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Chapter 16

Are Arterial, Muscle and Working Limb Lactate Exchange Data Obtained on Men at Altitude Consistent with the Hypothesis of an Intracellular Lactate Shuttle?

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Abstract: The "Lactate Shuttle" hypothesis posits that lactate removal requires exchange among producing and consuming cells. The "Intra-cellular Lactate Shuttle" hypothesis posits that lactate exchange occurs among compartments within cells, and that mitochondria are the major sites of cellular lactate disposal. Thus, cells with high mitochondrial densities (hepatic, myocytes, hepatocytes) are those which participate in lactate clearance. The model of an Intracellular Lactate Shuttle recognizes that the $K_m$ for LDH is $3.6 \times 10^{-3} M$; thus, glycolysis results in cytosolic lactate production regardless of the intracellular PO$_2$. The model also requires presence of a mitochondrial monocarboxylate transporter (MCT) that allows uptake of lactate as well as pyruvate, and intra-mitochondrial LDH whose function is linked to the ETC, and which permits lactate-pyruvate conversion and oxidation. Recently, we have shown that liver, heart and muscle mitochondria readily oxidize lactate and contain LDH and MCT1. Accordingly, we have concluded that lactate is the predominant monocarboxylate oxidized by mitochondria in vivo. The model of an "Intra-cellular Lactate Shuttle" is consistent with many of the observations on men at sea level and altitude. The observations include: oxidation is the primary fate of lactate disposal during rest and exercise; lactate production and oxidation occur simultaneously within resting and working muscle; increasing lactate, increases muscle lactate extraction, and that by increasing $O_2$ acclimatization reduces blood (lactate).
1. **INTRODUCTION**

Since the initial observations of Edwards (18), blood lactate responses to exercise at altitude have been observed to demonstrate several unexplained features. Not only has the term “lactate paradox” been used, but investigators have used different definitions for the “paradox” (24, 27, 34). However, since the initial observations and subsequent articulation of terms, our understanding of carbohydrate metabolism at altitude has grown immensely (5-9, 12, 27, 36, 37). Rather than dwell on any specific term whose utility may have passed, in this report the effort will be to use new knowledge in an attempt to explain blood metabolite responses to altitude.

Commemcing with brief reviews and presentation of data necessary to illustrate presence of the “Cell-Cell” and “Intracellular Lactate Shuttles,” an attempt will be made to evaluate whether these concepts are of use in understanding apparently disparate data observed on men at altitude.

2. **PHENOMENA TO EXPLAIN:**

- Why is arterial [lactate] elevated during exercise at a given power output at altitude compared to sea level?
- Is the elevation in arterial [lactate] at altitude attributable to increased production and appearance in the blood (Ra), or are lactate disposal (Rd) and clearance rates (MCR) inhibited at altitude?
- How can working human muscle display a “Stainsby Effect” at altitude wherein the start of contractions results in muscle net lactate release. Thereafter, net lactate release follows the fall of lactate [v-a] with net release declining to zero even as arterial [lactate] is elevated and constant?
- If there is an “Stainsby Effect” at altitude, what explains the elevation in arterial [lactate]?
- How can working muscle simultaneously release, consume and oxidize lactate at altitude?

3. **WHAT IS THE CELL-CELL LACTATE SHUTTLE?**

The original “Cell-Cell” lactate shuttle postulated that shunting of lactate through the interstitium and vasculature provides a significant carbon source for oxidation and gluconeogenesis during rest and exercise (4, 5). In Figure 1, Type IIB (fast-glycolytic) fibers are indicated to be lactate producing...

Intracellular Lactate Shuttle

Figure 1. Model of the "Cell-Cell" Lactate Shuttle. Lactate is formed in fast-twitch white type (Ib) skeletal muscle fibers, or transiently in populations of red fibers at the start of exercise. Lactate released into the interstitium from cellular sites of production can be taken up and oxidized by adjacent lactate consuming (type I) muscle fibers. Alternatively, during exercise some lactate released into the circulation reenters 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 be taken up by the heart and oxidized, or taken up by the liver and kidneys where lactate serves as a gluconeogenic precursor. Redrawn from (4).

cells, whereas Type I fibers are indicated as sites of lactate oxidation. This hypothesis was developed at a time when fiber heterogeneity in skeletal muscle was recognized (1, 3). Further, the hypothesis was based on results of rats and dogs made to exercise and infused with radioactive glucose and lactate tracers (10, 11, 16, 17). On those species it was shown that during exercise lactate turnover and oxidation exceed glucose turnover and oxidation. Subsequently, the same phenomena of lactate flux and oxidation were demonstrated in humans (30, 43). Data in Figure 2 shows the direct relationships between lactate turnover and metabolic rate and between lactate oxidation and metabolic rate (30). Lactate turnover is high at rest and increases as a direct function of exercise intensity and metabolic power output. Further, lactate disposal through oxidation (approximately 50% at rest), increases both absolutely and relatively during exercise such that oxidation accounts for 75% of lactate disposal during exercise.

While systemic lactate flux and whole-body oxidation data supported the concept of a lactate shuttle, the technology could not be used to demonstrate tissue lactate exchange. Therefore, studies were conducted to determine the lactate concentration differences across active (leg) and inactive (arm)
10. CONCLUSION

The Cell-Cell and Intramuscular Lactate Shuttle Concepts appear be of assistance in understanding several phenomena observed in men exercising at altitude. Further work is needed to evaluate the effects of hypoxia on expression of mitochondrial and extra-mitochondrial lactate transporters and lactate dehydrogenase isoforms.

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REFERENCES

extraction and oxidation by working muscle beds. The Lactate Shuttle Concepts accommodate all of these phenomena.

Arterial [lactate] is elevated at altitude because lactate Ra (production) is increased. However, it is clear working muscle is not the sole contributor to the circulating lactate load. Non-working muscle, skin, adipose and liver are all candidates as contributors to the circulating lactate load during exercise at altitude. Lactate production in non-contracting tissues appears to be under β-adrenergic control.

Lactate production is increased at altitude because glycolysis is stimulated. This stimulation occurs transiently in working muscle, but persists elsewhere in the body.

Working human muscle displays a "Stainsby Effect" during exercise at altitude. The start of contractions results in net lactate release followed the fall of lactate [v-a] and net release to zero. However, measurements of working limb (muscle) lactate concentration differences are inadequate to reveal active intramuscular production and oxidation.

Working skeletal muscle can consume and oxidize lactate at altitude. Further, working muscle lactate extraction and oxidation depend on the arterial lactate concentration. Because lactate extraction and oxidation are related to arterial [lactate], the greatest intramuscular lactate oxidation is observed during exercise upon acute altitude exposure.

It is now known that muscle possesses at least two MCT isoforms that facilitate sarcomere exchange of lactate; these are MCT1 and MCT4. The effects of chronic hypoxia on expression of these lactate transport proteins is unknown. However, the model (Figure 4) indicates a major role of sarcomere lactate transporters at altitude. Probably, MCT1 is present in mitochondria and sarcomerenal membranes whereas MCT4 is the constitutive muscle cell membrane isoform.

In addition to skeletal muscle, numerous other cells and tissues (erythrocytes, heart, liver, kidneys, brain, testes) express lactate transporter isoforms. Again, the effects of hypoxia are unknown.

Intra-myocyte, -hepatocyte, and -cardiocyte lactate oxidation depends on the presence of mitochondrial lactate-pyruvate transporter. When cytosolic [lactate] is high, lactate enters mitochondria where it is oxidized. Apparently, lactate oxidation in muscle is facilitated by high mitochondrial density and oxygen consumption rates.

Intra-myocyte, -hepatocyte, and -cardiocyte lactate oxidation depends on the presence of mitochondrial LDH. In mitochondria, LDH probably exists in the inter-membrane space as well as on the inner membrane. Mitochondrial LDH is essential for the oxidation of lactate.

and coronary blood flow indicated that exogenous lactate becomes the major fuel for the heart during exercise (22). Further, in those studies the appearance of 13C and 14C in blood glucose following tracer-lactate infusion indicated participation of the Cori Cycle in lactate removal (41, 43). Thus, skeletal as well as cardiac muscle and liver participate in lactate exchange and metabolism during exercise.

With regard to the issue of oxygen insufficiency as the cause of lactate production in muscle, the results obtained on resting, as well as contracting muscle indicate that lactate production occurs in well perfused and fully oxygenated tissues (47). Not only is lactate always present in resting muscle, but lactate release and oxidation occur simultaneously (6) while cardiac output and limb blood flow rise so that oxygen transport meets demand, and significant O2 remains in femoral venous blood. Data on intact functioning humans as well as animal muscle preparations indicate that lactate production occurs under fully aerobic conditions (14, 15, 25).

4. WHAT IS THE INTRACELULAR LACTATE SHUTTLE?

To resolve the dilemma of tissue lactate production under fully aerobic conditions, the "Intracellular Lactate Shuttle" was posited. While there was good evidence to support activity of the "Cell-Cell Lactate Shuttle" during exercise, at rest recruitment of Type IIB fibers could not be invoked. A better model that allowed for lactate production in all fiber types during rest and exercise was necessary (6). The "Intracellular" Shuttle (Figure 4) allows that glycolysis in the cytosol results in lactate production, and lactate shuttles to mitochondria within the cell of production for oxidative removal (7).

That glycolysis inevitably results in cytosolic lactate production relates to the energetics of the terminal enzyme, lactate dehydrogenase (LDH). The K_m for LDH is 3.6 x 10^-4 M^-1, and the free energy change (ΔG°) approximates −6 kcal/mol. This means that glycolysis inevitably proceeds to lactate production, and that its reversal in the cytosol is unlikely in vivo. How then is lactate oxidized?

To evaluate the role of mitochondria in balancing lactate production and oxidation as part of an "Intra-cellular Lactate Shuttle," isolated rat cardiac, skeletal muscle and liver mitochondria were respiring with lactate and pyruvate in presence or absence of known inhibitors of metabolism. As well, efforts were made to detect the presence of LDH isomers in mitochondria by electrophoresis and electron microscopy. Results support the conclusion of a mitochondrial role in cellular lactate oxidation (6, 7), and provide an
explanation for the high correlation between lactate clearance during exercise and muscle mitochondrial respiratory capacity (2, 31).

In one experiment, mitochondria were isolated and respirated with pyruvate-malate and lactate-malate as substrates. Respiration was stimulated by the presence of ADP. Figure 5 shows that maximal, ADP-stimulated respiration rates were obtained with both lactate and pyruvate as substrates (7). Further, the figure shows that addition of the known LDH inhibitor oxamate, blocks mitochondrial lactate oxidation. Thus, LDH is necessary for mitochondrial lactate oxidation.

To determine if LDH existed in mitochondria, organelles from rat liver, heart and skeletal muscle were isolated. Aagarose gel electrophoresis of LDH isoenzyme patterns in cytosolic fractions of different tissues (Figure 6) are consistent with results of previous investigations showing tissue specificity (e.g., 29). However, mitochondrial fractions also revealed presence of LDH isoenzymes. Further, LDH isoenzyme patterns differed among tissues, and between mitochondria and the surrounding cytosol in each tissue. Heart and

**Intracellular Lactate Shuttle**

![Graph](image)

Figure 12 Log blood lactate extraction as a function of arterial lactate concentration during rest (closed symbols) and leg cycling exercise (open symbols) at sea level (squares), upon acute exposure to 4,300 m altitude (circles), and after 3-wk acclimatization (triangles). Each point is n = 6, mean ± SEM. Lactate extraction is highly correlated with arterial concentration. From Brooks et al. (13).

Although the results portrayed in Figure 12 are unique to men working at altitude, they are not unique in nature. With canine muscle preparations made to contract *in situ*, Gladden and associates (23) showed that by raising the arterial [lactate], working muscle switches from net release to uptake. Similarly, by adding arm to leg exercise (arm exercise raises the arterial [lactate]), Richter et al. (35) showed that leg muscle switches from net release to uptake. Thus, based on either chemical balance (Figures 3 and 9), or isotope tracers (Figure 12), muscle is capable of lactate exchange and oxidation.

9. **RESOLUTION OF QUESTIONS:**

The Cell-Cell and Intramuscular Lactate Shuttle Concepts may be of assistance in understanding several phenomena observed in men exercising at altitude. Such phenomena of interest include: elevated arterial lactate levels, response of circulating lactate concentration to β-blockade, temporal variation in lactate [t-a] and net release, and simultaneous lactate release,
muscle mitochondria were noted by prevalence of both LDH-1 (H4) and LDH-5 (M4), while liver mitochondria were distinguished by presence of LDH-5 (M4) (7).

Figure 11. Mean (± SEM) leg net lactate venous-arterial concentration differences at rest and over time during exercise at sea level, upon acute exposure to 4,300 m altitude, and after a 3-wk acclimatization period: (A) control (n = 5), and (B) block-bled subjects (n = 6). Corresponding mean (± SEM) net lactate release from the two legs over time during rest and exercise time in the same: (C) control, and (D) block-bled subjects. From Brooks et al. (6).

Figure 12 portrays lactate extraction by leg muscles of men during rest and altitude at sea level, upon acute exposure to 4,300 m, and after a 3-week acclimatization period (9). The values were calculated from the decrease in tracer (3-14C) lactate mass across limbs. Not shown is that during exercise extraction equaled oxidation (i.e., the rise in 14CO2 equaled the decrease in 14C-lactate). As shown in Figure 12, lactate extraction and oxidation are directly related to arterial lactate concentration. Thus, greatest lactate extraction and oxidation rates were observed upon acute altitude exposure when the arterial lactate concentration was highest.

Figure 5. Depiction of a Clarke-O2, electrode tracing showing that oxamate blocks lactate, but not pyruvate oxidation. Such experiments indicate that the ability of mitochondria isolated from muscle, liver and heart to oxidize lactate requires LDH for conversion to pyruvate within mitochondria. Data from Brooks et al. (7).

Even though studies with oxamate indicated functionality of mitochondrial LDH (Figure 5), and electrophoresis showed mitochondrial LDH (Figure 6), to further exclude the possibility of isolation artifacts, antibodies to LDH isozymes were used to detect presence of mitochondrial LDH, in situ. The electron micrograph in Figure 7 clearly shows a mitochondrial location of LDH (7).

The model of an "intracellular Lactate Shuttle" (6, 7) depends heavily on presence of a mitochondrial lactate-pyruvate, or monocarboxylate transporter (MCT) (38, 39). To demonstrate the effects of inhibiting the mitochondrial lactate-pyruvate transporter, mitochondria were respired in the presence or absence of the known MCT inhibitor, cinnamate (CINN); results are shown in Figure 8. CINN blocked respiration of both substrates. However, in the presence of CINN, State 3 respiration was restored with
addition of either succinate or glutamate. Restoration of respiration with succinate or glutamate indicates that CINN does not block mitochondrial respiratory Complex I (glutamate, an NADH-linked substrate), or Complex II (succinate, an FADH$_2$-linked substrate). Upstream inhibition of mitochondrial lactate and pyruvate oxidation, such as a transport limitation, by CINN is indicated. Thus, consistent with the concept of an “intracellular Lactate Shuttle” (Figure 4), results as in Figures 5 and 8 show that mitochondrial lactate oxidation proceeds very rapidly requiring a transporter and LDH.

To date, preliminary studies have been conducted to identify the mitochondrial lactate-pyruvate transporter. Several possibilities exist. The cDNA of the first sarcosomal lactate transporter from the hamster ovary cells was isolated by expression cloning (28). The protein was expressed in several tissues including heart, red skeletal muscle and erythrocytes (21). The cDNA was subsequently used for the screening of hamster liver cDNA library and resulted in isolation of the second transporter isoform which was expressed mainly in liver (20). These isoforms shared 60% identity with each other. The third MCT isoform was initially isolated as a protein exclusively expressed in the chicken retinal epithelium (32). All three isoforms stimulate proton-coupled lactate and pyruvate uptake when expressed in a heterologous system and show high affinities for propionate and ketone bodies (20, 21), and were named monocarboxylate transporters (MCT1, MCT2 and MCT3). More recently, four new mammalian monocarboxylate transporter (MCT) homologues were cloned and sequenced (33), and other candidates exist (Avazadehvi and Brooks, unpublished). Although scattered and partial homology exists among the

Figure 6. Agarose gel electrophoresis of LDH from rat liver and heart. LDH isozyme patterns differ between cytosol and mitochondria in both tissues. From Brooks et al. (7).

8. LACTATE EXTRACTION AND OXIDATION DURING NET RELEASE:

Previously, Stanley et al. (42) demonstrated that working human muscle is capable of simultaneous lactate extraction and release with almost all of the extraction attributable to oxidation in working muscle. Leg extraction and oxidation can be calculated in two ways, which usually yield good agreement in the steady state. One method is to compare the amount of tracer lactate leaving the limb with that entering. The difference (i.e., the decrease in tracer mass leaving the leg) is quantitatively related to the increase in labeled CO$_2$ release. The alternative method is to compare the mass of tracer CO$_2$ entering and leaving the leg.
7. ILLUSTRATION OF THE "STAINSBY EFFECT" BY WORKING HUMAN MUSCLE AT ALTITUDE

To evaluate the role of working muscle in contributing to the circulating lactate load during exercise at high altitude, femoral arterial and venous catheterizations were performed and limb blood flow rates as well as [v-a] lactate differences were determined. In this way, net lactate release rates (L) were determined during rest and exercise under each environmental condition (12). That canine muscle preparations made to contract in situ release lactate on a net basis when exercise starts, and then switch to zero release or net consumption as contractions progress was first made by Stainsby and associates (40, 45). A similar "Stainsby Effect" is observable in

MCT isoforms, it is unlikely that all isoforms are lactate-pyruvate transmitters. Most probably, MCT1 and MCT4 are the lactate transporters present in muscle (46).

Figure 7. Electron micrograph of high pressure frozen rat liver showing mitochondria, rough ER, and cytosol. Immunolocalization of anti-LDH-S (M44) antibodies is indicated by the 15 nm gold particles. Magnification = 54,100 X and scale bar = 400 nm. Note presence of LDH-5 in mitochondria and surrounding matrix and organelles. From Brooks et al. (7).

Figure 8. Citrulline blocks oxidation of lactate and pyruvate. Because muscle, liver and heart mitochondria can respire both glutamate (donates electrons at Complex I) and succinate (donates electrons at Complex II), the blockage of citrulline is upstream of PDH, GDH or SDH. The essential role of a mitochondrial MCT (lactate/pyruvate) transporter is illustrated. Data from Brooks et al. (7).
Because there was a good relationship between the oxidative capacity of muscle and the abundance of MCT1, we raised an antibody to the C-terminus of rat MCT1. Western blots of cardiac and skeletal muscle mitochondria prepared from rats show presence of MCT1 in striated muscle mitochondria. Further, electron microscopy and immunolocalization techniques show MCT1 on inner mitochondrial membranes (Brooks, et al., unpublished). Thus, MCT1 is a candidate mitochondrial lactate/pyruvate transporter.

5. STUDIES ON MEN EXERCISING AT ALTITUDE

Having now introduced the Cell-Cell and Intracellular Lactate Shuttle Concepts, the pertinent data on men exercising at altitude will be reviewed and presented with an eye toward evaluating whether the concepts are of assistance in evaluating the data.

When men are acutely exposed to moderate altitude (e.g., 4,300 m on Pikes Peak), the circulating [lactate] is elevated during exercise compared to during the same, sub-maximal power output as at sea level. In each environmental condition portrayed (sea level, acute and chronic altitude exposure), the arterial [lactate] is elevated, and stable during exercise (Figure 9A). On acute altitude exposure, the greatest lactate concentration response is observed. With acclimatization, the circulating [lactate] declines even though whole body and working limb \( V_O_2 \) are unchanged at altitude compared to sea level. Thus, there can be no possibility of a change in lactate production due to \( O_2 \) lack.

Figure 9B shows the sympathetic effect on circulating lactate level. \( \beta \)-adrenergic blockade with the non-specific \( \beta \)-antagonist propranolol decreases arterial [lactate] by approximately 60%, a result most obvious on acute altitude exposure. At altitude, \( \beta \)-blockade attenuates, but does not completely block the blood lactate response to exercise. What is the source of circulating lactate during exercise at altitude?

6. LACTATE TURNOVER (PRODUCTION) AT ALTITUDE

In the 1988 Pikes Peak study, fluxes of glucose and lactate were determined simultaneously using primed-continuous infusions of \([6,6-^{14}C]glucose \) and \([3-^{13}C]lactate \). The results for sea level and acute exposure to 4,300 m are shown in Figure 10. During rest at sea level, glucose disposal (Ra) exceeds lactate appearance (Ra). Upon altitude exposure, glucose Ra rises significantly, but lactate Ra rises relatively more. During exercise at 50% \( V_{O_2\text{max}} \) at sea level, glucose flux rises compared to rest, and lactate flux rises to approximate glucose flux. Then, during exercise upon acute altitude exposure, lactate flux exceeds glucose flux. Thus, it is clear that altitude exposure causes a shift to carbohydrate metabolism, with both glucose and lactate fluxes exaggerated compared to sea level. Most importantly, blood lactate flux, a key measure of tissue lactate production, is increased significantly at altitude.