Commentary

Lactic acid: New roles in a new millennium

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The study of lactic acid (HLa) and muscular contraction has a long history, beginning perhaps as early as 1807 when Berzelius found HLa in muscular fluid and thought that "the amount of free lactic acid in a muscle [was] proportional to the extent to which the muscle had previously been exercised" (cited in ref. 1). Several subsequent studies in the 19th century established the view that HLa was a byproduct of metabolism under conditions of O_2 limitation. For example, in 1891, Araki (cited in ref. 2) reported elevated HLa levels in the blood and urine of a variety of animals subjected to hypoxia. In the early part of the 20th century, Fletcher and Hopkins (3) found an accumulation of HLa in anoxia as well as after prolonged stimulation to fatigue in amphibian muscle *in vitro*. Subsequently, based on the work of Fletcher and Hopkins (3) as well as his own studies, Hill (and colleagues; ref. 4) postulated that HLa increased during muscular exercise because of a lack of O_2 for the energy requirements of the contracting muscles. These studies laid the groundwork for the anaerobic threshold concept, which was introduced and detailed by Wasserman and colleagues in the 1960s and early 1970s (5–7). The basic anaerobic threshold paradigm is that elevated HLa production and concentration during muscular contractions or exercise are the result of cellular hypoxia. Table 1 summarizes the essential components of the anaerobic threshold concept.

However, several studies during the past ≈30 years have presented evidence questioning the idea that O_2 limitation is a prerequisite for HLa production and accumulation in muscle and blood. Jöbsis and Stainsby (8) stimulated the canine gastrocnemius *in situ* at a rate known to elicit peak twitch oxygen uptake ($\dot{V}O_2$) and high net HLa output. They (8) reasoned that if the HLA output was caused by O_2-limited oxidative phosphorylation, then there should be an accompanying reduction of members of the respiratory chain, including the NADH/NAD^+ pair. Instead, muscle surface fluorometry indicated NADH/NAD^+ oxidation in comparison to the resting condition. Later, Connett and colleagues (9–11), by using myoglobin cryomicrospectroscopy in small volumes of dog gracilis muscle, were unable to find loci with a PO_2 less than the critical PO_2 for maximal mitochondrial oxidative phosphorylation (0.1–0.5 mmHg) during muscle contractions resulting in HLa output and an increase in muscle HLa concentration. More recently, Richardson and colleagues (12) used proton magnetic resonance spectroscopy to determine myoglobin saturation (and thereby an estimate of intramuscular PO_2) during progressive exercise in humans. They found that HLa efflux was unrelated to muscle cytoplasmic PO_2 during normoxia. Although there are legitimate criticisms of these studies, they and many others of a related nature have led to alternative explanations for HLa production that do not involve O_2 limitation. In the present issue of PNAS, two papers (13, 14) illustrate the dichotomous relationship between lactic acid and oxygen.

First, Kemper and colleagues (13) add further evidence against O_2 as the key regulator of HLa production. They (13) used a unique model, the rattlesnake tailshaker muscle complex, to study intracellular glycolysis during ischemia in comparison to HLa efflux during free flow conditions; in both protocols, the muscle complex was active and producing rattling. In their first experiment, rattling was induced for 29 s during ischemia resulting from blood pressure cuff inflation between the cloaca and tailshaker muscle complex. In a second experiment, measures were taken during 108 s of rattling with normal, spontaneous blood flow. In both experiments, ^{31}P magnetic resonance spectroscopy permitted measurement of changes in muscle levels of PCr, ATP, Pi, and pH before, during, and after rattling. Based on previous methods established in their laboratory, Kemper and colleagues (13) estimated glycolytic flux during the ischemic and aerobic rattling protocols. The result was that total glycolytic flux was the same under both conditions! Kemper and colleagues (13) conclude that HLa generation does not necessarily reflect O_2 limitation.

To be fair, there are potential limitations to the excellent paper by Kemper and colleagues (13). First, and most importantly, they studied muscle metabolism in the transition from rest to rattling (29 s during ischemia and 108 s during free flow). Some investigators argue that oxidative phosphorylation is limited by O_2 delivery to the exercising muscles during this nonsteady-state transition even with spontaneous blood flow (for review, see ref. 15). This remains a matter of debate, and the role of O_2 in the transition from rest to contractions may depend on the intensity of contractions (16, 17). Of course, it is possible that the role of O_2 in the transition to rattling may be tempered by the high volume density of mitochondria and the high blood supply to this unique muscle complex (13, 18). Second, there could be significant early lactate production within the first seconds of the transition (19). Third, it would have been helpful to have measurements of intramuscular lactate and glycogen concentra-

Table 1. Anaerobic Threshold Concept

1. Muscle hypoxia occurs at ≈50–70% of $\dot{V}o_{2max}$
2. Inadequate O_2 ⇒ inhibition of the electron transport chain ⇒ insufficient ATP generation via oxidative phosphorylation and ↑ mitochondrial [NADH]
3. Mitochondrial NADH build-up inhibits NADH shuttles (malate-aspartate and glycerol-phosphate) and ⇒ ↑ cytosolic [NADH] and ↓ cytosolic [NAD]
4. ↑ Mitochondrial [NADH]/[NAD] inhibits TCA cycle ⇒ ↓ pyruvate use ⇒ ↑ mitochondrial [pyruvate] ⇒ ↑ cytosolic [pyruvate]
5. Pyruvate becomes H^+ acceptor in cytosol ⇒ ↑ HLa production
6. Inadequate aerobic ATP generation ⇒ ↓ [ATP], ↓ [PCr] and ↑ [ADP], ↑ [Pi], ↑ [AMP] ⇒ ↑ glycolytic rate, and ↑ HLa production
7. ↑ HLa production ⇒ ↑ muscle and blood [HLa]

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See companion articles on pages 711 and 723.

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COMMENTARY

Table 2. Increased HLa accumulation in the absence of O_2-limitation

1. a. HLa production depends on a competition for pyruvate and NADH between LDH and the NADH shuttles (malate-aspartate and glycerol-phosphate) and the pyruvate transporter (MCT)
   and/or
   b. High activity of LDH and Keq of pyruvate to lactate reaction guarantees HLa production particularly with increasing glycolytic rate
2. Phosphorylase is activated by increased work rate probably due to ↑ [Ca^{2+}], ↑ [Pi], and ↑ [AMP]; this increases glycolytic rate ⇒ ↑ HLa production
3. With increased exercise intensity; [ATP] ↓, [ADP] ↑, [AMP] ↑, [Pi] ↑, and [ammonia] ↑ ⇒ phosphofructokinase (PFK) activation and ↑ HLa production
4. ↑ [Ca^{2+}] may act in a feed-forward manner to activate phosphorylase and PFK independently of metabolic feedback
5. Sympathoadrenal activity increases with work rate—epinephrine activates phosphorylase and thereby ↑ glycolysis and HLa production
6. Sympathoadrenal activity also causes vasoconstriction and ↓ blood flow to liver, kidney, and inactive muscle ⇒ ↓ HLa oxidation and removal
7. Fast twitch fiber recruitment increases HLa production
8. HLa production exceeds removal ⇒ ↑ muscle and blood [HLa]

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tions for confirmation of the glycolytic balance sheet. Fourth, there was no verification that the work or force output was identical between the ischemic and aerobic rattling conditions. Finally, some measure of muscle PO_2 could have provided additional certainty. Clearly the degree of difficulty of the experiments would have escalated with these extra measures. Despite these potential shortcomings, it seems almost certain that O_2 limitation was greater and more prolonged during ischemic rattling, and yet glycolytic flux was the same as under spontaneous blood flow conditions.

If cellular hypoxia is not the cause of HLa production and accumulation, then what is? Table 2 summarizes numerous interacting factors that could contribute to cause increased HLa production, decreased HLa clearance, and increased concentrations of HLa in tissue and blood. Numerous studies using a wide variety of techniques and protocols support the scheme outlined in Table 2. Current understanding of the metabolic role of HLa is embodied in the "lactate shuttle hypothesis" developed by Brooks (20). This hypothesis holds that HLa formation and its subsequent distribution throughout the body is a major mechanism whereby the coordination of intermediary metabolism in different tissues, and cells within those tissues, can be accomplished. In this context, HLa is a useful metabolic intermediate because it can be exchanged rapidly among tissue compartments. Once formed in muscle cells that may have high glycogenolytic and glycolytic rates, HLa can be transported to other sites where it may serve as an energy source and a gluconeogenic precursor. HLa oxidation can occur in nearby oxidative muscle cells, or at other sites such as the heart or other oxidative skeletal muscles that might be either at rest or engaged in light to moderate exercise (21, 22). At the same time, HLa can be taken up in the liver and used for glucose production or liver glycogen storage.

A further refinement of the lactate shuttle hypothesis includes an intracellular component (23), as illustrated in Fig. 1. A central tenet of this intracellular shuttle is that HLa is an inevitable product of glycolysis, particularly during rapid glycolysis; this is so because lactate dehydrogenase (LDH) has the highest V_{max} of any enzyme in the glycolytic pathway and the Keq for pyruvate to lactate is far in the direction of lactate (20, 23). Further evidence shows that LDH is located in both the cytosol and in mitochondria and that a monocarboxylate transporter (MCT1) in the inner mitochondrial membrane can readily transport HLa (23, 24). The strong implication is that HLa produced in the cytosol by glycolysis can be taken up directly into mitochondria and oxidized. During exercise with accelerated glycolysis and elevated HLa concentration, the intracellular lactate shuttle may also account for most of the shuttling of reducing equivalents (NADH) into the mitochondria. The transfer of HLa into the mitochondria has the advantage of shuttling NADH across the mitochondrial membrane and also providing substrate for mitochondrial oxidation in the same step. This scheme fits very well with the detection, by Kemper and colleagues (13), of significant aerobic glycolysis in the rattlesnake tailshaker muscle complex.

Despite challenges to the paradigm that lactate hails hypoxia, it nevertheless remains clear that if mitochondria are O_2-limited, then there will be an enhanced

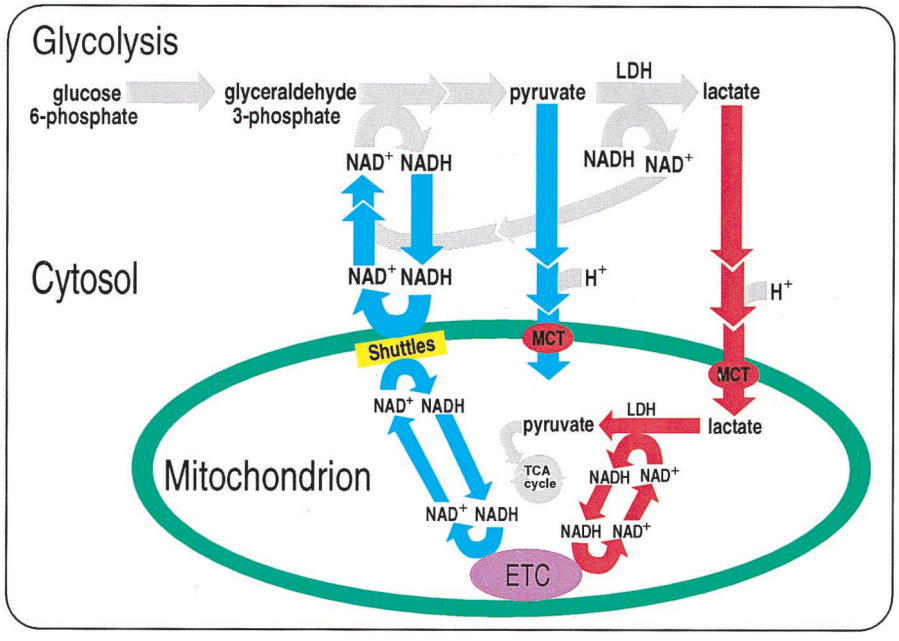

Fig. 1. Illustration of the essential elements of the recently introduced intracellular lactate shuttle (red) in comparison to the more well-known malate-aspartate and glycerol-phosphate NAD^+/NADH shuttles (blue). LDH, lactate dehydrogenase; MCT, monocarboxylate transporter; ETC, electron transport chain; Shuttles, malate-aspartate and glycerol-phosphate NAD^+/NADH shuttles. The H^+ ions for pyruvate and lactate are inserted to emphasize that the MCT symports a proton; the same MCT carrier can transport both pyruvate and lactate. Note that the mitochondrial LDH may actually be in the intermembrane space of the mitochondria and on the outer surface of the inner membrane. Note also that operation of the intracellular lactate shuttle delivers both reducing equivalents and substrate for oxidation to mitochondria. The intracellular lactate shuttle explains HLa production and accumulation under aerobic exercise conditions.

HLa production and accumulation. This leads us to a second paper in this issue (14). Sutherland and colleagues (14) report several types of evidence that together strongly indicate that the acid-sensing ion channel, ASIC3, mediates the acid sensitivity of rat cardiac sensory neurons. ASIC3 is a cloned channel that shares the following characteristics with the native channel much more closely than four other cloned ASICs: (*i*) high proton sensitivity, (*ii*) fast gating between closed, open, and desensitized states, and (*iii*) permeation and inhibition by Ca^{2+} (14). The extrapolation is that myocardial ischemia causes myocardial O_2 limitation, resulting in accelerated HLa production and accumulation (25); the HLa then moves across the sarcolemma via facilitated diffusion into the extracellular fluid where the decreased pH is detected by ASIC3, resulting in signals along cardiac sympathetic afferents which cause the sensation of chest pain.

Western and Northern blotting, immunofluorescence microscopy, and immunogold electron microscopy have shown that cardiac muscle cells contain MCT1 and a second, as yet unidentified MCT in the sarcolemma (26, 27). These transporters enhance HLa translocation under both aerobic conditions in which HLa is taken up for use as a respiratory fuel, and under ischemic or hypoxic conditions in which HLa must be removed from the cell to minimize deleterious effects on glycolysis and contraction (28).

At the dawn of a new millennium, we can say that the older view of lactic acid and hypoxia is correct in the sense that anaerobic glycolysis, HLa production, and HLa accumulation are increased under conditions that engender O_2-limited oxidative phosphorylation in mitochondria. In other words, it remains true that tissue hypoxia does indeed lead to increased HLa concentration. However, the induction that elevated HLa production and accumulation necessarily indicate the presence of hypoxia is not correct. This is the dichotomy of HLa in metabolism: on the one hand, an end product of anaerobic glycolysis, but on the other hand, an important metabolic intermediate of aerobic glycolysis.

I thank Drs. George A. Brooks and Richard A. Howlett for their helpful comments on the manuscript.

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