membrane.[20] In this latter example, a delay in the membranous translocation of lactate may appear and the transport system would be involved; however, it is generally agreed that the sarcolemma is a barrier for both the influx and efflux of muscle lactate. For a number of years, lactate was thought of as simply diffusing across the sarcolemmal membrane; however, research in the last 5 years has demonstrated that the removal and elimination of lactate was facilitated by a protein attached to the sarcoplasmic membrane.

In 1990, Roth and Brooks[69] described the lactate transport probably by a lactate carrier linked to the sarcolemmic vesicles of the skeletal muscles. In 1991, McDermott and Bonen[70] considered that the presence of the lactate carriers in the membrane matrix of the skeletal muscle is highly important in the movement of lactate. In 1993, these same authors[71] compared the capacity of the lactate transport in the sarcolemmal membranes of the skeletal muscles of trained and non-trained rats in endurance exercise. The K_m for the transport of lactate is reduced in trained rats indicating either a greater affinity for the substrate carriers or the existence of several families of transporters (isoforms) having different levels of affinity for the substrate and which would demonstrate different levels of sensitivity to endurance training.[71] Therefore, monocarboxylates such as lactate and pyruvate play a central role in cellular metabolism and metabolic communication between tissues.[72] Essential to these roles is their rapid transport across the plasma membrane that is catalysed by a family of proton-linked MCTs. Nine MCT-related sequences have so far been identified in mammals each having a different tissue distribution.[73,74] MCT1 is ubiquitously expressed but is especially prominent in heart and red muscle where it is regulated in response to increased work, suggesting an important role in lactic acid oxidation.[73,75-78]

In contrast, MCT4 is more evident in white muscle and other cells with a high glycolytic rate such as white blood cells, suggesting an important role in lactic acid efflux.[73,79] The affinity of MCT1 for lactate is greater (K_m = 5 mmol/L) than that of MCT4 (K_m = 20 mmol/L). MCT1 is correlated with the mitochondrial enzymatic activity of citrate synthase. The inward transport of lactate by oxida-

tive skeletal muscle, for consumption as a respiratory fuel, appears to be catalysed by MCT1 whereas the extrusion of this intermediate from glycolytic fibres may be mediated by MCT4. The major role of MCT4 is to defend intracellular pH in glycolytic tissue by extruding lactate and H^+ to the interstitium.[80] MCT2 is less widely expressed and appears to be associated with tissues which demonstrate a high uptake affinity for lactate and pyruvate such as the kidney and liver (for gluconeogenesis) and neurons (for oxidation).[81] Therefore, the existence of numerous MCTs suggests that individual MCT isoforms possess substrate specificities and Michaelis-Menten kinetics (i.e. K_m and Vmax) which serve the metabolic needs of the tissues in which they are expressed.[80] The existence of MCT in the mitochondrial membrane suggests that these mitochondrial MCTs are not only involved in the transport of pyruvate and ketone bodies but also are linked to the transport of lactate.[39,46,82]

Brooks et al.[46] demonstrated that the concentration of cytosolic lactate exceeds the concentration of pyruvate by 10-fold. Therefore, lactate rather than pyruvate is the main substrate involved in the mitochondrial respiration. Furthermore, these carriers play an important role in the regulation of intramuscular pH. They also play an important role in the regulation of the exchange of lactate between the different tissues and cells.

From plasma, lactate may be transported into liver, heart and noncontractive tissues for oxidation, gluconeogenesis, and glyconeogenesis, or it may be transported into red blood cells (RBCs). Poole and Halestrap[72] demonstrated that lactate transport in erythrocytes was mediated by the MCT1 isoform and that lactate and hydrogen ions are released into circulation by a carrier-mediated system that minimises the accumulation of lactate and protons in muscles and prolongs the muscle's capacity for anaerobic work. In horses, it has been speculated that during maximal exercise, when the rate of lactate oxidation is exceeded, RBCs act as a 'sink' for lactate and thus contribute to the plasma-muscle concentration difference that is the driving force for lactate efflux from working muscle.[83-85]

Lactate is transported across the RBC membrane by three distinct pathways: (i) non-ionic diffusion of the undissociated lactic acid; (ii) an organic exchange system (band-3 protein); and (iii) monocarboxylate specific carrier system. The undissociated lactic acid may diffuse across the cell membranes, whereas the lactate anion needs a carrier protein. Two carrier proteins have been identified in the RBC membrane: (i) the MCT1, which co-transports a lactate anion and a proton; and (ii) the inorganic anion-exchange transporter (band-3 protein), which is an antiport transporter and exchanges lactate for Cl^- or HCO_3^-.[72] MCT1 represents the main route for lactate transport in dogs and humans.[86,87]

Recently, Vaïhkönen et al.[88] showed no correlation between MCT1 activity and the athletic ability of certain species (dogs, horses, reindeer). Furthermore, both in humans and horses, training had no effect on lactate RBC transport. They showed that only in horses was the distribution of lactate-transport activity bimodal, and the possible connection between RBC lactate and performance capacity especially in this species warrants further studies.

Skelton et al.[87] reported that in humans, total lactate influx into RBC from the more endurance-trained athletes was significantly faster at 1.6 mmol/L lactate concentration when compared with the influx into RBC in untrained participants. However, there were no significant differences among the trained (sprint and endurance groups) and untrained participants with regards to the total influx of lactate at 4.1, 8.1 and 20 mmol/L. There were more similarities than differences in lactate influx into RBC between the subject groups based on types of training (sprint vs endurance). No studies have considered the training effect on the lactate influx into RBC using a longitudinal approach. Therefore, we shall consider the training effects on the muscle MCTs that have been studied in greater detail.

2. MLSS Concentration (MLSSc) and MLSSw Determination

2.1 The Estimation of MLSS by Incremental Methods

The MLSS concept was previously proposed by Margaria et al.[34,89] in the 1960s using five to eight independent constant load exercise tests to determine MLSS. To spare some time, this long procedure was replaced by the determination of the ventilatory threshold of Wasserman and McIlroy[90] and by the determination of the lactate threshold evaluated by determining blood lactate concentration using graded load protocols or single steps at a constant load of long duration (≥10 minutes) and near

complete recovery between the steps.[26] However, the duration and size of the intensity increments have been found to influence the value of the lactate threshold.[91]

Baldarini and Guidetti[12] have suggested that MLSS could probably also be determined by the so-called 'individual lactate threshold (IAT)' determined according the protocol of Stegmann et al.[92] providing that the IAT was determined by attributing blood lactate value to the antecedent stage during the incremental test rather than to the same stage of its measurement. The individual anaerobic threshold is defined as the metabolic rate where the elimination of blood lactate during exercise is both maximal and equal to the rate of disappearance of lactate into blood.[92] The calculation of IAT involves the measurement of blood lactate during a stepwise-increasing exercise followed by a passive recovery when the blood lactate level equals the value measured at the end of highest exercise intensity is assumed to represent a time when the elimination of lactate is maximal and equal to the rate of disappearance. A line drawn from this time during the recovery period that intercepts the exercise blood lactate curve will produce a time coordinate and, therefore, a power output that defines the IAT. Schnäbel et al.[93] have reported that after the initial adjustment arterial lactate stabilised at different levels between 2.7 and 6.0 mmol/L in a treadmill run of 50 minutes at the IAT (75 ± 2% of $\dot{V}O_{2max}$). However, they did not attest the fact that IAT was the 'maximal' lactate steady state. To accurately determine and not overestimate, the determination of MLSS needs long (20- to 30-minute) stages interspersed by 40 minutes to several hours of rest.[9]

2.2 The Estimation of the Maximal Lactate Steady Long Stages

The work load of stages is increased until blood lactate concentration increases continuously during the constant load. Measurements of MLSS demand several subsequent independent constant load tests that have to be performed at different work loads on different days. By definition, MLSS is attained when the blood lactate concentration varies by less than 1 mmol/L during the final 20 minutes of constant intensity.[15] To achieve a true MLSS, it has been found that it was necessary to have four or five prolonged exercise sessions of up to 30 minutes' duration performed at exercise intensities between 50% and 90% of $\dot{V}O_2$.[10,94-96] The accumulation of lactate is only evident after 10 minutes if the running speed is only slightly greater than at the velocity associated with maximal lactate steady state (vMLSS).[6,97,98] The determination of MLSS can be standardised by using 3 grades of 30 minutes' duration each separated by 1 hour of rest, according to the algorithm of McLellan and Jacobs.[99]

To avoid such a long procedure, Billat et al.[8] and later, Bacon and Kern[100] have validated a protocol allowing an immediate estimation of the exercise intensity corresponding to MLSS using only two 20-minute sub-maximal intensities in long-distance athletes.[8,9] These two tests were performed between 60 and 90% of the power at $\dot{V}O_{2max}$ and were separated by 40 minutes of complete rest. The work rates of the two stages were set one stage below and one above the onset of blood lactate accumulation determined in a previous incremental test which also determined $\dot{V}O_{2max}$. This approach has been validated by checking MLSS during an exercise lasting 1 hour.[8] Stages of 16- and 15-minutes' duration were used to determine MLSS in children.[101,102] If the test to determine MLSS is carried out in field conditions, the choice of speeds must take into account the non-linear relationship between the $\dot{V}O_2$ and the movement speed due to the important aerodynamic component affecting energy cost (swimming, cycling, speed skating, rowing).[103]

2.3 MLSSc

The determination of MLSSw is associated with those of the blood lactate concentration that shows great inter-individual difference. The average blood lactate concentration onset of blood lactate accumulation measured in a given population is 4 mmol/L.[15] However, Urhausen[96] determined the 4 mmol/L lactate threshold, during incremental tests and the lactate accumulation at different fixed work loads which their subjects attempted to maintain for 30–45 minutes. They found that 87% of the subjects (n = 30) could complete the endurance test at the work load corresponding to the lactate threshold. Interestingly, the average blood lactate accumulation during the 45-minute endurance test at the lactate threshold was not much higher than 4 mmol/L (4.2 mmol/L). However, 43% (6 of 14) of the subjects could complete the 45-minute endurance treadmill test whilst accumulating a stable blood lactate concentration (mean of approximately 5 mmol/L) at

a work load higher than the pre-determined lactate threshold intensity (105% of the lactate threshold and equivalent to the 4 mmol/L threshold). This has also been confirmed by other studies reporting an average MLSSc close to 4 mmol/L but with a wide inter-individual variation.[9,104] The blood lactate remained constant during exercise and ranged from 2.2–6.7 mmol/L (mean value of 3.9 ± 1 mmol/L) in the study of Billat et al.,[8] and 1.9–7.5 mmol/L in the study of Beneke et al.[6] both in capillary blood samples.

Recently, Myburgh et al.,[105] using a time trial format exercise test, reported a plasma MLSSc of 7.6 ± 2.1 mmol/L (with a range from 5.0–12.3 mmol/L) and Harnish et al.[106] obtained a MLSSc of 6.7 ± 0.7 (with a range from 3.2–10.5 mmol/L). This plasma lactate concentration is equivalent to the blood lactate concentration previously reported in capillary blood[107-109] since the RBC : plasma ratio is 0.58 and remains constant during constant-load exercise performed below or above the lactate threshold.[108] Beneke et al.[110] reported a maximal stable lactate equal to 4.2 ± 0.7 mmol/L with extreme values of 2.8–5.5 mmol/L, without this value varying with age in adolescents (from 10–20 years). Mocellin et al.[101] reported a MLSSc of 4.6 ± 1.3 mmol/L in boys of 11 years of age and Billat et al.[102] a MLSSc of 3.9 ± 1.1 mmol/L in 12-year-old boys and girls. To our knowledge, no studies have reported data on MLSS in elderly persons.

All these studies determined MLSSc using constant work rates far from race conditions where the aim is to produce the fastest time to perform a set distance. When racing, the work rate is variable, and this can affect the physiological response compared with exercise performed at the same average power output but in a constant way.[111,112] Zamparo et al.[113] reported that self-selected running speed during 1 hour (13.6 ± 1.4km) in 40-year-old recreational distance runners was not significantly different compared with the speed at 4 mmol/L (determined in an incremental test). However, the self-selected speed and the average heart rate during the race showed significantly lower variance compared with speed and heart rate at 4 mmol/L. This suggests that, as well as the need for avoiding lactate accumulation in blood, other factors must be involved in the choice of speed in running.[113] Using time-trial exercises, Myburgh et al.[105] clearly demonstrated that a blood lactate concentration of around 4 mmol/L does not correspond to the maximal sustained blood lactate during free-pace exercise for a large number of athletes. For this, performance tests (i.e. time trial tests) were used to determine MLSSc to simulate racing conditions.

2.4 The Time to Exhaustion at MLSSw

Many studies have focused on the impact of glycogen depletion on the anaerobic and ventilatory threshold determination[114] and performance (endurance time at the lactate threshold);[115] however, no studies have attempted to measure the endurance time at MLSS. Therefore, the intensity involving MLSS has often been overestimated, especially in highly trained endurance athletes using the lactate threshold as part of their training.[92,116,117]

Mognoni et al.[116] examined physiological responses during prolonged exercise at a power output corresponding to the capillary blood lactate concentration of 4 mmol/L. Out of 34 moderately fit men, 20 were exhausted at a mean time of 38.2 minutes instead of the predicted 60 minutes with a final blood lactate concentration equal to 5.3 ± 2.3 mmol/L; however, this was probably above MLSS for some of these participants.

We have seen from Schnäbel et al.[93] that it was possible for 12 male physical education students to sustain their IAT during 50 minutes with a blood lactate steady state between 2.7 and 6 mmol/L according to the individual differences. The following question is to be sure that the blood lactate level at the steady state is really the 'maximal' lactate steady state. Endurance time at MLSS has not yet been reported and is speculated to be an average of about 1 hour.[9] Unpublished data recently collected by our team confirmed this estimation in non-elite middle-aged (41 ± 5 years) long-distance runners. Time to exhaustion at MLSS was increased after 6 weeks of training (two sessions of 30–50 minutes at MLSS per week) from 40 ± 10 to 62 ± 16 minutes (+50%) while MLSSw was only increased by 3.4%. MLSS is below the world hour record and above the marathon pace especially in non-elite runners as the use of a carbohydrate energy source (glycogen, glucose, lactate) is the most important substrate utilised at this speed (the respiratory exchange ratio = 1).[6]

As described in section 2.3, Myburgh et al.[105] reported that the plasma lactate concentration for self-selected maximal effort lasting 1 hour (speed between 37–44 km/h at the average speed of 40.8 ± 2.2 km/h) was 7.6 ± 2.1 mmol/L (from 5–10 mmol/

L). This may suggest that MLSS was not far from this duration despite the fact that a constant power output is hardier to sustain than a variable pace for the same work rate performed.[111] At a given work load, high endurance performance reduces the glycolytic rate, increases the rate of oxidation and leads to a lower blood lactate concentration. However, MLSS does not indicate a given work load but rather an exercise-intensity above which metabolism changes qualitatively.[6] According to this theory, the duration of aerobic exercise up to MLSS intensity is limited by the stored energy.

2.5 The Substrates Used at MLSSw

vMLSS corresponds to a speed at which the rate of the expiratory ratio equals 1.00 ± 0.05.[6] The corresponding glycogen cost for exercise lasting 45 minutes at a MLSS work load of 3.4 W/kg and a working efficiency of 20% depletes muscle and liver glycogen by approximately 50%.[6] Therefore, the glycogen reserves could be one factor in exhaustion at MLSS since glycogen depletion is heterogeneous between muscle fibres and glycogen depleted muscle fibres do exhibit more pronounced energy deficiency evidenced by higher post-exercise inosine monophosphate.[118] Even at MLSS, speed elicits only 20% of the maximal voluntary contraction; however, glycogen is initially used as a net fuel providing the rapid release of energy needed to support muscle contractions that last around 20–100 m/sec.[119] Glycogen phosphorylase, the so-called 'fight-or-flight' enzyme which controls the rate of glycogenolysis, is well suited for this purpose because its rapid activation is triggered by calcium and a phosphorylation cascade. The subsequent conversion of glucose-6-phosphate to lactate produces the adenosine triphosphate needed to fuel the contraction. Between muscular contractions, any net phosphocreatine that is broken down for short-term requirements is resynthesised by oxidation of the generated lactate.[58] Therefore, glycogen is needed to provide the rapid burst of glycogenolysis required for contraction. At MLSS, rather than the increase in H^+, fatigue would be the result of an inability to provide the very high initial power requirements of the contraction through glycogenolysis.[119]

MLSSw can be sustained for about only 60 minutes and hence is faster than the marathon speed. Good marathon runners rely predominantly (80%) on body carbohydrate stores during marathon running because during the race the respiratory exchange ratio almost equals 1 (0.99 ± 0.01 in 2-hour 45-minute marathon runners versus 0.90 ± 0.01 in 3-hour 45-minute marathon runners.[120] Respiratory exchange ratio values observed when running at marathon pace range from 0.85 to close to 1.0.[121] This was also confirmed in elite marathon runners (<2 hours 11 minutes for males and <2 hours 31 minutes for females).[3]

The pattern of substrate utilisation in an individual at any point in time depends on the crossover between the exercise intensity-induced responses (which increase carbohydrate utilisation) and the endurance training-induced responses (which promote lipid oxidation).[122] The crossover point is identified as the power output at which energy (kJ) derived from oxidation of carbohydrate-based fuels predominates over that derived from lipids.[122] Brooks and Mercier[122] refer to carbohydrates as endogenous energy sources that include muscle and liver glycogen, blood glucose, blood, muscle and liver lactate. By 'lipids' they refer to adipose and intramuscular triglycerides as well as blood-borne free fatty acids and triglycerides. They showed that endurance training results in muscular biochemical adaptations which enhances lipid oxidation and decreases the sympathetic nervous system activity in response to a given absolute exercise intensity. However, it remains to be determined which, if any, blood lactate concentration or change in blood lactate concentration will correlate with the crossover point.[121] Further studies are needed to compare MLSS work rate to the crossover point. O'Brien et al.[120] have shown that the marathon substrate was carbohydrate rather than lipids. It may be suggested that the average marathon speed could be equal to MLSS because of runners varying their pace alternating recovery at a speed that is just below the lactate threshold (at which the rate of lactate decline is great[123]) with more intensive periods.

3. Interest of the MLSS for Performance

3.1 Comparison of MLSSw with the Critical Power Determined by the Two- and Three-Component Models

In locomotion sports, performance is defined as the time taken to cover a given distance. The relationship between distance (metres) and time (seconds) is a regression line.[124] Therefore, the

slope of this regression is a speed (m/sec). This speed is called the 'critical speed' (or critical power when the distance is replaced by work in Joules).[125,126]

Time to exhaustion (time limit) has been modelled using the 2-parameters critical power model.[127,128] Critical power (watts) represents the boundary between the power outputs which theoretically could be sustained indefinitely and those for which exhaustion occurs in a finite time. Although the critical power test has been regarded as providing a reliable measure of the maximal fatigueless rate of work[129] it has also come under criticism. In fact, critical power can only be sustained for less than or around 30 minutes.[130-133] Consequently, Morton proposed a 3-parameters critical power model.[134] For this model he proposed to extend the equation of the 2-parameters model allowing a non-zero asymptote. The critical speed, determined from the 3-parameters critical power model, better reflects the capacity for prolonged exercise than the critical speed calculated with the 2- parameters model.[135] MLSS speed may probably be closer to the critical power determined with the 3- rather than the 2-parameters critical power model. MLSSw is correlated and close to the critical speeds[136] calculated from Monod and Scherrer's model,[127] this being recently confirmed by Smith and Jones.[98]

3.2 At MLSSw the Energy Cost of Locomotion Still Remains Constant with Time

The MLSS work rate is significantly lower than the critical power[137] and a $\dot{V}O_2$ steady state is still observed. Consequently, at power output less than MLSSw there is a steady state of the energy cost of locomotion that is not the case for supra MLSS work rate if we consider the last development of the oxygen kinetics measurement for cycling on an ergometer and running.[138-142] Between MLSS and critical power, a slow component of the $\dot{V}O_2$ kinetics causes $\dot{V}O_2$ to rise above the value expected from extrapolation of the sub/MLSSw power output relationship.[143,144] Therefore, MLSSw is the highest work rate at which it is still possible to measure the energy cost of running from the $\dot{V}O_2$ measurement. The consequence of this slow additional $\dot{V}O_2$ increase is that it is not possible to associate $\dot{V}O_2$ with power output at this exercise intensity.

3.3 MLSSw is Related to Performance

Although the competition is not run at a constant speed, the speed which is compatible with the lactate steady state is a key factor along with the speed associated with the $\dot{V}O_{2max}$ in the training of endurance athletes. As an absolute value, the maximal steady state speed not only allows the prediction of the speed records for 30–60 minutes of running exercise but also for all endurance-type sports based on human locomotion (e.g. triathlon, rowing, cycling).[4,6,9] However, the performance is dependant on power (speed) as it is measured by time over distance (time = speed × distance). It is therefore logical that a power or speed associated with a lactataemia value is correlated with performance and this being the case whatever the lactataemia value.[145] In contrast with the absolute value of MLSSw, when MLSSw is expressed as a fraction of $v\dot{V}O_{2max}$ it is not systematically related to the performance. On the other hand, the fraction used of the speed associated with the $\dot{V}O_{2max}$ at the lactic threshold or vMLSS is not always correlated with the performance. The very top-level marathon runners (<2 hours 11 minutes) differentiate from those who may be classed as high-level (2 hours 11 minutes 01 seconds to 2 hours 16 minutes) only by their $\dot{V}O_{2max}$.[3]

If MLSS allows the prediction of the performance for races lasting between 30 and 60 minutes, in turn, some authors have proposed to estimate the vMLSS in trained cyclists from the time trial to cover 5 and 40km. Harnish et al.[106] reported that MLSS speed (36.8 ± 1 km/h) in trained cyclists, corresponded to 92.1 ± 1.2% of 5km average speed and was not significantly different from the 40km average speed (36.8 ± 0.9 km/h).

3.4 MLSSc is not Related to Performance but is Affected by Training

If there is a clear direct relationship between MLSSw and performance this is not the case for MLSS blood lactate concentration. Beneke et al.[6] reported that MLSSc (4.9 ± 1.4 mmol/L) was independent of MLSSw (3.4 ± 0.6 W/kg) and peak work load (4.8 ± 0.6 W/kg). Myburgh et al.[105] demonstrated that there was no relationship between plasma lactate concentrations for self-selected maximal effort lasting 1 hour in cycling (40.8 ± 2.2km at an average of 83 ± 4% of the predicted maximum heart

rate) and performance in well-trained endurance cyclists or triathletes.

We have seen that MLSS varies between individuals. Although this maximum level of stabilisation is not correlated with performance, it depends on the muscular mass involved. In effect, Beneke[4] reported in a cross-sectional study on elite athletes that MLSS was significantly lower in rowers (3.1 ± 0.5 mmol/L), than in cyclists (5.4 ± 1.0 mmol/L) or in speed skaters (6.6 ± 0.9 mmol/L) [not significant between skaters and cyclists]. In 2001, Beneke et al.[146] showed that the MLLSc but not MLSSw depends on the motor pattern of exercise (rowers vs cyclists). MLSSc seems to decrease with the mass of the primarily engaged muscle since MLSSc was 2.7 ± 0.6 mmol/L in rowers versus 4.5 ± 1.0 mmol/L in cyclists. This indicates that task-specific levels of MLSSc occur at distinct levels of power output per unit of primarily engaged muscle mass.[146]

Rowing is a combination of leg, trunk and arm work which represents more than 85% of the total muscle mass. This is considerably more than the corresponding muscle mass in cycling, which is dominated by leg work. The fact that a different level of MLSSc appeared at a different level of $\dot{V}O_2$, relative exercise intensity and heart rate indicates that in rowers, the power output per unit of muscle mass is less than in cyclists (9.6 vs 12.2 W/kg). However, it must be noted that lactate levels at the end of the marathon (with no acceleration at the end) amount to 6–7 mmol/L in runners who complete marathons in less than 2 hours 30 minutes (Billat et al., during the 2000 marathon of Paris, unpublished data). Marathon runners demonstrate some variations in speed (±1 km/h, i.e. 10 sec/km between 19 and 20 km/h). However, the comparison of the final blood lactate concentrations at constant or variable marathon pace has never been studied.

The reasons for such a wide inter-individual variation in MLSSc remain unknown. Even in selected animals such as laboratory rats MLSSc has been reported to have a great inter-individual variability.[147] Gobatto et al.[147] recently reported a MLSSc equal to 5.5 mmol/L in rats at a MLSSw measured in swimming with overloads of 5% of bodyweight. This value was not affected by training despite a shift of MLSSw to a higher intensity (percentage bodyweight overload). However, amazingly this study did not mention the standard deviation for this MLSSc. In humans, the coefficient of variation for MLSSc equal 25% both in adults and children.[8,102] To our knowledge, no studies have yet been published on the affect of training on MLSSc. It seems that because of this great inter-individual variation the best approach to a better understanding of the physiological mechanisms of MLSSc could be to focus on the different types of training effects.

For 10 years, the knowledge of shuttle and lactate transporters has provided an understanding of the background of MLSS. However, Lokkegaard et al.[148] have reported that MLSSc is not correlated with the number of lactate transporters in already well-trained subjects ($\dot{V}O_{2max}$ = 61 ml/kg/min).[148] To date, no studies have focused on the effects of training on MLSSc. Therefore, we do not know whether training at MLSS increases or decreases MLSSc and whether there is a link with any modification in the lactate transporters or exchange.

A very interesting longitudinal study performed by Bergman et al.[41] showed that arterial blood lactate concentration and blood lactate kinetics in resting and exercising men has been compared before and after 10 weeks of endurance training. Subjects studied at 45% and 65% of $\dot{V}O_{2max}$ absolute before training and after training at the same absolute power output that elicited 65% of $\dot{V}O_{2max}$ before training (54% $\dot{V}O_{2max}$) and 65% of the post-training $\dot{V}O_{2max}$. This experimental design allowed examination of the influence of training on both the same absolute work load known to be linearly related with lactate turnover and on the relative work load which will influence the balance between lactate appearance and disappearance. This study showed that training reduces blood lactate concentration at given relative and absolute power outputs and that lactate appearance was not affected by training at the same metabolic rate ($\dot{V}O_2$); however, the MCR (the effective volume from which the lactate is cleared per unit time) was increased after training and did not level off after 3 mmol/L of blood lactate concentration. Since Bergman et al.[41] did not actually measure MLSS, we have at best, estimated MLSSc and MLSSw and their changes. MLSSc was higher before training (6 mmol/L) since blood lactate level decreased to 5.5 mmol/L after 15 minutes of exercise (and 4.2 mmol/L at 60 minutes) versus 3.5 mmol/L after training. This means that while MLSSw was increased by 17% after training, MLSSc was decreased by 36%. We are not aware of any other studies having focused on the modification of MLSSc with training.

A pilot study conducted in our laboratory has shown that 6 weeks of training at MLSS did not significantly increase MLSSc in middle-aged non-elite long-distance runners (3.7 ± 0.8 vs 4.3 ± 1.4 mmol/L, p = 0.09, n = 9) [figure 1, figure 2, figure 3].

4. The Effect of Training on MLSS and Muscle Lactate Transporters

As seen by Bergman et al.[41], inactive muscle and other tissues must release lactate during exercise at intensities below the lactate threshold to explain the maintenance of elevated arterial lactate concentration. This may be important in understanding the sensitivity of MLSSc for training as Sumida and Donovan[43] have demonstrated that lactate disappearance is not enhanced by training in quiescent skeletal muscle. It seems that fitness training plays an important role in the regulation of lactate metabolism. The intermediary of modifications in the MCTs may carry out this regulation. Until now, only 20 studies (mostly on rats) have examined the effects of strenuous exercise, short-term (7 days), medium-term (3 weeks for rats and 8 or 9 weeks on humans) and long-term (5 months) training on MCTs associated with lactate transport. These studies also looked at enzyme modifications in the aerobic and anaerobic metabolism as well as those involved in the transport of glucose by the GLUT 4 (glucose transporting proteins).

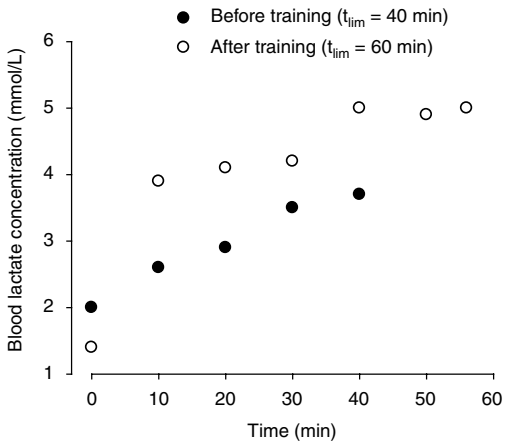

Fig. 1. The effect of 6 weeks of training (40 minutes twice a week) and blood lactate concentration in a 40-year-old sub-elite long distance runner during a time limit run at vMLSS (vMLSS increased by 1 km/h). $\mathbf{t}_{lim}$ = endurance time at vMLSS; **vMLSS** = the velocity associated with maximal lactate steady state.

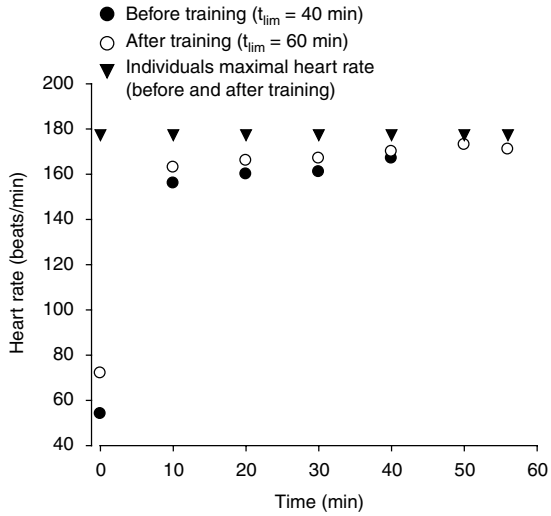

Fig. 2. The effect of 6 weeks of training (40 minutes twice a week) and heart rate in a 40-year-old sub-elite long distance runner during a time limit run at vMLSS. $\mathbf{t}_{lim}$ = endurance time at vMLSS; **vMLSS** = the velocity associated with maximal lactate steady state.

In a pioneer study on rats, Donovan and Pagliassotti[149] demonstrated that 8 weeks of endurance training, (1 hour per day at around 75% $\dot{V}O_{2max}$, 38 metres per minute with a slope of 10%) did not alter the resting lactate production but the rate of lactate clearance was two times greater in trained animals compared with non-trained. Furthermore, the trained rats showed an 25% increased gluconeogenesis. Therefore, for a given production of lactate, trained animals maintained a lower level of lactate through the increased elimination by gluconeogenesis and the oxidation of lactate.

A study on myocardium[147] showed that the trained subjects (rats) had a greater rate of lactate uptake by the heart compared with non-trained subjects. Training reduced this concentration to a given level of $\dot{V}O_2$ (absolute and relative) and it is possible that the lactate entering into the myocardium is reduced after training for a same absolute and relative $\dot{V}O_2$.

4.1 Monocarboxylate Transporter (MCT) Concentration Increases with Endurance Training: Animal Studies

Pilegaard et al.[150] showed in rats that 7 weeks of endurance training at 50% $\dot{V}O_{2max}$ (swimming) did not bring about any modifications in the transport of lactate. However, this is probably due to the rat

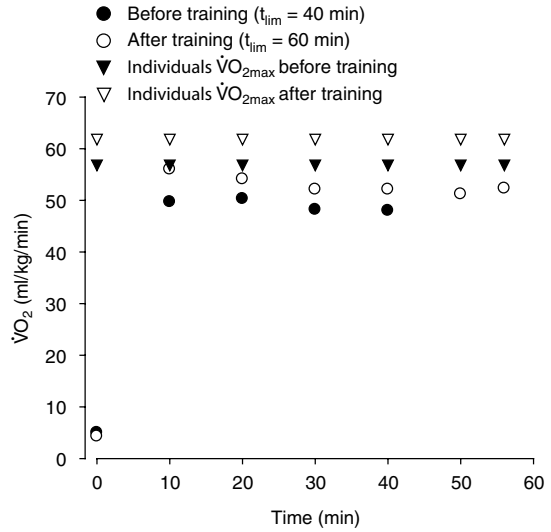

Fig. 3. The effect of 6 weeks of training (40 minutes twice a week) of oxygen uptake ($\dot{V}O_2$) in a 40-year-old sub-elite long distance runner during a time limit run at vMLSS (vMLSS increased by 1 km/h). $\mathbf{t}_{lim}$ = endurance time at vMLSS; **vMLSS** = the velocity associated with maximal lactate steady state; $\mathbf{\dot{V}O}_{2max}$ = maximal oxygen uptake.

preferring to float rather than swim and therefore not working at 50% of its $\dot{V}O_{2max}$. On the other hand, two types of interval training below 6 × 3 minutes at 90% of $v\dot{V}O_{2max}$) and above (6 × 1 minute 30 seconds at 112% of $\dot{V}O_{2max}$) the speed associated with $\dot{V}O_{2max}$ increased the number of MCT and their affinity for lactate (the K_m decreased after the two types of interval training). It is interesting to note that five non-training weeks are sufficient in dropping to pre-training levels. However, the time scale is not the same for a rat as its life span is inferior to humans. In a study in skeletal muscle in rats, Baker et al.[151] showed that the MCT1 and lactate uptake were also affected by treadmill training. However, the increases in MCT1 occurred independently of any changes in the heart's oxidative capacity as measured by citrate synthase activity. Training-induced increases in MCT1 occurred at a lower training-intensity in the heart than in skeletal muscle. Both heart and skeletal muscle lactate uptake were increased only when MCT1 was augmented; however, in the heart, lactate uptake was increased much more after high-intensity training compared with after moderate-intensity training, despite similar increases in heart MCT1 with these two training intensities. Eydoux et al.[152] were the first to show that rodent lactate transport protein saturation properties were modified after an acute bout of exhaustive exercise irrespective of the training state.

4.2 MCT Concentration Increase with Short- and Long-Term Endurance Training: Human Studies

It was supposed that endurance training could affect the concentration of MCT since in a transversal study, Pileggard et al.[153] comparing non-trained, trained and highly-trained subjects demonstrated that it is necessary to follow a frequent and intense training regime to obtain an increased lactate transport. Five years later, Pileggard et al.[154] showed that a local intense training of the quadriceps increased the transport capacity of both lactate and the H^+ proton (+12%) as well as the concentration of MCT1 (+76%) and MCT4 (+32%) in human skeletal muscle. Although the lactate gradient is weaker after training, the transport of lactate is greater. This signifies that training reduces the concentration gradient necessary to obtain the same transmembrane flow of H^+ in the trained muscle probably through an increased capacity of the exchange system of Na^+/H^+.[155]

In 2002, Green et al.[156] demonstrated that a single exercise session in humans performed at 60% of $\dot{V}O_{2max}$ for 6 hours resulted in elevations in MCT1 and MCT4 throughout the 6 days after exercise. They proposed that increases in MCT and plasma volume were at least partly involved in the lower muscle lactate content observed after the training session by increasing lactate membrane transport and removal respectively.

The short-term training model (7 days) allows the differentiation of the effects of the changes in fibre types from the modifications in the transporters. Furthermore, a week of training corresponds to the duration of a training microcycle commonly used by athletes. Phillips et al.[157] demonstrated that after 2 hours of daily cycling training over 7 days at 59% $\dot{V}O_{2max}$, the rate of appearance of lactate was unchanged despite a reduction in the concentration of lactate. Bonen et al.,[158] reported that 7 days training on a cycle ergometer (2 hours per day at 65% $\dot{V}O_{2max}$), increased the MCT1 content in the vastus lateralis muscle by 18%. Furthermore, the muscle and vein lactate concentrations were decreased at a given exercise intensity. However this adaptation was not related to the increase in MCT1.

Endurance training (9 weeks at 75% of $\dot{V}O_{2max}$) increases the expression of MCT1 with intervariable effects on MCT4.[82] This training led to a huge increase in $\dot{V}O_2$ peak (+15%). Dubouchaud et al.[82] had previously reported that the power output corresponding to the lactate threshold increased by 22%.[41] The same training brought about an increase in glyconeogenesis by 200% at rest and 300% at the same absolute and relative power output.[159] After training, the content of MCT1 increased significantly in all of the compartments, whereas the total muscle preparation content MCT4 was not significantly modified. The mitochondrial MCT1 pool increased by 78% after training, independently of the increase in the total mitochondrial density. These authors found an inverse relationship between mitochondrial MCT1 content and the net leg lactate release. These authors concluded that this inverse relationship could be interpreted as a proof that the mitochondrial MCT1 could participate in increased lactate oxidation after training.

In a recent study, Evertsen et al.[160] reported that already highly-trained cross-country skiers following 5 months of either 'moderate' (60–70% of $\dot{V}O_{2max}$) or 'high intensity' training (80–90% of $\dot{V}O_{2max}$ for 16% of the training, i.e. 1.6 hours over 10 hours per week) decreased or maintained the number of their amount of MCT1, respectively. The concentration of MCT4 did not change during the training period; however, as noted by the authors themselves, the 'pre-training' values of MCTs were in fact measured just after the competitive period and were already high due to the fact that the first biopsies were taken immediately after an intensive racing season. Intensive training carried out during the racing season might explain that the concentration of MCT1 decreased after moderate intensity training, probably because less high intensity training was carried out during the training period compared with the racing season.[160] Moreover, these authors noticed a lack of correlation between the MCTs and the other enzymes among elite skiers, probably because they represented a relatively homogeneous group, with quite similar muscle patterns. Interestingly, they demonstrated that these data were not affected by sex. This is the only study having focused on the relationship between MCTs and enzymes in elite athletes and despite the bias of the so called 'pre-training' biopsies, this opens the research field to the effects of intensive training on lactate transport in elite athletes. No studies have focused on the effects of training at MLSS on MCTs and it is important to note that probably more than 16% of training volume at least at MLSS is needed to obtain some modification of MCTs in already well-trained subjects.

Evertsen et al.[160] reported an enhancement of $3.2 \pm 0.9\%$ of the speed at the lactate threshold for the group who trained at the highest intensity. However, it is not specified whether this enhancement is due to the decreased energy cost of running or rather to an enhanced $\dot{V}O_2$ expressed as a fraction of $\dot{V}O_{2max}$ (to eliminate the running economy and $\dot{V}O_{2max}$ factors).

5. Conclusions

Lactate production, distribution and removal play equally important roles in blood lactate concentration.[16] This lactataemia at MLSS represents the highest point of equilibrium between the production and removal of lactate. If the lactate level is constant, then lactate appearance and disappearance are equal. However, there is a particular work rate at which the arterial lactate concentration is constant with time at its maximal level, which is termed 'MLSS'. The determination of MLSSw is correlated with performance (i.e. time sustained at a given speed), which is not the case for blood lactate concentration at MLSS that shows great inter-individual difference. Above MLSS intensity, contributing to the energetic needs of exercise, pyruvate production exceeds lactate clearance, muscular creatine-phosphate concentration, muscle and blood pH decrease, which causes the termination of exercise. However, no studies have focused on the reasons for discontinuing exercise at MLSS.

The comparison of MLSS before and after training determined by constant work rate or metabolic output ($\dot{V}O_2$) could be a way to appreciate the influence of additional type II fibres recruited in higher intensity stages (when work rate is constant) on MLSSw determination.[111] The transport of lactate was always considered passive and responsible for 80% of the transmembrane lactate transport (10% of the transport being assured by the sodium pump and 10% by the chloride/bicarbonate exchange). Since 1994, we know that lactate, like pyruvate is not only transported across the sarcoplasm but also across the mitochondrial matrix by the so-called 'MCT' proteins. Lokkegaard et al.[148] have reported that there was no correlation between

MCT and MLSS concentration. Moreover, we are also unaware if the level of stabilisation of the lactate concentration can influence the time sustained at MLSS.

The relationship between the enhancement of lactate transporters and MLSSw has not yet been demonstrated. Similarly, the effects of training on the value of blood lactate concentration at MLSS has not yet been clearly established since only two studies have reported contradictory data in swimming rats[147] and in humans cycling on an ergometer.[41] The adaptations brought by training at a speed associated with the lactate steady state for which the rate of lactate released into the blood is similar to the rate of lactate recovered by the tissues (liver, heart, oxidative skeletal muscle) are not yet known. Evertsen et al.[160] reported that the blood lactate concentration after exhaustive 20-minute treadmill running was correlated with the concentration of muscle MCT1 in each subject. The factors behind such a wide inter-individual difference in MLSSc remain unknown and have not yet been debated especially with different types of training performed below, at or above MLSS speed. In conclusion, the problem of MLSS is typical of the need to conciliate a systemic and molecular approach for solving the mechanisms of performance improvement by training.

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