Lactate Metabolism: Historical Context, Prior Misinterpretations, And Current Understanding

By: Brian S. Ferguson 1, **Matthew J. Rogatzki** 2, Matthew L. Goodwin 3 & 4, Daniel A. Kane 5, Zachary Rightmire 6, & L. Bruce Gladden 6

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Lactate (La−) has long been at the center of controversy in research, clinical, and athletic settings. Since its discovery in 1780, La− has often been erroneously viewed as simply a hypoxic waste product with multiple deleterious effects. Not until the 1980s, with the introduction of the cell-to-cell lactate shuttle did a paradigm shift in our understanding of the role of La− in metabolism begin. The evidence for La− as a major player in the coordination of whole-body metabolism has since grown rapidly. La− is a readily combusted fuel that is shuttled throughout the body, and it is a potent signal for angiogenesis irrespec- tive of oxygen tension. Despite this, many fundamental discoveries about La− are still working their way into mainstream research, clinical care, and practice. The purpose of this review is to synthesize current understanding of La− metabolism via an appraisal of its robust experimental history, particularly in exercise physiology. That La− production increases dur- ing dysoxia is beyond debate, but this condition is the exception rather than the rule. Fluctuations in blood [La−] in health and disease are not typically due to low oxygen tension, a principle first demonstrated with exercise and now understood to varying degrees across disciplines. From its role in coordinating whole-body metabolism as a fuel to its role as a signaling molecule in tumors, the study of La− metabolism continues to expand and holds potential for multiple clinical applications. This review highlights La−'s central role in metabolism and amplifies our understanding of past research.

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Lactate metabolism: historical context, prior misinterpretations, and current understanding

Brian S. Ferguson[1] · Matthew J. Rogatzki[2] · Matthew L. Goodwin[3,4] · Daniel A. Kane[5] · Zachary Rightmire[6] · L. Bruce Gladden[6]

Abstract

Lactate (La^-) has long been at the center of controversy in research, clinical, and athletic settings. Since its discovery in 1780, La^- has often been erroneously viewed as simply a hypoxic waste product with multiple deleterious effects. Not until the 1980s, with the introduction of the cell-to-cell lactate shuttle did a paradigm shift in our understanding of the role of La^- in metabolism begin. The evidence for La^- as a major player in the coordination of whole-body metabolism has since grown rapidly. La^- is a readily combusted fuel that is shuttled throughout the body, and it is a potent signal for angiogenesis irrespective of oxygen tension. Despite this, many fundamental discoveries about La^- are still working their way into mainstream research, clinical care, and practice. The purpose of this review is to synthesize current understanding of La^- metabolism via an appraisal of its robust experimental history, particularly in exercise physiology. That La^- production increases during dysoxia is beyond debate, but this condition is the exception rather than the rule. Fluctuations in blood [La^-] in health and disease are not typically due to low oxygen tension, a principle first demonstrated with exercise and now understood to varying degrees across disciplines. From its role in coordinating whole-body metabolism as a fuel to its role as a signaling molecule in tumors, the study of La^- metabolism continues to expand and holds potential for multiple clinical applications. This review highlights La^-'s central role in metabolism and amplifies our understanding of past research.

Keywords Lactate metabolism · Lactate shuttle · Hypoxia · Glycolysis · Cancer metabolism · Astrocyte–neuron lactate shuttle · Lactate threshold · Mitochondria · Fatigue and lactic acidosis · Cytosolic redox

Abbreviations

ADP	Adenosine diphosphate
ANLS	Astrocyte–neuron lactate shuttle
ATP	Adenosine triphosphate
C	Cytochrome c
CD147	Chaperone protein for MCT1
cLDH	Cytosolic L-lactate dehydrogenase
CO_2	Carbon dioxide
CoA	Coenzyme A;
COXIV	Cytochrome oxidase complex IV
D_{max}	Method for determination of lactate threshold
EAATs	Excitatory amino acid transporters
GET	Gas exchange threshold
GLUT	Glucose transporter
GPR81	HCA1 G-protein coupled receptor 81
GS	Gastrocnemius-superficial digital flexor muscle complex
H^+	Hydrogen ion, proton
^{1}H-MRS	Proton magnetic resonance spectroscopy
$H^{13}CO_3^-$	Isotopic bicarbonate
HIF-1	Hypoxia-inducible factor-1
I	Complex I/NADH oxidoreductase of the mitochondrial electron system

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III	Complex III of the mitochondrial electron transport system
IV/COX	Complex IV/cytochrome c oxidase
K_m	Michaelis–Menten constant for concentration of substrate at half-maximal speed of a reaction or transport process
La^-	Lactate anion
$[La^-]$	Lactate anion concentration
LDH	Lactate dehydrogenase
LPH	Lactate-protected hypoglycemia
LT	Lactate threshold
LT_D	Lactate threshold as determined by the D_{max} method
MAS	Malate–aspartate shuttle
MCT	Monocarboxylate transporter
mLDH	Mitochondrial lactate dehydrogenase
MLSS	Maximal lactate steady state
MPC	Mitochondrial pyruvate carrier
NAD^+	Oxidized nicotinamide adenine dinucleotide
NADH	Reduced nicotinamide adenine dinucleotide
NADPH	Reduced nicotinamide adenine dinucleotide phosphate
O_2	Oxygen
OBLA	Onset of blood lactate accumulation
PDH	Pyruvate dehydrogenase
PDK1	Pyruvate dehydrogenase kinase 1
PGC-1	αPeroxisome proliferator activated receptor gamma coactivator-1α
Pi	Inorganic phosphate
P_iO_2	Intracellular partial pressure of oxygen
Pyr^-	Pyruvate
Q	Quinone
SLC16	Solute Carrier Family 16 proteins
TCA	Tricarboxylic acid cycle
UCP3	Uncoupling protein 3
V	Complex V/ATP synthase
$\dot{V}CO_2$	Carbon dioxide output per minute
$\dot{V}O_2$	Oxygen uptake per minute
$\dot{V}O_{2LT}$	Oxygen uptake per minute at the lactate threshold
$\dot{V}O_{2max}$	Maximum oxygen uptake per minute
$\dot{V}O_{2peak}$	Peak oxygen uptake per minute

Introduction

Within the physiological pH range of muscle and blood, lactic acid (HLa) is more than 99% dissociated into lactate anions (La^-) and protons (H^+). Therefore, it will generally be referred to as "La^-" in this review. Unless otherwise specifi the l(+) enantiomer of La^- is implied. Enantiomers are a pair of stereoisomers which are mirror images of each other. In the case of La^- (Fig. 1), the C2 carbon

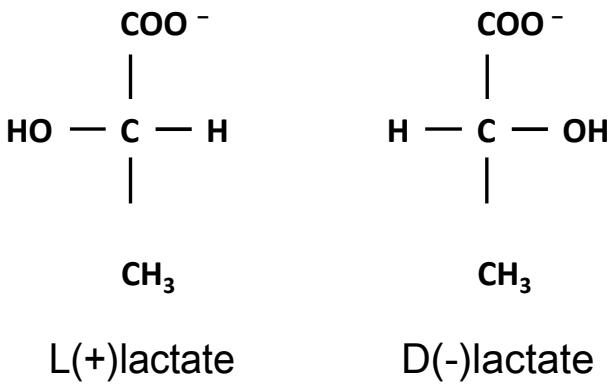

Fig. 1 Enantiomers of lactate. Enantiomers are a pair of stereoisomers which are mirror images of each other. While there is some

d(−)La in human tissues and blood from both endogenous and exog-

is asymmetrical (Chen et al. 2015) leading to enantiomers that are sometimes designated as l(+)La^- and d(−)La^-, although perhaps more correctly as (S)-La^- and (R)-La^-, respectively (Cahn et al. 1956). While there is some d(−)La^- in human tissues and blood from both endogenous and exogenous sources, the l(+) form is by far the most predominant and the one produced by glycolytic activity. Serum d(−)La^- concentration is between 0.013 and 0.2 mM in healthy humans (Hasegawa et al. 2003; Filiz et al. 2010) in comparison with l(+)La^- concentration on the order of 1.0 mM at rest and more than 15.0 mM after short duration maximal effort exercise.

The oft extolled, and persistent beliefs surrounding La^- can be found in numerous places, including popular fitness magazines, scientific literature, the athletic and coaching community, and clinical treatment. All too often, the framework of understanding has stemmed from centuries-old conclusions which initially found elevated [La^-] in both tissues and blood when O_2 levels were lower than normal. Along the way, La^- has been accused of roles in muscle soreness and fatigue, some of which are debatable or demonstrably incorrect. In acute care situations, [La^-] remains a frequently utilized prognostic tool to stratify trauma patients (Parsikia et al. 2014) as well as to identify risk of mortality in the critically ill (Nichol et al. 2010), with many clinicians viewing La^- as a waste product of hypoxia. This misinterpre- tation regarding the source and/or cause of hyperlactatemia can have profound implications. These misconceptions stem from long-standing assertions that have pervaded the scientific literature for more than a century. The paradigm of "low O_2 evokes high La^-" led to a simplistic converse deduction that "high La^- denotes low O_2", a position that was formed on the strength of research by Nobel laureates, Fletcher and Hopkins (1907), Hill et al. (1924), and Meyer- hof (1930b, 1942) in the early twentieth century. Because

La^- accumulates during strenuous exercise, the study of La^- metabolism has historically been closely tied to research into exercise energetics and fatigue. The "anaerobic threshold" concept (Wasserman and McIlroy 1964) reinforced this approach as well as the high La^--low O_2 paradigm. Today, despite numerous studies demonstrating the role of La^- as a metabolic fuel, and the discovery of many causative factors for La^- accumulation other than low O_2, the lactate-O_2 dogma persists in several research enclaves.

The aim of this paper is to review La^- through a historical lens, thereby gaining context for a broadening set of modern investigations and understanding. In many cases, the fundamental research that has brought our understanding to this point, and expanded that understanding into numerous disciplines, originated in the experiments of exercise physiologists investigating muscle energetics, most prominently the work of the George Brooks laboratory. This review will include: (1) a reevaluation of the recalcitrant view that La^- is always a product of inadequate tissue or cellular O_2; (2) highlighting recent work which recognizes La^- as a viable metabolite for the brain; (3) a summary of the elegant linking of substrate utilization and metabolism now known as the astrocyte–neuron lactate shuttle that is built upon the foundation of the original cell-to-cell lactate shuttle composed by Brooks (1985b); (4) an examination of additional roles which elevate La^- beyond a simple metabolite to include signaling functions as a "Lactormone"; and (5) assessment of a resurgent interest in the role of La^- in cancer, in tumorigenesis, and in tumor metabolism.

In contrast to the dogma that has plagued La^- as a dead-end, fatigue-causing waste product, we will provide evidence of La^- as an energy intermediate that can be formed in tissues undergoing accelerated glycolysis and subsequently distributed throughout the body to be taken up as a combustible substrate by oxidizing tissues, or as a precursor for gluconeogenesis (e.g., in liver) or glycogenesis (e.g., in liver or resting, glycolytic skeletal muscle). Our current understanding is that La^- is a ubiquitous metabolic intermediate operating simultaneously as both the terminal product of glycolytic metabolism and the intermediary to complete oxidation of carbohydrates by oxidative phosphorylation. A consistent, key finding that we hope to impart from these diverse studies is the centrality of La^- as a metabolic intermediate within and between cells. A particularly unique contribution of this review is the inclusion of key historical developments and misinterpretations in the study of La^- metabolism as well as a critical appraisal of current, cutting-edge investigations in this area.

Early history of lactate

In 1780, a German-speaking Swedish apothecary and chemist, Carl Wilhelm Scheele (Fig. 2) discovered HLa/La^- in sour milk (Benninga 1990). The new acid was named ''Mjölksyra,'' meaning ''acid of milk'' (Benninga 1990). Scheele also discovered chlorine, manganese, arsenic acid, barium, molybdenum, tungsten, and fl ine along with numerous other compounds (West 2014). However, Scheele will likely be best remembered as the fi t "modern" discoverer of oxygen (O_2), which he fi t called "vitriol air" (West 2014), and later "fi e air" (Scheele and Bergman 1777; Scheele et al. 1780; Severinghaus 2016). He actually discovered O_2 as early as 1772, more than a year before Joseph Priestley. Unfortunately, his experiments were not published until 1777, probably because of procrastination by Scheele as well as his friend and advocate, Torbern Bergman, who wrote an introduction to the book (Williams and Moyer 1982). There is also a more intriguing subtext to the story. In his *Traité élementaire de chimie* (Lavoisier 1789), Lavoisier falsely claimed that O_2 was discovered at almost the same time "by Mr. Priestley, Mr. Scheele, and myself" (Severinghaus 2016). This was a lie, not only because Joseph Priestley had communicated his discovery to Lavoisier in 1774 in person, but also because Scheele had sent Lavoisier a letter in the same year describing his 1771 discovery of O_2 (Grimaux 1890; Severinghaus 2016). This letter from Scheele to Lavoisier was kept hidden by Lavoisier and then

Fig. 2 Carl Wilhelm Scheele (1742–1786), discoverer of lactic acid and oxygen. Retrieved from (Lundgren 2014). Encyclopedia Britannica, https://www.britannica.com/biography/Carl-Wilhelm-Scheele. Access data August 04, 2017

by Lavoisier's wife's brother's descendants for 219 years (Severinghaus 2016)! Amazingly, Scheele made his landmark discoveries over a short lifetime, dying at the age of 43 (West 2014). Severinghaus (2006) speculates that he died as a result of tasting his discoveries, including one which was sold widely as Scheele's green cake frosting. The green color was due to arsenic, one of Scheele's discoveries.

Despite the fact that von Muralt (1950) called the intervening years between the discovery of La^- in 1780 and the muscle studies of Fletcher and Hopkins in 1907 (Fletcher and Hopkins 1907), "the prelactic acid era," there were some notable La^--related discoveries during this period (Brooks and Gladden 2003). Certainly, the fi most relevant to exercise physiology was the result by Berzelius in 1807 (Berzelius 1848; Needham 1971) that La^- concentration ($[La^-]$) was elevated in "the muscles of hunted stags" (Gladden 2008a). Quoting Berzelius (Berzelius 1848), "As early as 1807, I analyzed the meat of freshly slaughtered animals, and found a free acid in the liquids drawn from it." Apparently, Berzelius also convinced himself that the $[La^-]$ in a muscle was proportional to the amount of exercise that the muscle had performed (Needham 1971). Berzelius and others believed that the La^- found in muscle was the same as that found in sour milk by Scheele in 1780 (Brin 1965). However, in 1848, Engelhardt (1848), and later Wislicenus (1873) reported diff ent properties for muscle versus milk La^- (Brin 1965). Of course, we now know that animal tissues produce almost exclusively l(+)lactate, whereas microorganisms can produce d(-)lactate or sometimes a combination of d(−) and l(+); this can then lead to racemic mixtures in, for example, fermented milk (Lockwood et al. 1965; Caplice and Fitzgerald 1999).

As detailed by Kompanje et al. (2007), the presence of La^- in human blood under pathological conditions after death was first noted in 1843 and again in 1851 by Scherer (1843, 1851). Mosler and Körner (1862; Kompanje et al. 2007) note that blood from a living patient with leukemia was reported positive for La^- by Folwarczny in 1858; 5 years later, similar data were reported for blood from living patients with a number of different medical conditions (Folwarczny 1863; Kompanje et al. 2007). Failing to acknowledge prior research, Gaglio (1866) detected La^- in arterial blood freshly drawn from dogs and rabbits. In addition, neglecting previous work, Berlinerblau (1887) confi med the report of Gaglio in mammalian and human venous blood (Kompanje et al. 2007).

The nineteenth century saw progress in understanding the metabolic sources of La^- in animals. In 1845, Hermann von Helmholtz (1845) reported findings that were consistent with La^- formation at the expense of glycogen (von Muralt 1950). In 1847, German chemist, von Liebig (1847), founder of the world's fi t school of chemistry, noted the inevitable presence of La^- in muscle tissue from dead organisms (Kompanje et al. 2007). Interestingly, Claude Bernard (Bernard 1855), the discoverer of glycogen in 1859 (Bernard 1877; Needham 1971), found release of La^- from muscles excised from calf fetuses and placed in water at 15–20 °C and discussed its possible origin (Needham 1971). Shortly thereafter, as described by Fletcher and Hopkins (1907), Kompanje et al. (2007), and Needham (1971), du Bois-Reymond (in several 1859 publications) noted that activity caused muscles to become acidic and actually related this finding to the increased $[La^-]$ reported by Berzelius. Soon after (1864), Heidenhain (von Muralt 1950) reported that the amount of La^- increased with the amount of work done. In the next year, as reported by Fletcher and Hopkins (1907), Ranke concluded that resting muscle is alkaline but becomes acid after excision. Based on his experiments in 1877 and 1879, Nasse ultimately concluded that La^- is derived from glycogen (Nasse 1877, 1879; Needham 1971).

What appears above as a relatively linear, logical progression in the study of La^- in skeletal muscle is created by selective hindsight viewed through the lens of our current understanding. Quoting from the classic study of Fletcher and Hopkins (1907), just as Needham (Needham 1971) did: "there is hardly any important fact concerning the lactic acid formation in muscle which, advanced by one observer, has not been contradicted by some other." As support for this assessment, Fletcher and Hopkins (1907) cite numerous studies reporting that La^- was not formed in response to either muscle contraction or rigor, and did not accompany muscle fatigue. The paper by Fletcher and Hopkins (1907) is a landmark classic that will be discussed in the next section on the relationship between La^- and hypoxia. Fletcher was mentor to A. V. Hill and in 1929, Hopkins was awarded the Nobel Prize for "his discovery of growth-stimulating vitamins" (Levinovitz and Ringertz 2001).

The "high La^- denotes low O_2" paradigm

Research on disparate cell types established the fundamental concept that fermentation was markedly enhanced in the absence of O_2. In 1861, Louis Pasteur observed that the growth of yeast per gram of sugar consumed was much greater under aerobic than anaerobic conditions (Pasteur 1861; Racker 1974; Barnett 2003). Subsequently, most investigators focused on the measurement of the products of fermentation (alcohol for yeast and La^- for skeletal muscle) under O_2 versus no-O_2 conditions (Racker 1974). In yeast, the decrease in sugar consumption upon the introduction of O_2 was accompanied by a decrease in fermentation and, therefore, alcohol production. In skeletal muscle, this same phenomenon was later recognized as a decrease in glycogen breakdown and a decrease in La^- formation under aerobic as compared to anaerobic conditions (Meyerhof 1930b; Barnett 2003). Although

Pasteur's initial discovery occurred in 1861, it was not until 1926 that Warburg gave this phenomenon its name, the "Pasteur Eff (Warburg 1926; Krebs 1972). The intermixing of metabolic research in diff ent cell types in the 1920s is illustrated by the fact that Meyerhof and Warburg, to mention two giants in the field, both examined this Pasteur Eff in yeast, muscle, and other tissues (Barnett and Entian 2005). As quoted by Barnett (2003), von Euler and colleagues wrote in 1925, "…for some time we have been busy with developing the analogy between fermentative catabolism in yeast and anaerobic catabolism of carbohydrates in muscle" (von Euler et al. 1925).

The early twentieth century view of hypoxia as the necessary cause of La^- accumulation was promoted and reinforced by several notable contributions. Much of the uncertainty surrounding La^- formation in muscle at the end of the nineteenth century was due to methodological problems mainly associated with muscle glycogenolysis occurring during the muscle sampling and preparation process, prior to experimental study. In the aforementioned landmark study, Fletcher and Hopkins (1907) developed a method which prevented significant La^- formation in resting muscles before the extraction and analysis of the La^-. Subsequently, they were able to show that: (1) freshly excised resting muscle contains a low [La^-]; (2) [La^-] increases in excised, resting, anaerobic muscles; (3) La^- accumulates and [La^-] increases to high levels during stimulation of muscles to fatigue; and (4) most importantly in the present context, when fatigued muscles are placed in O_2-rich environments, La^- disappears (Brooks and Gladden 2003). As noted above, Nobel Prize winner Otto Meyerhof also observed La^- accumulation in the absence of O_2 and its removal upon the return of O_2 (Meyerhof 1930a; Krebs 1972). Therefore, it was likely that in the context of the Pasteur Eff in skeletal mus- cle, Hill et al. (1924) postulated that La^- increased during muscular exercise because of a lack of O_2 to remove the requisite La^- produced by the contracting muscles. This view of hypoxia as a cause of La^- accumulation was likely reinforced by the earlier studies of Araki and also Zillessen [cited by (Kompanje et al. 2007)] who had shown that interruption of the O_2 supply to the muscles of mammals and birds promoted an increase in [La^-].

To be fair, we should note that researchers at that time thought that La^- formation was of a necessity an anaerobic process. Specifically, it was believed that O_2 was required to combust some of the La^- that was produced during muscle activity back to some form of carbohydrate. If O_2 was not available for this combustion, then [La^-] would rise. The state-of-the-art view of La^- metabolism in humans in the late 1920s is exemplifi by Jervell's experiments (Jervell 1928). He (Jervell 1928) reported numerous experiments in which he investigated the concentration of "lactic acid" in blood and urine under both physiologic and pathologic conditions. Two quotes serve to illustrate the prevailing idea that a lack of O_2 was the reason for elevated [La^-]:

> "During my clinical work, I was naturally induced to supplement the experimental investigations by examination of the lactic acid's behavior in various pathological conditions. It was then deemed necessary to limit these examinations to those conditions, where owing to impeded intake of oxygen, we might expect to find hyperlactacidaemia." And "If the oxygen supply…is for one or other reason too small, we may expect to fi an increase in the quantity of lactic acid in the muscles."

From the idea that La^- production and accumulation was due to inadequate O_2 supply, it was only a simple, intuitive deduction to reach the converse conclusion that increased La^- production and concentration were the results of, and indicators of, hypoxia/dysoxia.

"Anaerobic" threshold

Some 40 years later, the idea that inadequate O_2 causes La^- accumulation was the centerpiece of the "anaerobic threshold" concept as proposed by Wasserman and McIlroy (1964; Wasserman et al. 1973). Although overshadowed by Wasserman's efforts, similar ideas were advanced independently by Hollman, Kinderman, Keul, and others (Kindermann et al. 1979; Stegmann et al. 1981; Hollmann 1985). In a letter of February 21, 2000 to G. A. Brooks, Wasserman described his motivations for investigating the "anaerobic threshold," and specifically, non-invasive assessment techniques for pulmonary medicine (Brooks and Gladden 2003). In 1959, Wasserman was a postdoctoral fellow under Julius H. Comroe in the University of California, San Francisco (UCSF) Cardiology Research Institute. Recognizing the "epidemic" in cardiovascular disease, in November of 1960 Comroe challenged Wasserman to develop procedures for early detection of heart disease. Wasserman's response was that evaluation "would be best done during exercise when the heart was being stressed…. The first sign of heart failure would be refl in the failure of the circulation to deliver adequate O_2 to the metabolizing tissues (exercising muscles). Since the muscle O_2 requirement would be markedly increased by exercise, the failure of the heart to transport O_2 adequately would result in lactic acidosis (Pasteur Effect)." (Wasserman letter of 2/21/2000 to Brooks). Aware of the earlier reports of Harrison and Pilcher (1930a, b) who showed increased CO_2 production and reduced O_2 uptake in heart-failure patients, Wasserman thought "it possible to investigate how to detect the $\dot{V}O_2$ at which lactic acidosis developed during exercise, using non-invasive gas exchange techniques." His experience at UCSF led to an appointment at Stanford, where Wasserman co-authored a paper with

Malcolm B. McIlroy (Wasserman and McIlroy 1964) in which "anaerobic threshold" was determined as the $\dot{V}O_2$ at which the respiratory exchange ratio (R) increased abruptly during a progressive incremental exercise test. It is informative to note that Wasserman discussed his research with D.B. Dill and credits Dill with inspiring the term "anaerobic threshold." Wasserman showed Dill his data and the method of gas exchange he was using to "detect the work rate at which O_2 transport was inadequate to prevent an exercise lactic acidosis." Wasserman further states, "As close[ly] as I can remember, his [Dill's] comment was that I was detecting the threshold of anaerobic metabolism during exercise by measuring exercise gas exchange. Thus, he put into words succinctly what I was trying to measure" (Wasserman letter of 2/21/2000 to Brooks), (Brooks and Gladden 2003). A fascinating aspect of Wasserman's personal account of the "anaerobic threshold" concept is its connection to the 1920s concept of the Pasteur Effect and to the person of D. B. Dill who directed the Harvard Fatigue Laboratory from 1927 to 1947. In 1973, Wasserman et al. (1973) refined the concept in their classic paper, defining "anaerobic threshold" as the "level of work or O_2 consumption just below that at which metabolic acidosis and the associated changes in gas exchange occur." This non-invasive measure was in turn asserted to correspond to the work rate or $\dot{V}O_2$ above which lactate systematically increased in response to an increase in work rate. This 1973 paper (Wasserman et al. 1973) generated great interest which continues to the present day with over 2,200 citations according to Google Scholar as of August 2, 2017.

For the subsequent span of over three decades, Wasserman and colleagues vigorously pursued the development of non-invasive technologies to identify the presence of lactic acidosis in graded clinical exercise protocols. Numerous gas exchange measures have been used over the years, and a full discussion is beyond the scope of the current review. In brief, the so-called V-slope method (Beaver et al. 1986) has become the preferred method for determining what is currently most appropriately referred to as the gas exchange threshold (GET). For the V-slope method of threshold detection, carbon dioxide output ($\dot{V}CO_2$) is plotted against $\dot{V}O_2$ and a computerized regression analysis detects the breakpoint in the relationship (Fig. 3). The coincidence of the GET with the lactate threshold (see below) as determined directly from the pattern of blood [La^-] has been heavily debated (Caiozzo et al. 1982; Davis et al. 1983; Green et al. 1983; Simon et al. 1983; Yeh et al. 1983; Gladden et al. 1985; Plato et al. 2008) and is also beyond the scope of the present review.

While the Wasserman lab emphasized the non-invasive measurement of the "anaerobic threshold", many other researchers focused on a direct assessment of the blood La^- response to exercise of varying intensities. As presaged

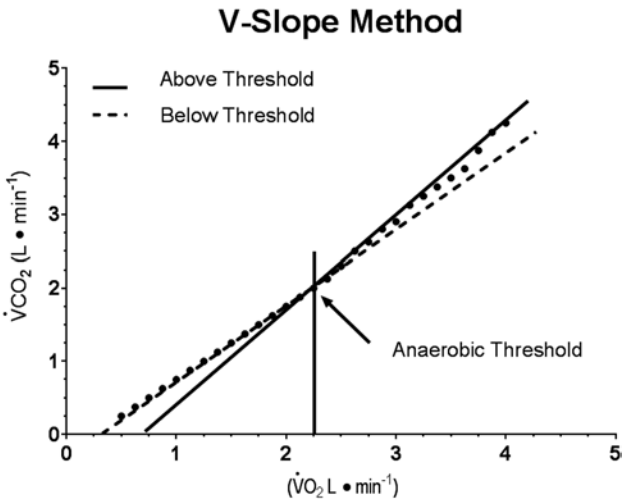

Fig. 3 V-slope method for determining the gas exchange threshold, redrawn with permission from (Beaver et al. 1986). Carbon dioxide output ($\dot{V}CO_2$) is plotted against simultaneously determined oxy- gen uptake ($\dot{V}O_2$) in a progressive incremental exercise test. The gas exchange threshold is detected by a mathematical algorithm that determines the intersection of the two straight lines that provide the best fit to the overall data set

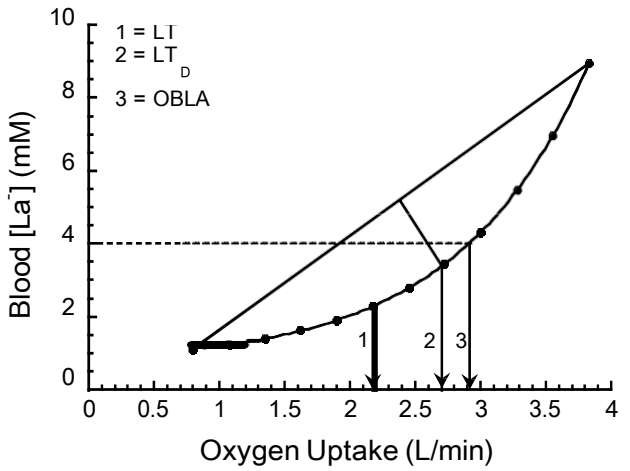

Fig. 4 Idealized blood [La^-] response to progressive incremental exercise plotted against oxygen uptake ($\dot{V}O_2$). Also illustrated are three single point measures often used to describe the response: the visually determined lactate threshold (LT) (Gladden et al. 1985), LT as determined by the D_{max} method (LT_D) (Cheng et al. 1992), and the onset of blood La^- accumulation (OBLA = 4 mM) (Heck et al. 1985). Reprinted with permission from Gladden (2004a) Lactate metabolism during exercise. In *Principles of Exercise Biochemistry*, 3rd edn, ed. Poortmans JR, pp. 152–196. Karger, Basel

by Owles (1930), during progressive, incremental exercise, blood [La^-] rises gradually at first and then more rapidly as the exercise becomes more intense; this idealized response is shown in Fig. 4. A primary issue in the study of data of this type has been the identification and interpretation of a single

value that best represents this curvilinear blood La^- profile. As examples, Fig. 4 illustrates three of these methods; the lactate threshold (LT), the onset of blood La^- accumulation (OBLA), and D_{max} (Wasserman et al. 1973, 1986; Gladden et al. 1985; Heck et al. 1985; Cheng et al. 1992; Bishop et al. 1998; Jones and Doust 1998; Nicholson and Sleivert 2001; Gladden 2004a; Faude et al. 2009). While these methods often do not provide the same quantitative value, they generally correlate well with each other, and each is highly correlated with endurance performance (Bishop et al. 1998; Jones and Doust 1998; Nicholson and Sleivert 2001). Depending on the particular protocol, the pattern of increase in blood [La^-] can differ from the idealized response shown in Fig. 4. As one example, some researchers report a second "breakpoint" in the response (e.g., Skinner and McLellan 1980). Nevertheless, we contend that the various indicators are correlated with each other.

Biopsies from exercising muscles have shown that muscle [La^-] shows similar patterns to those of blood [La^-] although the exercising muscle [La^-] is generally higher than that in blood (Green et al. 1983; Gollnick et al. 1986). The blood [La^-] response to progressive exercise has in turn been related to the blood [La^-] levels that are measured in more prolonged, constant work rate exercise (Wasserman et al. 1967; Pringle and Jones 2002), and are, therefore, more closely related to endurance performance ability. Figure 5 provides a schematized view of this response. Exercise intensities which are below the LT typically result in a transient elevation of blood [La^-] which then declines to a lower level, sometimes returning to near the resting [La^-]. During exercise that elicits metabolic rates between LT ($\dot{V}O_{2LT}$) and 50% of the difference between $\dot{V}O_{2LT}$ and $\dot{V}O_{2peak}$ {$50\%\Delta = \dot{V}O_{2LT} + 0.5\ (\dot{V}O_{2peak} - \dot{V}O_{2LT})$}, many subjects reach their maximal lactate steady state (MLSS) (Pringle and Jones 2002). MLSS is defined as the highest intensity of exercise that can be performed while maintaining a constant, but elevated blood [La^-] (Beneke and von Duvillard 1996; Jones and Doust 1998). Operationally, exercise intensities that are greater than the MLSS are identified as metabolic rates that result in an increase of more than 1.0 mM in blood [La^-] during the last 20 min of a 30-min constant work rate trial (Beneke and von Duvillard 1996; Jones and Doust 1998; Pringle and Jones 2002; Gladden 2004a). At work rates exceeding the MLSS, both blood [La^-] and $\dot{V}O_2$ drift upward until exhaustion occurs. Although the drifts in [La^-] and $\dot{V}O_2$ share a similar time course, there is apparently no cause-and-effect relationship between the two (Poole et al. 1994; Gaesser and Poole 1996; Billat et al. 1998). Despite the idealized representation in Fig. 5, there is a surprising range of blood [La^-]s at the MLSS; e.g., 1.5–6.3 mM (Pedersen et al. 2001). Several indices of physiological thresholds of exercise intensity occur at the same metabolic rate (i.e., $\dot{V}O_2$) (Keir et al. 2015) or at least are highly correlated

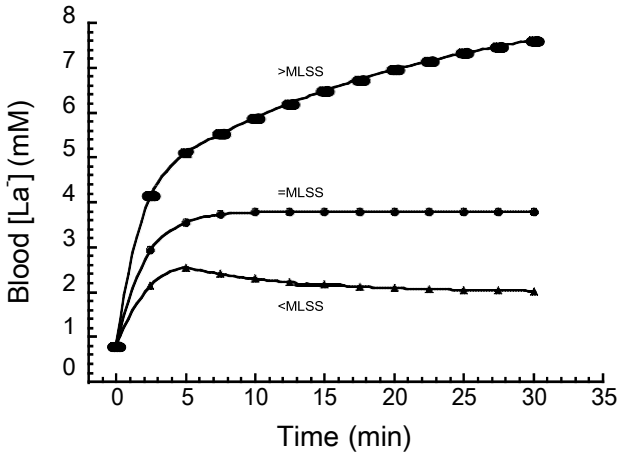

Fig. 5 Blood [La^-] response to constant work rate exercise at different intensities. Idealized data based on the reports of Wasserman et al. (1967, 1986) and Pringle and Jones (Pringle and Jones 2002) are plotted. Blood [La^-] responses for work rates below (<MLSS), at (=MLSS) and greater than (>MLSS) the maximal lactate steady state intensity are shown. Although the usual blood [La^-] at the MLSS is approximately 4 mM, this concentration can vary widely among individuals (Pedersen et al. 2001). Reprinted with permission from Gladden (2004a) Lactate metabolism during exercise. In *Principles of Exercise Biochemistry*, 3rd edn, ed. Poortmans JR, pp. 152–196. Karger, Basel

(Pringle and Jones 2002); these include critical power, muscle deoxyhemoglobin breakpoint, and the MLSS.

Essentially, Wasserman's anaerobic threshold hypothesis implies that the accumulation of La^- during muscular exercise is due to "dysoxia." Dysoxia as defined by Connett et al. (1990) is a sufficiently low level of O_2, such that cytochrome turnover is limited, and therefore, there is O_2-limited oxidative phosphorylation. As will be detailed in the following sections, the preponderance of evidence argues against this paradigm, and therefore, whether determined either invasively via blood [La^-] or non-invasively via gas exchange, threshold measures should not be termed "anaerobic."

Prime evidence for causative factors other than low O_2

Today, it is well established that any increase in [La^-] typically represents something other than an O_2 limitation; hypoxia-driven La^- accumulation is very much the exception rather than the rule. As reviewed by Barnard and Holloszy (2003), results of experiments conducted in 1885 by Rubner and Frey should have raised questions about the relationship between La^- and O_2. These investigators perfused mammalian muscles with oxygenated blood and observed La^- release despite the presence of apparently suffi
O_2. Zuntz (1911; Barnard and Holloszy 2003) actually discussed these results in 1911, but remained convinced that

hypoxic conditions were a prerequisite for La^- accumulation. Intriguingly, Zuntz proposed that some parts of the muscles might have been hypoxic because of arterial blood clots or narrowing of blood vessels (Zuntz 1911; Barnard and Holloszy 2003); perhaps, an early suggestion of "occult hypoperfusion" (see below). In a different arena of research in the 1920s, the Cori's (Cori and Cori 1925) and Warburg (Warburg et al. 1927) carried out whole animal experiments, demonstrating that limbs bearing tumors had high venous [La^-] despite presumed normoxic conditions. Glucose-avid, La^--producing tumors in normoxic conditions were eventually labeled as demonstrating a "Warburg eff " Recent interest in tumor metabolism has proven this to be much less straightforward than originally described, as it is now proposed that there may be thousands of cancer genotypes (Lundberg 2011) yielding a much smaller but still large number of cancer phenotypes, and even among the same tumor type, La^- uptake or output can vary markedly with arterial [La^-] (Sauer and Dauchy 1985). However, the Cori's and Warburg (Cori and Cori 1925; Warburg et al. 1927) provided early experimental animal evidence that La^- production and/or accumulation need not be linked obligatorily with hypoxia. The Warburg eff was thought to be characteristic of some tumors, but not reflective of any normal physiology (after all, why would there be increased [La^-] in normoxia?). As a result of this thinking and perhaps the isolation of research into silos, the Cori's and Warburg's intriguing results were never transposed to the field of exercise metabolism as a challenge to the prevailing dogma of the next approximately 60 years.

It was not until the 1960s that evidence against the notion of low O_2 as a prerequisite for La^- production and accumulation began to gain traction. During the same era, when Wasserman and colleagues were detailing the "anaerobic threshold," other investigations were underway that argued against an O_2 limitation as the causative factor for La^- accumulation. Utilizing a canine gastrocnemius-superficial digital flexor complex (GS) in situ, Stainsby and Welch (Stainsby and Welch 1966) aimed to investigate La^- production in response to various steady contraction rates. Surprisingly, they found that net La^- output by the contracting GS was always transient (Fig. 6). During contractions over periods of 40–60 min, net La^- output peaked and declined within 15–20 min, even becoming net La^- uptake as the contractions continued at a constant stimulation rate. If hypoxia were the cause of La^- production in contracting muscle, then why would net La^- output decline to near zero or even revert to net La^- uptake with continued contractions at an unchanged metabolic rate and unchanged O_2 delivery (Brooks and Gladden 2003)?

To answer this question, Stainsby forged a collaboration with Frans Jöbsis who, with Britton Chance, had invented the surface fluorescence method for NAD^+/NADH detection

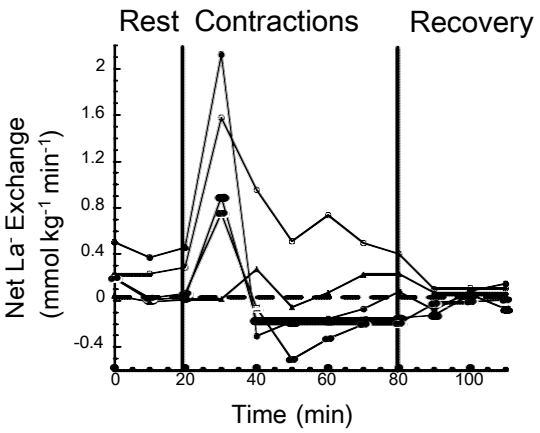

Fig. 6 Net La^- output and uptake across an isolated, blood-perfused, contracting skeletal muscle (canine gastrocnemius-superficial digital flexor complex; $N=5$) at rest and during contractions at 5 twitches per sec over a period of 1 hour, followed by 30 min of recovery. Net La^- output is positive on the *y*-axis and net La^- uptake is negative. Note that net La^- output during contractions peaks at approximately 10 min and then declines and often becomes net uptake as the contractions continue. See text for additional details. Redrawn with permission from Welch and Stainsby (1967)

(Brooks and Gladden 2003). Using this NAD^+/NADH fluorescence technique in the isolated canine GS, Jöbsis and Stainsby (1968) found that the redox pair was highly reduced at rest but became highly oxidized quickly after the onset of contractions, suggesting an adequate O_2 supply for oxidative phosphorylation. If O_2 is inadequate, oxidative phosphorylation will be restrained and the electron transport chain will "back up" thus causing a reduction in the NAD^+/NADH redox couple. Therefore, the net La^- output at contraction onset that Stainsby and Welch had previously observed, could not be explained by hypoxic/dysoxic stimulation of anaerobic glycolysis (Jöbsis and Stainsby 1968). Certainly, these experiments are subject to criticism on several grounds (Gladden 1996) including the fact that the surface fluorometry technique is unable to distinguish between NADH and NADPH (Akerboom et al. 1979; Unkefer et al. 1983; Katz and Sahlin 1988) or to distinguish between redox changes within the mitochondria versus the cytosol (Kushmerick 1983; Paddle 1985; Katz and Sahlin 1988), the former being much more reduced (Williamson et al. 1967; Jones and Sies 2015). Nevertheless, the totality of the Stainsby studies promoted a re-consideration of the relationship between La^- production and hypoxia.

The next major blows against dysoxia as the cause of La^- production by contracting/exercising skeletal muscle involved measures of intracellular partial pressure of O_2 (P_iO_2). Granted that a sufficiently low O_2 level resulting in dysoxia will limit mitochondrial oxidative phosphorylation, a prime question results: how much O_2 is enough to prevent inhibition and allow normal mitochondrial function? Studies

in isolated mitochondria and whole cells contend that PiO_2 in the range of 0.5 to 2.0 Torr is sufficient for maximal mitochondrial oxidative phosphorylation (Chance and Williams 1956; Wilson et al. 1988; Connett et al. 1990; Gnaiger et al. 1995; Gnaiger and Kuznetsov 2002). Direct exploration into potential O_2-limited muscle La^- production was initially provided by measuring myoglobin saturation (and thereby P_iO_2) via cryomicrospectroscopy combined with measures of [La^-] from approximately the same cell populations in isolated, perfused dog gracilis muscle (Connett et al. 1984). At rest and during submaximal twitch contractions, approximating 10 and 70% of peak twitch $\dot{V}O_2$ in this muscle, myoglobin saturation did not decrease in the fi t seconds of stimulation, remained greater than 5 mmHg at all sampling locations during transition from rest to contraction, and remained greater than 2 mmHg in the steady state (Connett et al. 1984). Over the same time course, muscle [La^-] rose from 1.2 mmol kg^{-1} at rest to 2.0 mmol kg^{-1} at the 10% $\dot{V}O_{2peak}$ contraction rate and to 5.7 mmol kg^{-1} at the 70% $\dot{V}O_{2peak}$ contraction rate. While technical limitations of this cryomicrospectroscopy method were more fully revealed in later work (Voter and Gayeski 1995), the conclusions still stand. From their results, Connett et al. (1983, 1984, 1986) concluded that fully aerobic La^- accumulation occurs in red muscle and that the accumulation results from causes other than O_2-limitation of mitochondrial ATP production.

More recently, proton magnetic resonance spectroscopy (^{1}H-MRS) was used to determine myoglobin saturation during single-leg, maximal eff t, quadriceps contractions in humans, concluding similarly that increased La^- efflux from the exercising muscle did not result from inadequate muscle PiO_2 (Richardson et al. 1998). Even while breathing hypoxic gas (12% O_2), local O_2 tension was never low enough to unequivocally limit oxidative phosphorylation (Richardson et al. 1998). Furthermore, there was no correlation between net blood La^- efflux from the exercising quadriceps and PiO_2 across a range of metabolic rates from 50 to 100% of $\dot{V}O_{2max}$. The PiO_2 data from this study are discussed in more detail below.

This evidence strongly suggests that frank dysoxia is not one of the causes of a rise in muscle and blood La^- levels at submaximal exercise intensities. The major, legitimate underlying mechanisms for the increase in [La^-] with increasing exercise intensity have been discussed in detail elsewhere (Gladden 1996, 2004a, b) and include accelerating glycolysis, failure of La^- removal to keep pace with La^- production [e.g., (Brooks 1985a)], and recruitment of fast twitch muscle fibers (Ivy et al. 1980; Armstrong 1988). Accelerated glycolysis will always result in an increase in La^- production because of the near-equilibrium nature of the lactate dehydrogenase (LDH) reaction, and because LDH has a much higher activity than the key regulatory enzymes of the glycolytic and oxidative pathways; thus, La^- is always the end-product of glycolysis (Kane 2014; Rogatzki et al. 2015; Schurr 2017).

Is there any role for hypoxia in the rising [La^-] with increasing exercise intensity?

Despite the fact that La^- accumulation has been observed consistently in the absence of dysoxia, an important question remains: does O_2 play any role whatsoever in increasing [La^-] with increasing metabolic intensity? The short answer is that La^- production is not associated with a clearly defined dysoxic condition in skeletal muscle, but in an apparent inconsistency, La^- accumulation during exercise is significantly affected by perturbations in O_2 delivery during heavy exercise. A fuller answer to the question follows.

Even though exercising skeletal muscle P_iO_2 does not decrease to the level of absolute inhibition of mitochondrial oxidative phosphorylation function, it is nevertheless true that skeletal muscle P_iO_2 declines with increasing exercise intensity. P_iO_2 is a diffi measurement to make during exercise. The most convincing data rely on derived values from myoglobin desaturation as determined via proton nuclear magnetic resonance spectroscopy (^{1}H NMRS) along with accurate values of myoglobin P_{50}. Using best evidence from this technique, how does P_iO_2 change as a function of exercise intensity? In studies of plantar flexion exercise in Jue's laboratory (Molé et al. 1999; Chung et al. 2005), P_iO_2 was reported to decline progressively and linearly with increasing work rate, down to ≈3.1 Torr. However, in the majority of studies, most directed by Richardson and colleagues employing single-leg knee-extensor exercise, which limits activity to the quadriceps femoris muscle group, the nadir is reached at approximately 50–60% of $\dot{V}O_{2max}$ and remains there with further increases in exercise intensity (Richardson et al. 1998, 1999, 2001, 2002). Similar data have been reported by Vanderthommen et al. (2003). Figure 7 (Clanton et al. 2013) combines data from Molé et al. (1999) with those of Richardson et al. (2001) including the most reliable data available for resting P_iO_2 in which Richardson, in collaboration with Carlier (Richardson et al. 2006), employed a 4.0 T magnet to measure myoglobin desaturation. Notably, considering the different muscle groups studied, and the diff ent protocols employed, the results are much more congruent than expected across laboratories and techniques. Overall, these data demonstrate that the largest reductions in P_iO_2 occur relatively early in incrementally increasing exercise, but reach a relative plateau in moderate-to-heavy exercise. Since we know that significant muscle La^- production occurs at exercise levels above a metabolic threshold of approximately 50–60% of $\dot{V}O_{2max}$, it appears that La^- production is not associated with a clearly defined dysoxic condition in skeletal muscle, at least during single muscle group exercise.

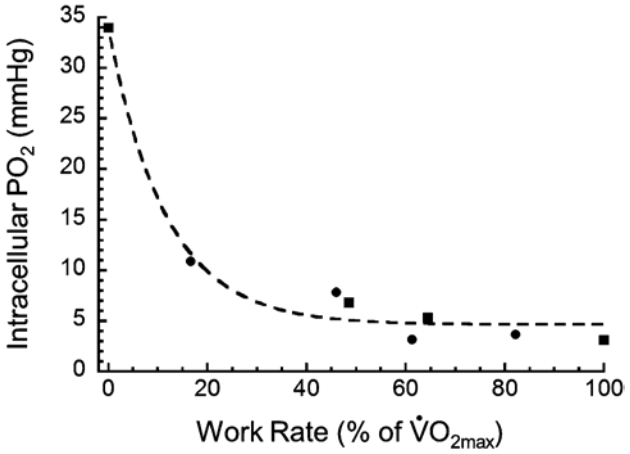

Fig. 7 Intracellular PO_2 (P_iO_2) versus metabolic rate using combined data of Molé et al. [(1999); solid squares] and Richardson et al. [(2001); solid circles]. Data from Richardson et al. (2006) working in Carlier's lab were used as the resting value for both sets of data, so the square is overlying a circle. In addition, note that the reported P_iO_2 value at 100% of $\dot{V}O_{2max}$ was the same for both data sets, so again, the square is overlying the circle. Reprinted with permission from Clanton et al. (2013)

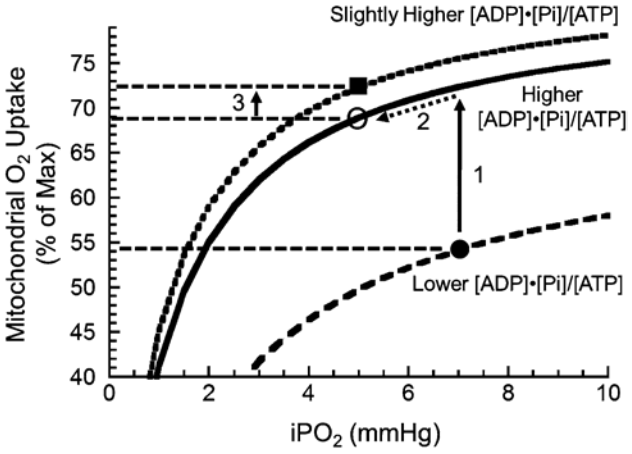

Fig. 8 Hypothetical pattern of metabolic dependence (La^- production) on intracellular PO_2 (PiO_2) during the transition from one exercise work rate to another. With increase in work rate (arrow 1), [ADP]·[Pi]/[ATP] increases. However, intracellular PO_2 will likely also decrease with increased work rate (arrow 2). As a result, O_2 uptake would be transiently insufficient to supply ATP at the required rate, leading to a further increase in [ADP]·[Pi]/[ATP] (arrow 3, from open circle to solid square) for adequate stimulation of oxidative phosphorylation. The connection to La^- metabolism is that the increases in [ADP]·[Pi]/[ATP] simultaneously accelerate glycolysis and, therefore, La production (see text for additional details).

Reprinted with permission from Clanton et al. (2013)

Yet, paradoxically, La^- accumulation during heavy whole-body exercise is signifi y aff by perturbations in O_2 delivery. Wasserman reviewed this evidence (Wasserman 1984; Wasserman and Koike 1992), much of which dates well back before the turn of the century. When subjects inspire gas with a lower PO_2, blood La^- levels are elevated during exercise [e.g., (Lundin and Ström 1947)]. For the same heavy work rate, blood [La^-] is higher in isovolemic anemia as compared to the normal, control state [e.g., (Woodson et al. 1978)]. Adding carbon monoxide (CO) to the inspired gas sufficient to bind 15–20% of the O_2 binding sites on hemoglobin also increases blood [La^-] during exercise (Vogel and Gleser 1972). A reduction in circulating blood volume in normal subjects results in an increase in exercise [La^-] [e.g., (Fortney et al. 1981)]. Patients with a low cardiac output due to disease (Huckabee and Judson 1958), as well as normal subjects subjected to beta-blockade via propranolol (Twentyman et al. 1981) also display an exaggerated blood [La^-] response to exercise. Administration of an inotropic drug to cardiac patients with low exercise tolerance reduces the blood [La^-] increase during exercise (Siskind et al. 1981). Finally, it has been well demonstrated that breathing hyperoxic gases will cause a reduction in blood [La^-] during exercise [e.g., (Hogan et al. 1983)].

Therefore, even in the absence of apparent dysoxia, glycolytic metabolism and La^- production are at least somewhat O_2 dependent. How could this be? Certainly, one possibility is that blood catecholamine concentrations are higher during hypoxic conditions (Mazzeo et al. 1991; Richardson et al. 1998; Mazzeo and Reeves 2003). However, an additional hypothesis relies on cellular changes in the [ADP]·[Pi]/[ATP] ratio that occur in high metabolic states as a mechanism for acute responses to hypoxia. This idea is illustrated in the hypothetical example shown in Fig. 8 (Clanton et al. 2013). Suppose an individual is exercising at approximately 55% of $\dot{V}O_{2max}$ (solid circle) and then transitions to a higher work rate, just above 70% $\dot{V}O_{2max}$. Abundant evidence demonstrates that at the higher work rate, cytosolic [ADP] and [Pi] will be elevated, while [ATP] will perhaps be slightly decreased, leading to an increase in [ADP]·[Pi]/[ATP]. As such, the $\dot{V}O_2$–P_iO_2 curve will be shifted upward, as indicated by arrow 1 and extensively reported by Wilson and colleagues (Wilson et al. 1979, 1988; Wilson 1994). However, based on the results of Molé et al. (1999) and Richardson et al. (2001), there will also be a decrease in P_iO_2 (arrow 2). This small reduction in P_iO_2 would then result in movement down the $\dot{V}O_2$–P_iO_2 curve, thus reducing $\dot{V}O_2$ (open circle). This reduction in $\dot{V}O_2$ below the required level would result in additional accumulation of ADP and Pi, elevating [ADP]·[Pi]/[ATP] further, shifting the mitochondria to yet a higher $\dot{V}O_2$–P_iO_2 curve (arrow 3). Ultimately, shifts to higher [ADP]·[Pi]/[ATP] ratios as energy consumption increases could lead to an equilibrium state, where P_iO_2 remains relatively constant, but $\dot{V}O_2$ can continue to increase. This is consistent with observations of the

relatively constant P_iO_2 in moderate-to-severe exercise, as shown in Fig. 7.

What connects the preceding discussion to increased glycolysis and La^- production in non-dysoxic muscle? The link is that any increase in [ADP]·[Pi]/[ATP] required to elevate $\dot{V}O_2$ is a very powerful stimulus for glycolytic activity, independent of any changes in P_iO_2. Furthermore, as one moves to higher [ADP]·[Pi]/[ATP] levels, the O_2 dependence of the $\dot{V}O_2$–P_iO_2 curve becomes greater, as reviewed by Gnaiger et al. (2001), and Scandurra and Gnaiger (2010). With changes in available P_iO_2, the system can sustain a constant $\dot{V}O_2$, but there will be resultant elevations in [ADP]·[Pi]/[ATP] which, in turn, stimulate glycolysis. The system is O_2 sensitive, because as available O_2 decreases or increases, it can increase or decrease the necessary elevations in [ADP]·[Pi]/[ATP] and thus also increase or decrease the glycolytic rate. There is experimental evidence to support this hypothesis as a mechanism for O_2 dependence of La^- production [e.g., (Hogan et al. 1992a, b; Haseler et al. 1998; Richardson et al. 1998)]. These data support the hypothesis that it is not a limited oxidative phosphorylation engendered by a low dysoxic PiO_2 that drives La^- production, but rather the elevations in [ADP]·[Pi]/[ATP] required to sustain metabolic rate and to drive mitochondrial respiration at the extant low P_iO_2.

One might ask if this requisite increase in La^- production and blood [La^-] represents an additional energy expenditure and, therefore, a decrease in efficiency. Quantification of energy expenditure due to La^- accumulation is fraught with diffi (Gladden 2004a). The [La^-] varies among muscles due to differing degrees of recruitment for a given activity and blood [La^-] varies depending on the tissue being drained. Even if an average [La^-] could be determined, the dilution space is undefi and non-uniform. Neverthe- less, using a series of assumptions, calculations based on the results of Bransford and Howley (1977) to account for energy provided by anaerobic glycolysis (i.e., net La^- accumulation) lead to an estimated decrease in effi of about 2% at approximately 90% of $\dot{V}O_{2max}$. Note, however, that this calculation does not mean that the use of ATP synthesized via anaerobic pathways is inherently less efficient than the use of aerobically synthesized ATP (Gladden and Welch 1978). Careful measures of delta efficiency (Gaesser and Brooks 1975) at submaximal work rates (almost entirely aerobic) versus very heavy work rates (signifi anaero- bic contribution) suggest that delta effi decreases with increasing work rate, regardless of the contribution of anaerobic energy pathways (Gladden and Welch 1978).

It is very important to note here that if an individual is in a steady state in which the [La^-] is unchanging, there is no additional energy being provided above that determined via O_2 consumption (Brooks 2012). Even though La^- is formed continuously from glycolysis, it is largely combusted as a fuel, thereby being accounted for by the measurement of O_2 consumption (Brooks 2012).

Lactate and fatigue

Exercise and skeletal muscle fatigue are complex processes with numerous potential causes ranging from central nervous system function to the activity of myosin at the individual cross-bridge level (Gladden 2016). However, during intense muscle contractions or exercise, [La^-] increases dramatically and contributes significantly to a decrease in pH within the active muscles and in the case of whole-body exercise, the blood. During intense dynamic exercise, intracellular water [La^-] can reach 30 mM and intracellular pH can decline to the range of 6.2–6.5 (Sahlin et al. 1976; Debold et al. 2016). For a discussion of La^-'s role in acidosis, see a following section in this review. A complete discussion of the potential role of La^- and pH in fatigue would require a full review of its own, so we offer only an abbreviated discussion here.

Classic support for lactic acidosis as a cause of fatigue in human exercise was provided by imperfect studies that employed prior exercise to elevate [La^-] and decrease pH [e.g., (Klausen et al. 1972; Karlsson et al. 1975; Bangsbo and Juel 2006)]. As a detailed example, Yates, Gladden and Cresanta (Yates et al. 1983) had participants perform a 1-min maximal eff t cycle ergometer task prior to isometrically maintaining 40% of maximum voluntary contraction (MVC) force to exhaustion with the elbow flexors. The prior exercise elevated blood [La^-] to 11 mM and decreased arterialized blood pH to 7.20. As a result, isometric endurance was reduced by 25%, although there was no effect of the prior exercise on MVC, peak rate of force increase, peak rate of force decrease, one-half contraction time, or one-half relaxation time.

Certainly, the lactate anion (La^-) could be a fatigue agent. For example, Hogan et al. (1995) reported that infusion of isolated canine gastrocnemius muscles with blood [La^-] at 12–15 mM caused a 15% decrease in twitch tension, even though muscle pH was not altered from control conditions. Supportive findings have been published for isolated muscles in vitro (Spangenburg et al. 1998), skinned muscle fi (Andrews et al. 1996), and sarcoplasmic reticulum vesicles [e.g., (Favero et al. 1995; Spangenburg et al. 1998)]. In addition, Samaja et al. (1999) found that La^- apparently depressed developed pressure irreversibly in Langendorff perfused rat hearts; overall, the eff of elevated [La^-] were more deleterious than the effects of high [H^+]. In con- trast, other investigators have reported effects of 5% or less on muscle contractility in skinned mammalian muscle fibers [e.g., (Posterino et al. 2001)]. In fact, Allen et al. (2008) have summarized evidence against La^- and dismissed its potential role as a fatigue agent, largely on the basis of studies with skinned muscle fibers. Our view is that while the impact of

high [La^-] *per se* in the fatigue phenomenon has been heavily challenged by investigations in vitro, it remains feasible that the La^- anion has a small but potentially significant deleterious effect in intact systems such as perfused skeletal muscle and heart and intact humans. We also note that while a 5% fatigue eff does not argue for a central role in the fatigue process, it is also not negligible.

What about acidosis and fatigue? In 2006 (Bangsbo and Juel 2006; Lamb and Stephenson 2006) and again in 2016 (Fitts 2016; Westerblad 2016), the role of decreased pH in fatigue was directly debated. Historically, lactic acid was viewed as one of the major causes of muscle and exercise fatigue (Fitts 1994). As early as 1929, Hill and Kupalov (Hill and Kupalov 1929) proposed lactic acid accumulation as the cause of fatigue during twitch contractions of frog sartorii at temperatures in the range of 14–20 °C. More recently, it has been noted that the negative effects of low pH on peak isometric force in studies of isolated muscle fibers are much smaller at higher temperatures such as 30 °C [see (Fitts 2016)]. However, research from the Fitts laboratory (Knuth et al. 2006; Nelson et al. 2014; Fitts 2016) has found that low pH (down to 6.2) at 30 °C depressed velocity and power of skinned muscle fibers by 30–35%! These deleterious effects were even greater in the presence of inorganic phosphate concentration ([Pi]) elevated to the level seen during intense exercise. The fatiguing effect of combined low pH and high [Pi] may be further increased in the presence of suboptimal [Ca^{2+}] such as occurs during fatigue (Fitts 2016). Debold et al. (2016) have recently reviewed the fatiguing effects of elevated [H^+], elevated [Pi], and decreased Ca^{2+} sensitivity determined from both isolated muscle fiber studies and investigations at the level of the contractile protein, myosin, and the muscle regulatory proteins, troponin, and tropomyosin.

Despite the evidence outlined in the previous paragraph and the prior dogma that acidosis is a major cause of fatigue, there is a plethora of data opposing this position (Lamb and Stephenson 2006; Westerblad 2016). Westerblad (2016) noted that there are several studies (Sahlin and Ren 1989; Westerblad and Allen 1992; Degroot et al. 1993) which failed to find a temporal correlation between acidosis and a decrease in muscle function and observed that fatigue can occur in the absence of acidosis. Another study from Westerblad's laboratory (Bruton et al. 1998) reported that mouse muscle fibers did not fatigue more rapidly when they had been pre-acidified by 0.4 pH units. However, the major challenge to the notion of lactic acid as a fatigue agent has come from studies of isolated rat soleus muscles (Nielsen et al. 2001; De Paoli et al. 2007) and mechanically skinned rat extensor digitorum longus (EDL) muscle fibers (Pedersen et al. 2004); [see (Lamb and Stephenson 2006) for additional references]. These studies propose that increased [La^-] actually counteracts fatigue. These investigations first note that in one type of fatigue K^+ builds up in the transverse-tubular (t) system and in the microenvironment of stimulated muscle fibers. This increase in [K^+] leads to sarcolemmal depolarization which may slow or even prevent the recovery of Na^+ channels from inactivation. This scenario would lead to a reduced amplitude of action potentials or maybe failure, thereby reducing muscle force production (Lamb and Stephenson 2006). In 2001, Nielsen et al. (2001) observed that incubation of isolated rat soleus muscles in 11 mM K^+ reduced tetanic force by 75%, but this deficit was completely eliminated upon addition of 20 mM lactic acid. In 2004, Pedersen et al. (2004) performed a sophisticated study of mechanically skinned rat EDL muscle fi fi that in the presence of Cl^-, acidosis decreased Cl^- permeability thus preserving the propagation of action potentials into the t-tubules despite Na^+ channel inactivation. Subsequently, de Paoli et al. (De Paoli et al. 2007) reported that a combination of 20 mM lactic acid and epinephrine off t an 85% reduction in force production by rat soleus muscles in the presence of an external 15 mM [K^+]. The overall conclusion of these researchers and others is that lactic acidosis is actually beneficial in delaying the onset of fatigue (Lamb and Stephenson 2006).

What is the bottom line? During exercise intensities up to maximal lactate steady state (approximately 4 mM blood [La^-]), La^- likely provides an advantage, since it offers a means of distributing substrate and perhaps coordinating redox state among tissues (see discussion in a later section) with little change in pH (Gladden and Hogan 2006). The ability to produce and accumulate La^- is also critical to maximal effort performance particularly during periods of approximately 30–90 s (Gladden 2004a; Brooks 2012). In this context, La^- plus NAD^+ formation from pyruvate and NADH via the LDH reaction regenerates the required substrate (NAD^+) for the glyceraldehyde dehydrogenase reaction, permitting continued rapid glycolysis with the production of 1.5 ATP per glucosyl unit derived from glycogen. However, what about the fatigue effects of La^- and its concomitant acidosis? In our view, many studies of isolated muscles and fi although not all, disagree not on whether lactic acidosis is a negative factor for muscle function but on the primacy of acidosis as a cause of fatigue during high intensity contractions. In other words, lactic acidosis is likely not the primary cause of fatigue, but it is nevertheless an important factor. For example, even as he argues against the role of acidosis in skeletal muscle fatigue, Westerblad (2016) notes decreases of about 10% in maximum isometric force in skinned fiber studies due to acidosis. As Cairns and Lindinger (Cairns and Lindinger 2008) have noted, high intensity fatigue is likely due to multiple ionic interactions. Perhaps, a rundown of the transsarcolemmal K^+ gradient is the major factor (Cairns and Lindinger 2008), but H^+ and even La^- likely have negative effects, particularly in

combination with other metabolites such as Pi (Debold et al. 2016; Fitts 2016). In addition, while there are views to the contrary, there is evidence that acidosis inhibits glycolysis (Parolin et al. 1999). At the whole-body level, it is possible that low tissue pH causes pain and that blood acidosis encourages arterial hemoglobin desaturation that can diminish aerobic performance (Gladden and Hogan 2006). Finally, alkalosis via sodium bicarbonate supplementation generally improves high intensity performance by 2–3% (Siegler et al. 2016). In toto, our conclusion is that both La^- and H^+ likely have some negative eff on exercise performance, albeit they are not necessarily the major causes of fatigue.

Cell-to-cell lactate shuttle

With evidence mounting that hypoxia was not the primary cause of La^- production, and that La^- could in fact be used as a fuel by numerous tissues including both resting and contracting skeletal muscle, a paradigm shift occurred in the conceptualization of La^- metabolism during exercise. This paradigm shift was impelled (Brooks 1985b) and expounded by Brooks (2007, 2009, 2016), beginning in 1985 (Brooks 1985b) when he introduced the lactate shuttle, now known as the intercellular or cell-to-cell lactate shuttle. Key support for the cell-to-cell lactate shuttle came from a plethora of isotopic tracer studies by the Brooks laboratory which tracked the fl w of carbon intermediates during rest, exercise, and recovery in combination with arteriovenous measures of net La^- exchange, and muscle biopsies (Bergman et al. 1999, 2000; Brooks 2002). In contrast to the dogma of the 19th and most of the twentieth century, which viewed La^- as a dead-end, fatigue-causing waste product, the now well-supported tenet of the cell-to-cell lactate shuttle emphasizes La^- as an energy intermediate that can be formed in tissues undergoing accelerated glycolysis and subsequently distributed throughout the body to be taken up as an energy substrate by oxidizing tissues or as a precursor for gluconeogenesis or glycogenesis. The whole of the evidence gleaned from studies of exercise metabolism is overwhelmingly supportive of this theory, and it is now the framework within which La^- metabolism is studied. Both the evidence and the concept have been extensively reviewed previously (Gladden 1996, 2004a, b, 2008b; Brooks 2000, 2002, 2007, 2009, 2016; Brooks and Gladden 2003), and will, therefore, be only summarized here. As extolled by Brooks (2007) and emphasized here, this theory is based on La^- exchanges: (a) between low-oxidative and high-oxidative skeletal muscle fi allow- ing La^- to be oxidized within individual working muscles; (b) among contracting skeletal muscles producing La^- and other distant skeletal muscles that are at a lower metabolic rate (rest or submaximal exercise); (c) between contracting skeletal muscles and the heart; and (d) between tissues of net La^- release and gluconeogenesis or glyconeogenesis. Essentially, La^- is exchanged by tissues throughout the body, importantly including the brain (see below) as illustrated in Fig. 9.

Approximately 30 years after the work of Stainsby, Welch, and Jöbsis (Stainsby and Welch 1966; Welch and Stainsby 1967; Jöbsis and Stainsby 1968), Pagliassotti and Donovan (1990) and Gladden et al. (Gladden and Yates 1983; Gladden 1991; Gladden et al. 1992, 1994; Hamann et al. 2001; Kelley et al. 2002) provided clear evidence of net La^- uptake and metabolism by isolated, perfused skeletal muscle under both resting and contracting conditions. Among many studies in humans also showing net La^- release by some muscles with net La^- uptake by other muscles, those of Richter et al. (1988) and van Hall et al. (2003) are illustrative. In contracting muscle displaying a net uptake of La^-, the vast majority of the La^- is oxidized as a fuel (Mazzeo et al. 1986; Stanley et al. 1986; Bergman et al. 2000; Kelley et al. 2002).

La^- clamp studies in humans further illustrated the importance of La^- as both a fuel and a gluconeogenic substrate. Using a combination of [3-^{13}C]lactate, $H^{13}CO_3^-$ and [6,6-$^2H_2$]glucose tracers, Miller et al. (2002a, b) maintained arterialized venous plasma [La^-] at approximately 4 mM in subjects exercising at a moderate exercise intensity (~ 55% $\dot{V}O_{2peak}$). Overall, they (Miller et al. 2002a) found a signifi increase in La^- oxidation accompa- nied by a decrease in glucose oxidation, suggesting that La^- competes successfully with glucose as a carbohydrate fuel source. In addition, the La^- clamp data of Miller et al. (2002b) and Roef et al. (2003) taken together demonstrated that La^- was an important gluconeogenic precursor at both low and moderate exercise intensities. These La^- clamp studies, along with many other investigations of diff ent types, emphasize the role of La^- as arguably the most important substrate for gluconeogenesis, and of course, the key role of the liver [e.g., (Connor and Woods 1982; Nielsen et al. 2002)] as an important player in the cell-to-cell lactate shuttle.

Studies of cardiac muscle, a highly oxidative tissue, show that under conditions of elevated blood [La^-], elevated myocardial blood flow, and elevated myocardial metabolic rate; La^- can become the preferred fuel, accounting for as much as 60% of the myocardial substrate (Gertz et al. 1988; Stanley 1991). Essentially, all of this La^- taken up by the heart is oxidized as a fuel (Stanley 1991). The paramount importance of La^- as a fuel source for the heart is further seen when the heart is stressed via β-adrenergic stimulation (Lopaschuk et al. 2010), increased afterload (Wyatt et al. 1977), chronotropic challenge (Camici et al. 1989; Bagger et al. 2000), and shock (Kline et al. 2000).

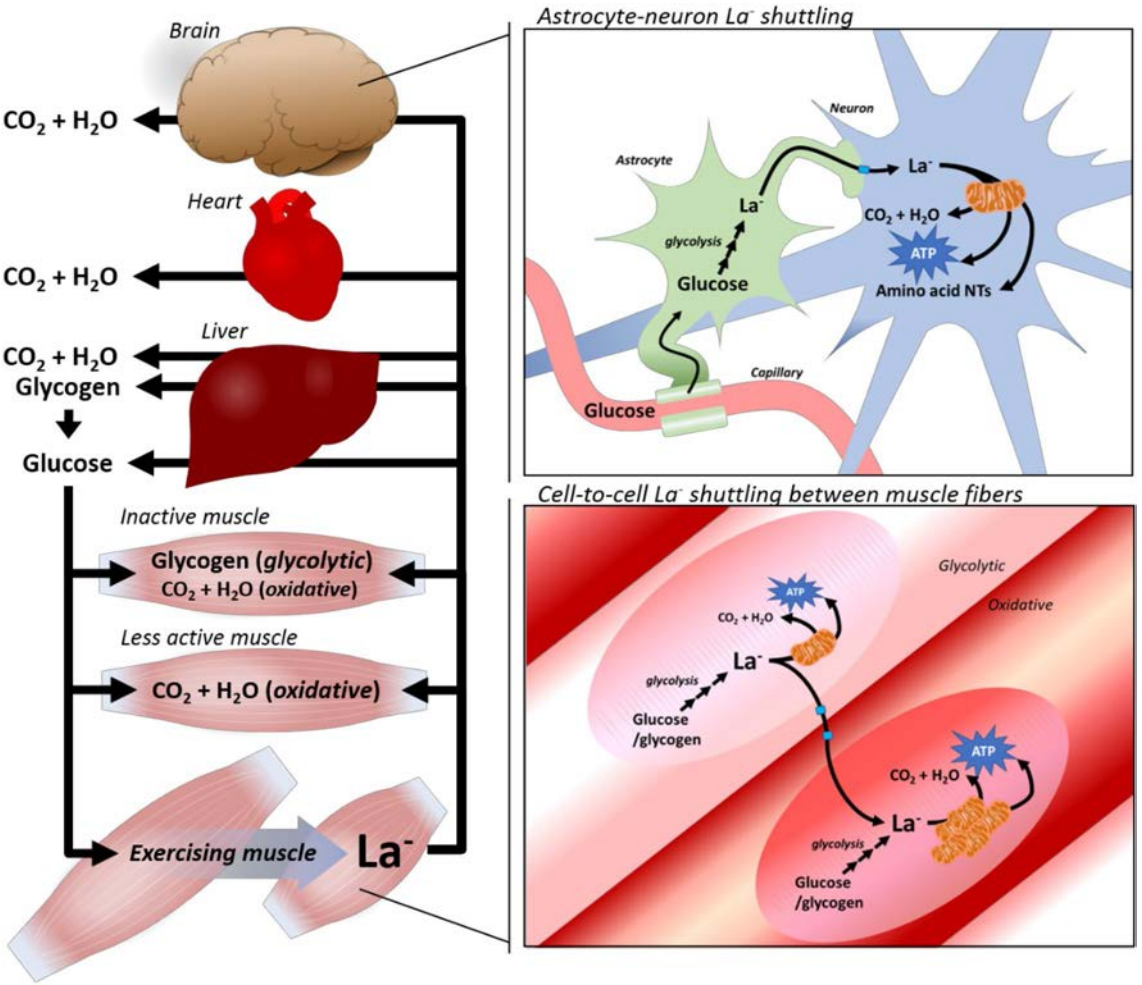

Fig. 9 Cell-to-cell lactate shuttle, and the astrocyte–neuron lactate shuttle. Based on (Pellerin et al. 1998, Brooks 2002). As the rate of demand for ATP increases in exercising skeletal muscle, La^- accumulates and efflux from muscle fibers increases by means of MCTs (lower left). Within the exercising muscle, La^- is shuttled among heterogeneous fiber types, from glycolytic to oxidative fibers via MCTs (small blue squares in lower right). Alternatively, La^- may be carried in circulation to less, or slightly active skeletal muscle, where it is oxidized ($CO_2 + H_2O$) in oxidative fibers. In inactive glycolytic skeletal muscle fibers, La^- is converted chiefly to glycogen, with some oxidized in oxidative fibers. La^- is also carried in the blood to the liver, where it is either oxidized, or converted to glucose via gluconeogenesis. The newly formed glucose is either stored as glycogen, or released back into circulation (Cori cycle), where it can return to, for example, exercising muscle. Circulating La^- is also readily oxidized in cardiac fibers and brain. Within the brain, the astrocyte–neuron lactate shuttle (upper right panel) is the process by which astrocytes take up glucose from the microcirculation, converting the glucose to La^- via glycolysis. This astrocytic La^- diffuses from the astrocyte to an adjacent neuron by means of MCTs. In the neuron, the La^- is oxidized at the mitochondria ($CO_2 + H_2O$) to resynthesize ATP, and/or used for the cataplerotic production of amino acid neurotransmitters (NTs; e.g., glutamate, aspartate, and GABA)

The brain: part of the cell-to-cell lactate shuttle and site of the astrocyte–neuron lactate shuttle

While myocardial tissue has long been appreciated as a metabolic omnivore, capable of readily utilizing a variety of substrates, the long-standing model for brain metabolism identifies glucose as the predominant energy substrate in the adult brain (Sokoloff 1989). However, the newborn mammalian brain has been shown to utilize elevated blood La^- as a substrate, and additionally, consume circulating ketones during breastfeeding to meet a substantial portion of energy requirements (Pellerin 2010). Subsequent investigations with ^{13}C nuclear magnetic resonance spectroscopy explored La^- metabolism in the resting brain, finding that with baseline physiological blood [La^-]s, approximately 10% of brain energy needs are met via oxidation of La^-, with the ability to oxidize more as circulating La^- levels rise (Boumezbeur et al. 2010).

In hindsight, the ability for neuronal tissue to function on alternate substrates seems intuitive for organismal success. As first suggested by the laboratory of Holmes and Holmes in the 1920s (Holmes and Holmes 1925a, b, 1926, 1927;

Holmes 1930), later including Ashford and Holmes (1929), and recently reviewed by Schurr (2017), it is also now clear that in addition to glucose, the brain takes up La^-. However, a full appreciation of La^- as a viable substrate for the brain has only recently been realized. In this context, the recycling of La^- in exercising skeletal muscle served as an important model for exploration within the brain. Global brain uptake of La^- during exercise was reported as early as at least 1972 (Ahlborg and Wahren 1972). Almost 30 years later, Ide et al. observed an increase in La^- uptake by the brain during both exercise (Ide et al. 1999) and recovery from exercise (Ide et al. 2000). It is now well established that with intense exercise, the brain is activated and this, in conjunction with the accompanying elevated arterial blood [La^-], leads to a large increase in net La^- uptake which in fact becomes greater than glucose uptake (Ide and Secher 2000; Quistorff et al. 2008). At the conclusion of exercise to exhaustion, blood [La^-] was shown to be increased, while concentrations within the cerebral spinal fl and brain were equivalent to baseline levels, ruling out La^- accumulation and indicating La^- utilization as a substrate (Dalsgaard et al. 2004). In euglycemic adult males, infusion of sodium La^- to levels mimicking mild exercise elicited net La^- uptake, with a concomitant reduction in glucose uptake of 17% versus control (Smith et al. 2003). Clearly, global brain La^- metabolism is a part of the cell-to-cell lactate shuttle. There is also evidence that regular physical activity slows age-related memory impairment (Tsai et al. 2016) and brain tissue loss with aging (Colcombe et al. 2003) leading to the notion that La^- may serve as a link between exercise and improvement of cognitive functions (Proia et al. 2016). Recently, Hashimoto et al. (2017) observed a significant correlation between executive function and La^- extraction by the brain following high intensity interval exercise. On the basis of their findings, they (Hashimoto et al. 2017) proposed that exercise training should be above the LT to foster brain activity.

Broadly, two conceptual hurdles hindered the consideration of La^- as a fuel for the brain. First, a limited appreciation that aerobic glycolysis, specifi y cerebral glycoly- sis, terminates in La^- formation, and second, that La^- is an important substrate for the brain. In consideration of the first point, work from investigators in both skeletal and cardiac tissue provided a framework by defining the cell-to-cell lactate shuttle hypothesis, stating that La^- is shuttled from its place of formation to distant sites for mitochondrial oxidation (Brooks 1985b, 1998; Brooks et al. 1999b; Van Hall 2000; Chatham et al. 2001; Gladden 2004b). Further contributions from the field of muscle metabolism affirmed that La^- is always the end-product of glycolysis (Gladden 2004b; Rogatzki et al. 2015), and as elegantly summarized by Schurr, is the most feasible path when considered thermodynamically, chemically, and spatially (Schurr 2006). A second hurdle to overcome was development of an experimentally supported framework to explain how La^- could be utilized in the brain. Following the footsteps of the cell-to-cell lactate shuttle framed by Brooks (see discussion above), this conceptual framework has emerged as the Astrocyte–Neuron Lactate Shuttle (ANLS) hypothesis (Fig. 9), and in recent decades a body of evidence has grown to support La^- as an oxidizable substrate that is not only taken up from the blood but one that is also shuttled within the brain. This is a major arena of scientific discovery for which studies of metabolism during exercise paved the way.

Following the introduction of La^- shuttling by the Brooks group, early evidence suggested that La^- could support both normal synaptic function, and reactivate glucose-depleted synaptic quiescence in hippocampal slices (Schurr et al. 1988). This was furthered to conclude that La^-, rather than glucose (Schurr et al. 1997b), is shuttled to post-hypoxic neurons to recover function (Schurr et al. 1997c) and that the La^- is produced in glial cells (Schurr et al. 1997a). At approximately the same time that the supportive role of La^- was being elucidated in the brain, the ANLS framework for the contemporary view of neuroenergetics was posited (Magistretti and Pellerin 1996). This hypothesis suggests that in support of increased neuronal activity, astrocytes increase their rate of glucose uptake, glycolysis, and the subsequent release of La^- into the extracellular space, where it is then taken up by neurons for oxidation.

The conceptual evolution of La^- shuttling in brain cells has grown in complexity over the last two decades. Conventional metabolic theory suggests that the action potentials of neurons result in an influx of Na^+ ions and an efflux of K^+ ions activating Na^+-K^+-ATPase pumps. This consumes ATP while producing ADP, inorganic phosphate, and AMP, each of which further stimulate glycolysis, the TCA cycle, and oxidative phosphorylation. In contrast, the ANLS hypothesis describes a supportive metabolic milieu in which stimulated neurons release glutamate as an excitatory neurotransmitter (Pellerin and Magistretti 1994, 2003; Pellerin 2003; Bélanger et al. 2011). Increased activity at glutaminergic synapses leads to glutamate uptake by astrocytes through a co-transporter mechanism that carries one glutamate, three Na^+, and one H^+ inward, while a single K^+ is exported out of the cell (Attwell 2000; Tsacopoulos 2002). In the new model, it is this transport activity which triggers the Na^+–K^+-ATPase activity to restore ionic balance, thus consuming ATP and triggering astrocyte metabolism. In the intracellular space, glutamate is converted to glutamine in an ATP-dependent reaction that is catalyzed by an astrocyte-specific glutamine synthetase (Mason 2017). Glutamine is released back to the extracellular space, where it is taken up by neurons and reconverted to glutamate by glutaminase (Bélanger et al. 2011). This process replenishes the pool of excitatory neurotransmitter, while the energy cost of the ATPase pumps and glutamine synthesis generate the

classic stimuli for glycolysis and subsequent La^- production: decreased [ATP], increased [ADP], increased [Pi], and increased [AMP].

Within the ANLS hypothesis, there is a division of labor, whereby the astrocyte may be viewed as a supportive cell type responsible for the glycolytic production of La^-, which is subsequently processed oxidatively by the neurons when energy demands are increased. Several tenets of the ANLS hypothesis are corroborated by the identification of key players in the shuttling mechanism. First, neuronal glutamate release is facilitated by the excitatory amino acid transporters (EAATs) with EAAT3 expressed exclusively in neurons and glutamate uptake via the glia-specific EAAT1 and EAAT2 transporters (Bélanger et al. 2011; Mason 2017). This neuro-stimulatory traffi king of glutamate provides the impetus for increased metabolism and the shuttling of La^- within the brain.

Lactate dehydrogenase (LDH), the enzyme responsible for the interconversion of pyruvate and La^-, is expressed in fi e isozymes ranging from oxidative (LDH-1) to glycolytic (LDH-5). The LDH-5 subunit possesses a higher maximal velocity (V_{max}) and is more commonly expressed in glycolytic tissues, often termed "muscle type". LDH-1 is inhibited by pyruvate; thus, increased La^- in the presence of the LDH-1 isozyme is posited to favor return to pyruvate and ultimately oxidation; this isozyme is often termed "heart type". LDH isozyme distribution correlates with substrate utilization with the LDH-5 isozyme predominating in astrocytes, while LDH-1 is more prevalent in neurons (Bittar et al. 1996) [note that a causative role for LDH isoforms remains a matter of debate (Rogatzki et al. 2015)]. Additional support is observed in the distribution of the monocarboxylate transporters (MCTs) in the brain. MCTs allow for monocarboxylate movement across cell barriers down their concentration gradients (see discussion below). MCT2 transporters are localized to neurons and associated with net La^- uptake, while the MCT4 subtype is expressed in astrocytes and associated with net La^- release. High-resolution immunocytochemical methods from rodent studies explored MCT distribution in distinctly oxidative versus glycolytic compartments and corroborated this MCT distribution lending support to the shuttling of glycolytically produced La^- from the astrocyte to the oxidative neuron (Bergersen 2007). However, to repeat a caveat from above, these locational–metabolic relationships are correlative and not necessarily causal.

A great deal of experimental support has been generated for the ANLS hypothesis of brain metabolism; however, it has not been unanimously accepted (Chih et al. 2001; Chih and Roberts 2003). A detailed summary was provided by Pellerin and Magistretti (Pellerin and Magistretti 2011) with review of both supportive, and critical, investigations concerning the ANLS hypothesis concluding that the ANLS model is a useful framework for further investigation to better understand the coupling of neuronal activity and energetics.

Lactate and volume transmission

In the CNS, focused synaptic action via "wired" transmission of electrical–chemical signals is a well described means by which cells communicate quickly. Another widespread form of inter-CNS communication involves activation of neurotransmitter systems over relatively large volumes and distances of the brain (Agnati et al. 1995; Zoli and Agnati 1996). Dubbed "volume transmission" by Agnati and Fuxe in 1986 (Fuxe et al. 2010), this mode of communication relies on diffusion and convection of chemical modulators through the brain's extracellular fl and cerebrospinal fluid. As such, volume transmission is conceptually similar to paracrine/autocrine signaling, and tends to elicit changes more slowly than wired transmission.

The list of potential agents of volume transmission is long, and includes monamines (Fuxe et al. 2010), glutamate (Okubo and Iino 2011), and l-DOPA (Gjedde et al. 1993; Ugrumov 2009). Evidence also suggests that La^- may act as a volume transmitter (Lauritzen et al. 2013; Tang et al. 2014; Morland et al. 2015). Bergersen & Gjedde (Bergersen and Gjedde 2012) forwarded the idea that by acting as a volume transmitter through either the GPR81 receptor (and subsequently altering intracellular cAMP levels), or by adjusting intracellular NAD^+/NADH redox ratios (see section on whole-body cytosolic redox coupling via La^-), La^- could link the maintenance of brain energy status with cerebral blood flow. In this way, La^- would be viewed as a mediator of metabolic information rather than, or perhaps in addition to, a metabolic substrate.

Lactate transport across membranes

We now understand La^- as a ubiquitous metabolic intermediate operating as both the terminal product of glycolytic metabolism and the intermediary to complete oxidation of glucose by oxidative phosphorylation. In this framework, it is important to understand how La^- is rapidly mobilized from sites of production and taken up as a source of oxidizable or gluconeogenic/glyconeogenic substrate in remote tissues. For much of the last century, it was thought that La^- traveled in and out of muscle via simple diffusion [see (Gladden 1989) for review]. However: (1) large differences in [La^-] between blood and muscle during exercise and infusion; (2) saturation of transmembrane flux in response to an increasing [La^-] gradient; and (3) inhibition of La^- transport by chemical agents [see (Gladden 1989) for review] stimulated the search for a transporter. In the 1970s, the Halestrap laboratory reported that cyanohydroxycinnamate (CHC)

specifically inhibited the proton-linked transport of La^- and pyruvate into human red blood cells (Halestrap and Denton 1974; Halestrap 1976). Over succeeding years, kinetics of the transporter as well as substrate and inhibitor specificities were characterized in detail by both the Deuticke (Deuticke 1982) and Halestrap (Poole and Halestrap 1993) groups. In 1990, Roth and Brooks (Roth and Brooks 1990a, b) were the first to study La^- flux into sarcolemmal vesicles in skeletal muscle. They demonstrated that sarcolemmal La^- transport was concentration dependent, saturable, stereospecific, competitively inhibited by other monocarboxylates, blocked by known inhibitors of monocarboxylate transport, sensitive to temperature, and stimulated by $[H^+]$ gradients. Their studies were at the forefront of an exponential increase of research on membrane transport of La^-.

Abundant research has now revealed that transmembrane La^- movement is broadly accomplished by the Solute Carrier Family 16 (SLC16) proteins, one of 52 SLC families (Jones and Morris 2016) with over 300 members (Merezhinskaya and Fishbein 2009). Specifically, MCTs are a family of transporters including 14 related proteins, only the first four of which are particularly important in the transport of La^-, pyruvate, and ketone bodies (Halestrap and Wilson 2012; Halestrap 2013). The rapidity of La^- exchange is dramatically enhanced by the activity of the MCTs. MCTs 1–4 are energy independent (i.e., passive) and facilitate metabolite fl down concentration gradients in conjunction with H^+ ions (Halestrap and Wilson 2012). Concurrent advances in our understanding of the MCT family enabled the conceptual shift in our grasp of La^- as an intermediate in metabolism. Much of what is known concerning the transport of pyruvate and La^- into and out of cells came from investigations with red blood cells (RBCs) (Skelton et al. 1995, 1998), particularly the work of the Halestrap lab (Halestrap 1976, 2012; Poole and Halestrap 1992, 1993; Juel and Halestrap 1999; Halestrap and Meredith 2004; Ullah et al. 2006; Halestrap and Wilson 2012).

MCTs 1–4 are responsible for the proton linked exchange of MCTs across plasma membranes down their concentration gradients. Monocarboxylates; L–La^-, pyruvate, d-β-hydroxybutyrate, and acetoacetate are driven to enter or exit cells based on their concentration gradients as well as the H^+ ion distribution (i.e., from low pH toward high pH) (Halestrap and Wilson 2012). It is important to note that the MCT isoforms are not independently responsible for influx or efflux, but rather, facilitate concentration-driven transport with regulation guided by expression, tissue distribution, and cellular location. MCT1 has the broadest tissue distribution (Merezhinskaya and Fishbein 2009) and is often, but not always, most highly expressed in cellular locations where net La^- uptake occurs such as heart and oxidative skeletal muscle. As noted by Halestrap and Wilson (2012), the major differences among MCTs 1–4 are their substrate and inhibitor affinities, the tissues in which they are expressed, and where they are located within cells. The predominant isomer of La^- found in humans is L–La^- for which MCT1 is stereoselective with K_m approximating 3–5 mM for L–La^- and tenfold higher for d-lactate (Poole and Halestrap 1993). In comparison with MCT1, MCT2 has a higher affi for both pyruvate (K_m = 0.1 mM) and L–La^- (K_m = 0.74 mM) (Bröer et al. 1998). MCT3 has similar kinetic properties to MCT1; however, it is restricted to the basal membrane of retinal pigment epithelium and choroid plexus epithelium (Bergersen et al. 1999; Philp et al. 2001). MCT4 is also widely expressed in tissues and has higher expression in tissues associated with net export of La^- such as fast glycolytic skeletal muscle, astrocytes, white blood cells, and chondrocytes (Juel and Halestrap 1999; Bonen 2001; Halestrap and Meredith 2004). The functional diff ence of MCT4 from MCT1 can be appreciated by the K_m values for pyruvate and L–La^- of about 150 and 28 mM, respectively (Bröer et al. 1998; Halestrap and Wilson 2012), which presumably aids in cellular retention of pyruvate and limits loss of La^- to higher physiological concentrations.

In general, MCT1 expression is highly correlated with indices of oxidative metabolism in skeletal muscle, while MCT4 expression is correlated with indices of glycolytic metabolism (Juel and Halestrap 1999; Brooks 2000; Halestrap and Wilson 2012). It is also generally true that chronic changes in muscle activity cause corresponding changes in La^- transport. Endurance exercise training and chronic electrical activity increase La^- transport capability, whereas inactivity, due to denervation or hindlimb suspension as examples, causes a decrease in La^- transport capability [see (Juel and Halestrap 1999; Bonen 2001; Gladden 2004a; Halestrap and Wilson 2012) for review]. The main change in MCT isoform profile with exercise training or a high energy demand stimulus is an increase in MCT1 with MCT4 changes being much less (Gladden 2004a; Halestrap and Wilson 2012; Thomas et al. 2012). However, in some research, hypoxia appears to be a stimulus for increased MCT4 mRNA and protein expression in a variety of cells, an effect that is likely regulated by HIF-1α (Ullah et al. 2006; Halestrap and Wilson 2012). The response of MCTs to an acute exercise bout is less clear with some studies reporting an increase in MCT content (Green et al. 2002, 2008; Coles et al. 2004; Bickham et al. 2006), while others have reported either no change or a decrease in MCT content (Tonouchi et al. 2002; Bishop et al. 2007). Overall, Thomas et al. (2012) propose that there is a rapid decrease in membrane MCT1 and MCT4 content between 45 s and 10 min after exercise onset, no change from 30 to 80 min and an increase in MCT1 content 2 h after exercise onset. However, they (Thomas et al. 2012) correctly note that these MCT responses to acute exercise are based on only a small number of studies, thus further research is needed. There is also

some evidence that MCT content and maximal La^- transport capability are not always in perfect concordance, and may be independently regulated (Thomas et al. 2012). As one considers the distribution of MCT isoforms and changes in their density and distribution with various stimuli, two caveats should always be considered: (1) the MCTs are not independent causative factors in La^- transport, because they are passive facilitators, regardless of isoform and (2) cells with a preponderance of MCT1 such as oxidative skeletal muscle fi tend to have a much greater maximal La^- transport rate than do cells with a preponderance of MCT4 such as glycolytic skeletal muscle fi (Juel et al. 1991; Bonen 2001; Juel 2001; Gladden 2004a). In this context, it is noteworthy that while MCT4 is associated with La^- effl its kinetic properties are such that it will likely be limiting to transmembrane transfer, leading to a [La^-] gradient from one side to the other (e.g., intracellular to extracellular in a La^--producing cell).

Cytosol-to-mitochondria lactate shuttle

The cytosol-to-mitochondria lactate shuttle (Rogatzki et al. 2015) describes the continuous glycolytic production, and mitochondrial oxidation of La^- within the cell of its origin (Fig. 10) (Stainsby and Brooks 1990; Hashimoto et al. 2006; Gladden 2008a; Rogatzki et al. 2015). In this model, mitochondria oxidize La^- at or near the inner mitochondrial membrane, possibly by a La^- oxidation complex (Hashimoto et al. 2006), but not in the mitochondrial matrix. The NADH produced by mitochondrial LDH (mLDH) is reoxidized by the malate–aspartate shuttle (MAS) (Kane 2014), or possibly other electron shuttles (see below). While evidence for mitochondrial MCT1 (as per a La^- oxidation complex) is not unanimously accepted (Halestrap and Wilson 2012), pyruvate transport could be similarly accomplished via the mitochondrial pyruvate carrier. As mentioned previously, because of the high LDH activity in a diversity of tissues, as well as the equilibrium constant for LDH greatly favoring La^-, the predominant result of glycolysis is La^- (Kane 2014; Rogatzki et al. 2015; Schurr 2017). However, it should be noted that formation of La^- is not synonymous with La^- accumulation and increased [La^-]. In a manner analogous to the subcellular distributions of the cytosolic versus mitochondrial creatine kinase of the phosphocreatine shuttle, the mitochondrial reticulum (Glancy et al. 2015) (and its resident intermembrane or juxta-inner membrane LDH) serves as a sink for La^-, maintaining La^- flux irrespective of accumulation (Kane 2014; Rogatzki et al. 2015).

Despite the seemingly straightforward concept of cytosol-to-mitochondria La^- shuttling, the nature of mitochondrial La^- metabolism remains a subject to which the following quotation of Fletcher and Hopkins (1907), cited earlier, might equally apply today: “there is hardly any important fact concerning the lactic acid formation in muscle which, advanced by one observer, has not been contradicted by some other.” A comprehensive treatment of this contentious issue is beyond the scope of this review (Brandt et al. 1987; Brooks et al. 1999b; Rasmussen et al. 2002; Sahlin et al. 2002; Ponsot et al. 2005; Hashimoto et al. 2006; Atlante et al. 2007; Schurr and Payne 2007; Yoshida et al. 2007; Lemire et al. 2008; Passarella et al. 2008, 2014; Gallagher et al. 2009; Elustondo et al. 2013; Jacobs et al. 2013; Schurr 2014; Herbst et al. 2017; Paventi et al. 2017). Suffi it to say that at present, discord exists about the precise functional location of mitochondrial LDH, but that the majority of the evidence indicates that LDH is not in the mitochondrial matrix (Baba and Sharma 1971; Brandt et al. 1987; Rasmussen et al. 2002; Sahlin et al. 2002; Ponsot et al. 2005; Hashimoto et al. 2006, 2008; Yoshida et al. 2007; Hashimoto and Brooks 2008; Elustondo et al. 2013; Jacobs et al. 2013; Herbst et al. 2017), and therefore, evidence does not favor an intracellular lactate shuttle as originally conceptualized (Brooks et al. 1999a, b).

However, it is important to recognize that progress around mitochondrial La^- metabolism has been complicated for two main reasons: (1) technical limitations in subcellular/cellular studies and (2) the potential differences among tissue and cell types. Spectrophotometric monitoring of NADH change, for example, has been used repeatedly in the study of La^- metabolism in mitochondria of diverse origin (Valenti et al. 2002; De Bari et al. 2004, 2010; Atlante et al. 2007; Pizzuto et al. 2012; Passarella et al. 2014; Paventi et al. 2017). This approach is problematic, not least, because NAD(H) is a cofactor in a myriad of biochemical reactions (Ying 2008), but also because the La^-/pyruvate redox couple is so closely linked to cytosolic, and not mitochondrial matrix NAD^+/NADH; not to mention that the latter is orders of magnitude more reduced relative to the cytosol (Williamson et al. 1967; Jones and Sies 2015); see Sahlin et al. 2002 for discussion (Sahlin et al. 2002). Furthermore, the ability of intact mitochondria to oxidize exogenous NADH (to which the inner mitochondrial membrane is impermeable) is a consistent observation, first reported by Lehninger over 65 years ago (Lehninger 1951). Similarly, spectrophotometric monitoring of NADH change has been used to study the rotenone-insensitive, aerobic reoxidation of cytosolic NADH via cytochrome b_5 reductase in cerebellar granular cells (Atlante et al. 1999) and liver outer mitochondrial membranes (Bernardi and Azzone 1981; Abbrescia et al. 2012), as well as the rotenone-sensitive, “exo-NADH oxidase system” in isolated cardiac (Rasmussen 1969; Jørgensen et al. 1985; Rasmussen and Rasmussen 1985; Nohl 1987) and skeletal muscle mitochondria (Szczesna-Kaczmarek et al. 1984; Rasmussen et al. 2001, 2003a, b). Interpreting NADH changes in a system in which LDH is not the exclusive NADH oxidoreductase is further complicated in the study

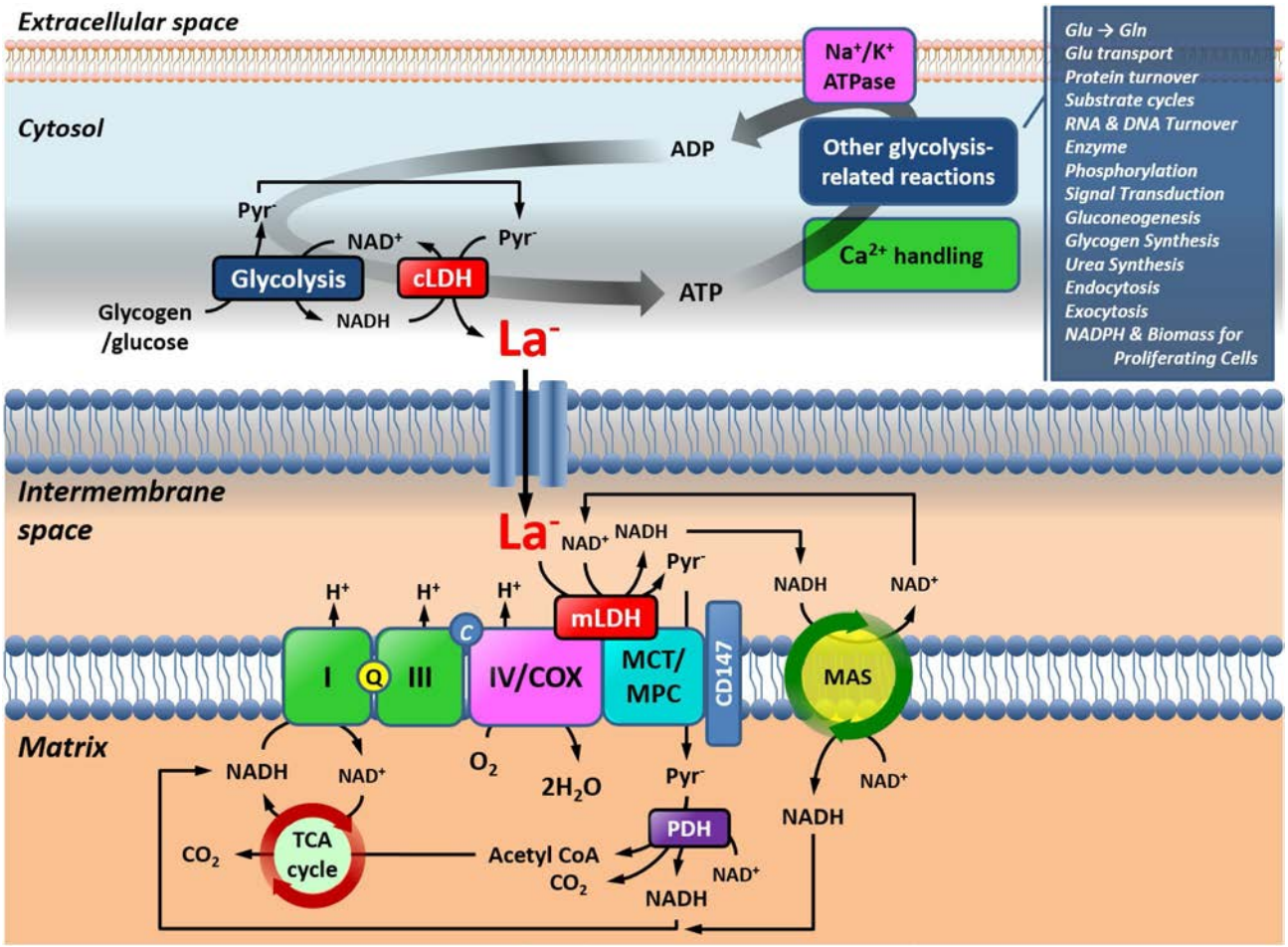

Fig. 10 Cytosol-to-mitochondria lactate shuttle (Rogatzki et al. 2015). Based on (Stainsby and Brooks 1990; Hashimoto et al. 2006; Gladden 2008a; Rogatzki et al. 2015) and combined from (Kane 2014; Rogatzki et al. 2015). The prominent activity of the near- equilibrium LDH ensures cytosolic La^- formation during most con- ditions, with rates increasing in parallel with increased glycolytic activity. The figure depicts a skeletal muscle, and not all cells nec- essarily exhibit all the processes depicted in the upper right. Com- partmentations with glycolytic activity include association with the sarcolemmal Na^+/K^+ ATPase, glycolysis-related reactions, and the Ca^{2+}-ATPase of the sarcoplasmic reticulum. La^- enters the mitochon- drial intermembrane space through porins in the outer mitochondrial membrane. Here, mLDH oxidizes La^- to Pyr^-, reducing NAD^+ to NADH. Pyr^- crosses the mitochondrial inner membrane via the MPC and/or MCT1, stabilized by CD147. Note: arguments against mito- chondrial MCT have been made; see (Halestrap and Wilson 2012). The NADH generated by mLDH is reoxidized by the MAS, which shuttles reducing power across the inner mitochondrial membrane. This is necessary, because the inner mitochondrial membrane is impermeable to NAD^+ and NADH. In the matrix, Pyr^- is oxidized by PDH to acetyl coA, which enters the TCA cycle, generating addi- tional NADH. Oxidation of NADH at Complex I is a primary entry point of electrons into the mitochondrial electron transport system (ETS). The electrons are then passed to the mobile electron carrier Q, transported through Complex III, and then to Complex IV/COX via cytochrome *c*. The final electron acceptor at Complex IV in this schematic is molecular O_2. The drops in free energy occurring across the ETS at complexes I, III, and IV are sufficient to drive the extru- sion of H^+ from the matrix into the intermembrane space, generating the proton motive force necessary to drive the synthesis of ATP at Complex V/ATP synthase. Omitted for clarity: Complex II/succinate dehydrogenase of the TCA/ETS, the glycerol 3-phosphate shuttle, the NAD(P) transhydrogenase, and stoichiometries of reactants and prod- ucts for all reactions. *ADP* adenosine diphosphate, *ATP* adenosine triphosphate, *C* cytochrome c, *CD147* chaperone protein for MCT1, *cLDH* cytosolic L-lactate dehydrogenase, CO_2 carbon dioxide, *CoA* coenzyme A, H^+ hydrogen ion, H_2O water, *I* Complex I/NADH oxi- doreductase of the mitochondrial electron system, *III* Complex III of the mitochondrial electron transport system, *IV/COX* complex IV/ cytochrome c oxidase, La^- L-lactate; MAS malate–aspartate shuttle, *MCT* monocarboxylate transporter, *mLDH* mitochondrial L-lactate dehydrogenase, *MPC* mitochondrial pyruvate carrier, NAD^+ oxidized nicotinamide adenine dinucleotide, *NADH* reduced nicotinamide adenine dinucleotide, O_2 molecular oxygen, *PDH* pyruvate dehydro- genase complex, Pyr^- pyruvate, *Q* quinone, *TCA* tricarboxylic acid cycle, *V* Complex V/ATP synthase

of mitochondrial La^- metabolism by the liberal employment of the LDH inhibitor oxamate which also inhibits pyruvate transport (Martin-Requero et al. 1986) and the aspartate aminotransferase of the malate–aspartate shuttle (Rej 1979; Thornburg et al. 2008). Among the potentially unintended effects of other agents used, mersalyl is an inhibitor of outer

mitochondrial membrane cytochrome b_5 reductase (Bernardi and Azzone 1981); digitonin increases exogenous mitochondrial NADH oxidation in rat liver (Bodrova et al. 1998); and Triton X-100 increases exogenous mitochondrial NADH oxidation in pigeon heart (Rasmussen and Rasmussen 1985). Ironically, Szczesna-Kaczmarek et al. (Szczesna-Kaczmarek et al. 1984) even suggested a relationship between extramatrix La^- oxidation and the external mitochondrial NADH oxidation pathway in muscle; such a system would help explain stimulation of NAD^+-dependent La^- oxidation with exogenous cytochrome *c* added after (Elustondo et al. 2013), but not before (Jacobs et al. 2013) adding La^- to permeabilized skeletal muscle fibers, as cytochrome *c* can transfer electrons between cytochrome b_5 reductase of the outer mitochondrial membrane and cytochrome *c* oxidase (Complex IV) of the electron transport system.

Recently, Patti and associates (Chen et al. 2016) took a more direct approach, using high-resolution mass spectrometry to study mitochondrial metabolism of La^- in cultured immortalized cells derived from cervical (HeLa) and lung cancer (H460), as well as 3T3-L1 fi oblasts. Their work (Chen et al. 2016) supports the contention that La^- plays a predominantly anaplerotic/cataplerotic role in proliferating cells [vis-à-vis the Warburg/reverse-Warburg eff (Warburg 1926)] and is consistent with notions of matrix LDH and a mitochondrial La^- carrier (Taylor 2017). Nevertheless, even they (Chen et al. 2016) admitted that they were unable to resolve exactly where La^- oxidation occurs in mitochondria. Because much of the previous research does not support matrix La^- oxidation, reconciling equivocal studies will require further investigation. As discussed in greater detail below, however (see "Lactate and cancer"), cancer and other proliferating cells exhibit metabolic phenotypes distinct from normal or differentiated tissue (Lunt and Vander Heiden 2011; San-Millán and Brooks 2017), and culture conditions do not replicate fully the tumor microenvironment in vivo (Goodwin et al. 2014). Nevertheless, a consistent, key finding that emerges among these diverse studies is the centrality of La^- as a metabolic intermediate within and between cells.

Whole-body cytosolic redox coupling via La^-

Recently, the Rabinowitz laboratory (Hui et al. 2017), using stable isotope techniques, rediscovered and reemphasized the cell-to-cell lactate shuttle. They (Hui et al. 2017) measured the turnover flux of 16 different carbon metabolites in mice under non-exercise conditions. Their major contribution was the observation that the circulatory turnover fl of La^- exceeds that of all other metabolites, being 10–150% greater than the rate of glucose turnover flux, depending on whether the mice were fed or post-absorptive. Furthermore, these investigators postulate that the rapid translocation of La^- across cell membranes (see discussion above) and its bulk circulation in the blood positions La^- to serve as a means of equating cytosolic NAD^+/NADH ratios across tissues throughout the body. This is an intriguing, albeit not novel (Brooks 2009; Halestrap 2013; San-Millán and Brooks 2017) concept. The Rabinowitz group (Hui et al. 2017) further postulates that this redox function of the cell-to-cell lactate shuttle would permit the use of carbohydrate-derived energy without the necessity of glycolysis in some tissues, while other tissues could modulate glycolytic activity to support other needs such as cell proliferation, NADPH production via the pentose phosphate pathway, activity of brain neurons, and systemic glucose homeostasis (Vander Heiden et al. 2009). Halestrap (Halestrap 2013) explains that this process is possible because of the exclusively cytosolic location of LDH and the fact that the LDH reaction is close to equilibrium in most cells. Furthermore, due to these relationships, changes in the cytosolic La^-/pyruvate ratio can cause changes in the NAD^+/NADH ratio and vice versa (Halestrap 2013).

We see merit in these proposals but note two additional, important factors. First, while La^- rapidly exchanges throughout the body, it equilibrates across permeable membranes in accordance with the $[H^+]$ (pH) gradient (Roos 1975; Gladden and Yates 1983; Roth and Brooks 1990a). Systemic pH is in turn regulated by a number of other factors [e.g., (Gladden 2008a; Stickland et al. 2013)]. Second, while the cell-to-cell lactate shuttle might coordinate cytosolic redox balance among tissues, it will not directly impact mitochondrial redox. This is because cytosolic and mitochondrial matrix redox states are not in equilibrium with each other (Sahlin et al. 2002). The major underlying reason for the separate NAD^+/NADH ratios between these two compartments is that there are nonequilibrium steps for electron transfer across the inner mitochondrial membrane. For the malate–aspartate shuttle, the nonequilibrium step is the exchange of glutamate plus a proton from outside the inner mitochondrial membrane for aspartate from the mitochondrial matrix (Nicholls and Ferguson 2013). For the *s,n*-glycerophosphate shuttle, the nonequilibrium step is at the flavoprotein-linked *sn*-glycerophosphate dehydrogenase reaction within the inner mitochondrial membrane (Nicholls and Ferguson 2013). With regard to La^- exchange across the inner mitochondrial membrane, this is not a mechanism for redox equilibration because of the absence of LDH in the mitochondrial matrix as discussed in the previous section.

Lactate as a signaling molecule

The lactate/pyruvate ratio is the predominant cytosolic redox couple for NAD^+/NADH. It should, therefore, not be surprising that the marked rises in $[La^-]$ during times of cellular stress constitute a signaling molecule—a "lactormone"

(Brooks 2009; San-Millán and Brooks 2017). In addition to serving as an oxidizable fuel itself, La^- also inhibits lipolysis (Gold et al. 1963), and proportionally down-regulates the oxidation and disposal of glucose [(Miller et al. 2002a, b); see discussion earlier]. It is thought that La^- achieves this lactormone-like regulation of metabolism by interacting with the G-protein coupled receptor GPR81 (a.k.a., HCA1) on fat cells to inhibit lipolysis (Liu et al. 2009). Though by comparison, its level of expression is much greater in adipose (Cai et al. 2008; Ahmed et al. 2010), GPR81 expression in brain (Bergersen and Gjedde 2012; Lauritzen et al. 2014), skeletal muscle (Ge et al. 2008; Kuei et al. 2011; Rooney and Trayhurn 2011), heart and other tissues (Liu et al. 2009), nevertheless, suggests important La^- signaling functions in these tissues which await complete description.

La^- can also elicit longer term influence over gene expression via hypoxia-inducible factor-1 (HIF-1), a transcription factor regarded as the master regulator of O_2 homeostasis (Semenza 2004). When activated by hypoxia, HIF-1 regulates the expression of several genes coding for proteins involved in glycolysis, La^- handling, and metabolism. For example, HIF-1 activation increases pyruvate dehydrogenase kinase 1 (PDK1) content in mouse embryo fibroblasts (Kim et al. 2006), which inhibits pyruvate dehydrogenase (PDH), and stems flux away from pyruvate oxidation at PDH, instead leading to LDH-dependent La^- accumulation. HIF-1 also increases the content of some cell membrane glucose transporters (Wood et al. 1998; Chen et al. 2001; Mobasheri et al. 2005; Sakagami et al. 2014), and 11 enzymes of glycolysis (Semenza 2001), including the main rate-controlling enzyme phosphofructokinase (PFK). Increased glucose transport and glycolytic activity promote the formation and accumulation of La^-. HIF-1 also increases MCT4 content (Ullah et al. 2006), the isoform associated with La^- effl from glycolytic skeletal muscle. Thus, HIF-1 activation leads to both the enhanced formation, and eff e effl of La^-. Interestingly, HIF-1 and La^- exhibit reciprocal activation, whereby La^- can activate HIF-1, and HIF-1 can promote the production of La^-. It has been proposed that reactive oxygen species may mediate the reciprocal activation of HIF-1 and La^- production (Nalbandian and Takeda 2016).

Peroxisome proliferator activated receptor gamma coactivator-1 α (PGC-1 α), a transcription coactivator widely regarded as the master regulator of mitochondrial biogenesis, also plays a role in regulating La^- metabolism. Upon binding to the estrogen-related receptor-α on an *ldhb* promotor, PGC-1 α promotes the transcription of lactate dehydrogenase isoform B (LDHB). Recently, Liang et al. (2016) demonstrated not only that exercise stimulated the expression of skeletal muscle LDHB, but that overexpression of *ldhb* in mice acts downstream of the PGC-1α/nuclear receptor regulatory circuit to increase markers of mitochondrial content. As with HIF-1, it has been theorized that La^--associated reactive oxygen species (ROS) mediates these PGC-1 α-dependent adaptations (Nalbandian and Takeda 2016); though unlike the upregulation of PFK with La^- in hypoxia, La^- appears to downregulate PFK under normoxic conditions (Leite et al. 2007, 2011). In what may be another example of reciprocal activation, La^- also appears to upregulate expression and content of some of the same proteins upregulated by PGC-1 α, including MCT1 (Hashimoto et al. 2007; Hoshino et al. 2014) and COXIV (Hashimoto et al. 2007), as well as PDK4 and UCP3 (Kitaoka et al. 2016).

The lactormone function of La^- adds to the long and growing list of ways in which nature has integrated this unassuming molecule into a diversity of physiological processes. It should come as little surprise that as the tools of scientific inquiry advance, La^- will continue to feature prominently in areas of both physiology and pathophysiology (e.g., see section on the role of La^- in cancer).

Lactate and cell volume regulation

There is also evidence of a role for La^- in fl volume regulation during heavy exercise. During moderate-to-intense exercise, hypotonic fluid is shifted out of the vascular compartment causing the plasma osmolarity to increase (Mack 2012). One of the main reasons for this fl shift involves net water movement into contracting muscles due to increases in intracellular and interstitial osmolarity caused by increases in skeletal muscle intracellular and extracellular fluid [La^-]. For example, in humans exercising at 75% of $\dot{V}O_{2peak}$ to exhaustion, a three-phase pattern of plasma volume shifts is observed, the first of which (first 2 min of exercise) is thought to be due to increases in capillary pressure and osmotic pressures within the contracting muscle (Lindinger et al. 1994). The second phase of plasma volume decrease is associated with an increase in muscle metabolites such as La^- due to increased rates of glycolysis (Lindinger et al. 1994). The increase in extracellular osmolarity results in a volume loss in non-contracting cells; this is hypothesized to attenuate plasma fluid losses early in exercise (Lindinger et al. 2011). Opposing this loss of fl inactive skeletal muscle fibers exhibit a regulatory volume increase mediated by ion transport using the electroneutral Na-K-2Cl co-transporter (Lindinger et al. 2011); however, inward transport of La^- via MCT1 also contributes (Lindinger et al. 2013). Whether a similar role for La^- as a mediator of cell volume regulation exists in cells of the central nervous system has, to our knowledge, not been addressed. Conceivably such a role could exist because of the ease with which La^- permeates the blood–brain barrier, and the ubiquity of MCTs in brain cells.

In the eye, it has been suggested that MCTs 1 and 3 facilitate the volume of the subretinal space via transport

of lactate and its attendant tonicity (Halestrap 2013), and evidence also supports direct transport of water with lactate (Zeuthen et al. 1996; Hamann et al. 2003; Li et al. 2016).

Lactate and cancer

The history of cancer is complicated and riddled with descriptions of masses dating back to as early as 1600 BC (The History of Cancer 2002). However, it was not until the 1920s that detailed experiments on tumor metabolism were fi t reported. As reviewed by Otto (Otto 2016), Warburg and Minami (Warburg and Minami 1923) placed tumors in Ringer solution and observed acidifi upon addition of glucose. The acidification was indicated by color change from organic pH indicators, while La^- was measured in the solution chemically. Subsequently, Warburg placed slices of a rat hepatoma in a modified manometer and calculated La^- from the increase in CO_2 formation during 30 min of incubation. Amazingly, the tumor slice produced La^- at a rate that was 70 times greater than that of normal liver, kidney, and heart tissue (Otto 2016)! This high La^- production rate was independent of the presence of O_2. In 1925, the Cori's (Cori and Cori 1925) reported that venous blood from a chicken wing with an implanted sarcoma had higher [La^-] and lower [glucose] than venous blood from the contralateral, non-tumor wing. Warburg (Warburg et al. 1927) next used a rat model to show that the artery feeding the tumor always had lower [La^-] and higher [glucose] than the vein draining it, consistent with La^- production by a tumor in presumably normoxic conditions. In 1972, Efraim Racker called this glucose-avid, La^--producing behavior of tumor cells in the presence of adequate O_2 supply, the "Warburg Eff (Racker 1972). This eff is now well known in the study of cancer, and research on the topic has skyrocketed since approximately 2000. It has also sparked interest among researchers and clinicians as a target for treatment (Nijsten and van Dam 2009; Goodwin et al. 2015; Martinez-Outschoorn et al. 2017).

Our current understanding of tumor metabolism is exemplifi by a report from Sonveaux et al. (Sonveaux et al. 2008). These researchers (Sonveaux et al. 2008) determined the metabolic characteristics of two tumor lines via measurement of O_2 consumption, glucose utilization, and La^- production. One line, SiHa human cervix squamous carcinoma cells, consumed O_2 rapidly, used glucose more slowly, produced La^- more slowly, and oxidized La^- as readily as glucose. The second line, WiDr human colorectal adenocarcinoma cells behaved conversely to the SiHa cells in that they consumed O_2 slowly, used glucose more rapidly, produced La^- more rapidly, and were much less effective at oxidizing La^-. In addition, the tumor lines differed in terms of MCT expression in that the SiHa cells preferentially expressed MCT1, whereas the WiDr cells expressed MCT4. Following up on these results in vitro, Sonveaux et al. (2008) obtained both SiHa and WiDr tumor biopsies from tumors grown in mice from the corresponding cancer cells injected intramuscularly in the rear leg. The SiHa tumors were particularly interesting. Within the SiHa tumors, two tumor cell subpopulations were documented; one group was located in a well-vascularized, well-oxygenated area of the tumor and the other was located in a poorly vascularized, hypoxic area. MCT1 was expressed in the oxygenated cells but not in the hypoxic cells. Again, following in the footsteps of exercise metabolism research, Sonveaux et al. viewed the whole of their results and hypothesized a La^- shuttle in which La^- is produced by the hypoxic tumor sites and oxidized as a fuel in the normoxic sites. In a commentary further detailing the potential facets of such a shuttle, Greg Semenza, himself the discoverer of HIF1-α (Semenza and Wang 1992), stated, "Was there any precedent that should have alerted us to the existence of this symbiotic relationship between aerobic and hypoxic cancer cells? Of course: the well-known recycling of lactate in exercising muscle" (Semenza 2008). This began the incorporation of knowledge gained from studying La^- metabolism in exercise physiology, particularly the work of G. A. Brooks, into tumor physiology.

The Warburg cancer cell profile is characterized by several features. First, there is an increase in glycolytic enzymes, sometimes by as much as 500-fold (Moreno-Sánchez et al. 2007; San-Millán and Brooks 2017). The ample carbohydrate fuel supply that is critical for Warburg cells is aided by an increased expression of glucose transporters, particularly GLUT1, a phenomenon that is associated with aggressive tumors and a poor prognosis [e.g., (Kunkel et al. 2003; San-Millán and Brooks 2017)]. Note that the basis for the whole-body non-invasive PET scan (^{18}F-fluorodeoxygluscose positron emission tomography) technique for diagnosing and monitoring cancer progression is based on the high glucose uptake by tumor cells (Jadvar et al. 2009). Since La^- is the terminal end-product, it is not surprising that MCT1 and MCT4 transporters are often overexpressed in many types of cancers [e.g., (Kennedy and Dewhirst 2010; San-Millán and Brooks 2017)], and are in fact, correlated with a poor prognosis and high mortality (Pértega-Gomes et al. 2011). The underlying causes of these changes and of cancer itself, are numerous, complicated, and incompletely understood [e.g., (San-Millán and Brooks 2017)]. Intriguingly, Sán-Millan and Brooks (2017) have even proposed that the elevated La^- production and subsequent increased [La^-] (up to 40-fold) are central signaling players in the generation of the Warburg cell profile. The primary LDH isoform in Warburg cells is LDHA (Semenza et al. 1996; Vander Heiden et al. 2009; Granchi et al. 2010; San-Millán and Brooks 2017) which has been argued to promote La^- production [e.g., (Everse and Kaplan 1973; Granchi et al. 2010)] and to negatively impact prognosis [e.g., (Sheng et al. 2012; Shi et al.

2014; Huang et al. 2016)]. Knockdown of LDHA has been an experimental approach to cancer treatment and it has met with some success [e.g., (Fantin et al. 2006; Vander Heiden 2011; Wang et al. 2015)]. However, it is not clear that LDHA has been inhibited independently of total LDH activity. We have previously expressed our skepticism that LDH isoform profile is a determinative factor in net La^- production and accumulation (Rogatzki et al. 2015). This reprogramming of cell metabolism in many, perhaps most cancer cells, is so dramatic and widespread that it has been proposed as a new hallmark of cancer (Hanahan and Weinberg 2011).

One might ask why cancer cells would gorge on glucose and only partially realize its full energy potential by emphasizing La^- production rather than full oxidation. An older idea (Potter 1958) that has been re-derived (Vander Heiden et al. 2009; Hanahan and Weinberg 2011; Martinez-Outschoorn et al. 2017) is that this metabolic scheme provides building blocks for proliferation. Because the organism absorbs the burden of regulating blood glucose concentration, the tumor cells are awash in fuel, thus negating any substrate limitation. Subsequent to the rapid use of glucose, glycolytic intermediates are diverted into numerous biosynthetic pathways. Included in these proliferative pathways are the generation of nucleosides and amino acids, providing the foundational materials for macromolecules and organelles which can ultimately constitute new cells via rapid cell division. Faster conversion of fuel into biomass maximizes the rate of growth; proliferation rather than effi may be the "goal" of the cancer cell (Vander Heiden et al. 2009). An additional benefit of increased La^- production and efflux to the cancer cell might be the acidification of the extracellular milieu, which is believed to facilitate cell invasion of surrounding tissue and metastasis (Gatenby and Gillies 2004; Gillies and Gatenby 2007).

Given the Warburg cell's dependence on glucose as a fuel, a clever notion is to deprive tumors of glucose and thereby either kill them, weaken them, or heighten their susceptibility to other treatments (Nijsten and van Dam 2009). It is well known that depriving cultured cancer cells of glucose is frequently detrimental to growth [e.g., (Aykin-Burns et al. 2009; Choo et al. 2010; Graham et al. 2012)]. However, how can the organism survive the glucose deprivation required to damage cancer cells? Nijsten and van Dam (Nijsten and van Dam 2009) proposed making patients severely hypoglycemic while using La^- as an alternative fuel; i.e., lactate-protected hypoglycemia (LPH) (Goodwin et al. 2015). As revealed in the cell-to-cell lactate shuttle, most normal cells (but not Warburg cells) can readily use La^- as a fuel. A particular concern, despite studies showing that the brain can oxidize La^-, is whether or not the brain can survive in the face of severe hypoglycemia (< 1 mM). A case study encouraged this idea. Oldenbeuving et al. (Oldenbeuving et al. 2014) reported the case of a patient arriving at the emergency room with what is usually considered nonsurvivable hypoglycemia (0.7 mM); however, his arterial [La^-] was 25 mM. The patient, suffering from paracetamol-induced acute liver failure, was ambulating and speaking, presumably due to the use of La^- as a fuel by his vital tissues, particularly the brain. Subsequently, Goodwin et al. (Goodwin ML, Rogatzki MJ, Sun Y, McDonald JR, Lee K, Oldenbeuving G, Nijsten MWN, Gladden LB, unpublished results) attempted LPH in an anesthetized canine model. As shown in Fig. 11, Panel A, three animals were made severely hypoglycemic via a combination of insulin, propranolol, and ethanol/phenformin; once severe hypoglycemia was reached, the anesthetized animals survived on the average about 30 min as indicated by isoelectricity on the electroencephalogram. In contrast, two animals were made hyperlactatemic ([La^-] = ≈ 10 mM; Fig. 11B) prior to initiating severe hypoglycemia. Those two animals survived at least 5 h before brain isoelectricity occurred. These results offer the tantalizing possibility that the brain can survive severe hypoglycemia for at least several hours in the presence of hyperlactatemia.

Unfortunately, it is possible, perhaps likely, that the idea of treating cancer with LPH is overly simplistic. Work from Lisanti's lab (Pavlides et al. 2009; Gladden et al. 2011; Whitaker-Menezes et al. 2011) found that when MCF7 breast cancer cells were co-cultured with normal fi oblasts, the fi oblasts began to express MCT4, something they failed to do when cultured alone. Furthermore, the breast cancer cells in this co-culture demonstrated an upregulation of MCT1. This group (Pavlides et al. 2009; Whitaker-Menezes et al. 2011; Witkiewicz et al. 2012) has proposed that these results are part of a shuttle between stromal fibroblasts which display aerobic glycolysis, producing La^-, which is subsequently taken up by the neighboring cancer cells and oxidized as a fuel. The behavior of the cancer cells in this scenario was described as 'Reverse Warburg' (Pavlides et al. 2009). Overall, these results are a warning to remember that tumors are a heterogeneous collection of normal tissue cells, immune cells, blood vessels, and cancer cells, some of which may be of a Warburg phenotype, while others are of a Reverse-Warburg type (Hanahan and Weinberg 2011; Martinez-Outschoorn et al. 2017; Potter et al. 2016; Lytovchenko and Kunji 2017). Some areas of the tumor may be hypoxic, while others are amply supplied with O_2. Still other areas are nutrient deficient or nutrient rich, and this landscape is likely to change as the tumor infiltrates and proliferates (Hanahan and Weinberg 2011; Martinez-Outschoorn et al. 2016; Potter et al. 2016; Lytovchenko and Kunji 2017).

As a result, it should be emphasized here that cell culture experiments in vitro can afford excellent control of the local environment, but also can be quite misleading, as so much of cancer cell behavior depends on a complex and poorly understood tumor microenvironment (Goodwin et al. 2015). In addition, an often-unrecognized shortcoming of

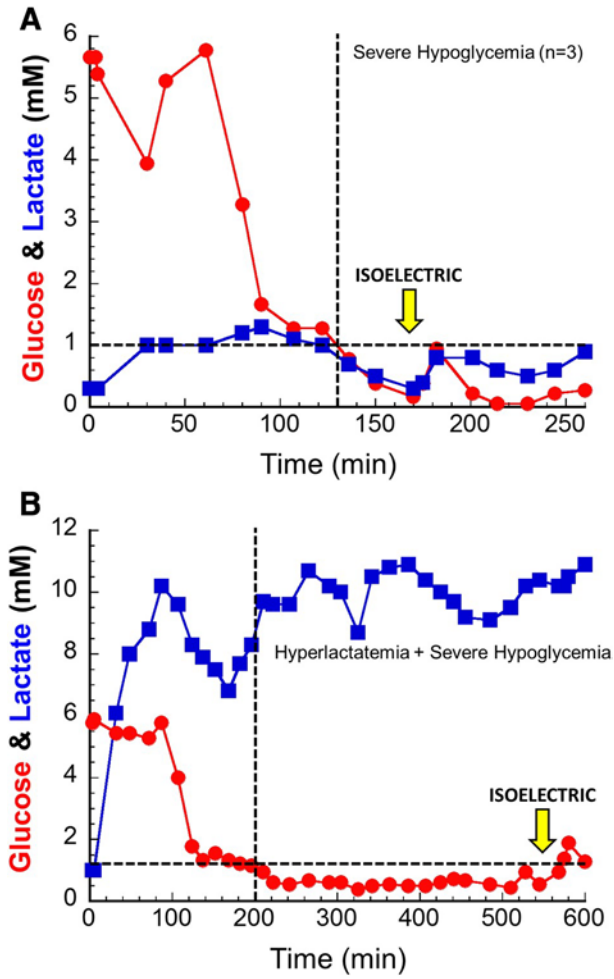

Fig. 11 Demonstration of lactate-protected hypoglycemia (LPH) in anesthetized dogs. **A** Experimental manipulation of [glucose] in anesthetized dogs (n = 3) as described in the text. Once blood [glucose] was reduced to <1 mM, time to brain isoelectricity (death) was ~0.5 h. In **B**, the same protocol was repeated (n = 2) but with prior and continued infusion of exogenous La^- to almost ten times normal and clamped there (~10 mM). Subsequently, time to isoelectricity was increased 10-fold (>5.5 h), substantiating for the first time in whole animals what had been suspected from clinical case reports (Oldenbeuving et al. 2014) and decades of data demonstrating La^-'s ability to be transported and utilized as a fuel throughout almost all tissues of the body (Gladden 2004b). This proposed LPH (Nijsten and van Dam 2009; Goodwin et al. 2015) might function for at least a period of hours. Note different scales on x- and y-axes in Panels **A** versus **B**. Data are from unpublished results of Goodwin ML, Rogatzki MJ, Sun Y, McDonald JR, Lee K, Oldenbeuving G, Nijsten MWN, Gladden LB

cell studies in vitro is that the cells are typically exposed to ambient O_2 levels; these levels are extremely hyperoxic in comparison with conditions observed in vivo. Accordingly, studies utilizing whole limbs or animals likely provide a more nuanced understanding of how the tumor interacts with its microenvironment, including its production and/or consumption of La^-. Sauer and Dauchy (1985) measured arteriovenous diff ences across tumors in their rat model and demonstrated that most tumors were net La^- producers when the arterial [La^-] was below 2.5 mM; they subsequently utilized La^- as a fuel when arterial [La^-] was above 2.5 mM (Sauer and Dauchy 1985). It is likely that the degree to which tumors globally utilize La^- as a fuel ultimately depends on cell type, available substrate, and specific microenvironment. Targeting this remarkably flexible metabolism as a means to combat cancer is a formidable challenge!

While targeting La^- metabolism *per se* may prove to be quite difficult, recent work suggests that La^- is also a potent signaling molecule (see above as well), driving HIF1-α, VEGF, and ultimately angiogenesis (Goodwin et al. 2014). Lactate's role as a signaling molecule was fi t described as a glycolytic metabolite that could, independent of O_2, induce angiogenesis (Murray and Wilson 2001); this has now expanded with both theoretical and experimental support into the cancer literature. A recent review (Goodwin et al. 2015) highlights this lactate-to-angiogenesis pathway. Briefl , LDH is near-equilibrium and any increase in glycolysis will increase both [La^-] and [pyruvate]. Increased [pyruvate] inhibits formation of 2-oxoglutarate, the molecule typically responsible for degradation of HIF1-α. Thus, increases in [La^-] and [pyruvate] independent of O_2 tension, can drive stabilization of HIF1-α and lead to angiogenesis. This has been demonstrated in several experimental designs but is yet to be implemented clinically. Most recently, the Kevin B. Jones lab in Utah introduced a novel transgenic mouse sarcoma model which resulted in mouse tumors that were indistinguishable from the human tumor (Goodwin et al. 2014). These tumors formed in areas of high [La^-] and expressed large amounts of HIF1-α despite normoxic conditions. As further confirmation of La^-'s potent role as a signal, daily exogenous La^- administration led to dramatic upregulation in the vascularity of these already well-vascularized tumors.

Research in skeletal muscle provided the early framework from which clinical application has now begun in both the central nervous system and tumor research. While many active clinical areas are currently under investigation (see section "Lactate in clinical applications"), research extending what is known about La^- metabolism in muscle to now include the brain and tumors has raised as many questions as it has answered. Given La^-'s central role as the metabolite by which whole-body metabolism is coordinated (Gladden 2004b), opportunities for clinical application are numerous.

Lactate in clinical applications

Given the confusion surrounding lactate research over the last 100 years, it is not surprising that in many ways clinical application has lagged even further behind. Originally thought of as a "hypoxic waste product", many clinicians

still view La^- in this manner. [La^-] is most often used by hospitals today in the trauma setting as an indicator of "resuscitation." While frank dysoxia will obligatorily engender an elevated [La^-], an elevated [La^-] clearly does not typically indicate clinical hypoxia or hypoperfusion, a point that continues to cloud clinical interpretation. To complicate matters, [La^-] during the first 48 h in the hospital has remained one of the best indicators of clinical outcomes, with persistently elevated [La^-] being a relatively good predictor of surgical complications and even mortality (Claridge et al. 2000). Although typically reported as "clearance" in multiple papers (and even in their titles!) (Odom et al. 2013), concentration and not clearance is almost exclusively what is actually measured in the trauma and critical care setting (Goodwin and Rothberg 2014).

Many large clinical trials have examined [La^-] and clinical outcomes in the trauma and critical care setting. However, careful review of the literature calls into question the rationale for La^--guided treatment in clinical practice. Pölönen et al. (Pölönen et al. 2000) used goals of [La^-] < 2.0 mM and SvO2 > 70% in the 8 h postoperative window in 403 bypass patients, utilizing additional IV fluids and dobutamine in an attempt to reach these goals. While the treatment group had less organ dysfunction and shorter length of stay by 1 day, only 57% actually met the goals (versus 42% in the control group) and [La^-] was not different between groups at any sampling time, suggesting that under-resuscitation/hypoperfusion is likely *not* the reason for continued La^- elevation in this setting (Pölönen et al. 2000). This type of goal-driven therapy has been repeated in septic shock patients, indicating some improvements with the additional treatments, but little to no difference in [La^-] between groups (Rivers et al. 2001; ProCESS 2014). Perhaps the most often cited study is from the Netherlands (Jansen et al. 2010), in which 348 ICU patients underwent goal-directed therapy in a randomized controlled trial with a goal of decreasing [La^-] by 20% every 2 h for 8 h utilizing fluids and vasodilators. The treatment group did receive significantly more fluid and medication, with a slight change in adjusted in-hospital and ICU (but not 28-day) mortality. However, [La^-] was not significantly different between the two groups at any time. Despite these results, the authors conclude that "lactate monitoring during the fi t 8 h of ICU admission, aimed at reducing lactate levels by 20% per 2 h, signifi y reduced ICU length of stay and also ICU and hospital mortality…" Despite no change in [La^-] between the groups, this study (Jansen et al. 2010) continues to be cited as a reason that La^--guided treatment should be utilized.

The question looming over these trials is why [La^-] remains elevated despite interventions that increase cardiac index and/or perfusion, and thereby global O_2 delivery. This has led many clinicians to use the phrase "occult hypoperfusion," in reference to the noted elevated [La^-] in patients in whom no perfusion deficit can be found and attempts to increase perfusion do not alter [La^-]. Clinicians often cite visceral organs as being the site of ischemia, yet this has been diffi to demonstrate in normal trauma or critical care settings. For example, clamping off the superior mesenteric artery in animals, making much of the bowel ischemic, fails to induce enough La^- production to increase arterial [La^-] to a significantly different level (Heino et al. 1997; Tenhunen et al. 2001). Consistent with this, improving the microcirculation of critical organs in critically ill patients does not alter arterial [La^-] (Trzeciak et al. 2008).

We are intensely skeptical of "occult hypoperfusion" in these cases, and propose instead that the increased [La^-] is due to nonhypoxic mechanisms. Clinically, increases in blood [La^-] due to hypoxia is likely the exception rather than the rule. Dysoxia is difficult to evoke, even during intense exercise. Richardson et al. (1995), cited earlier, had subjects inspire 12% O_2, yet muscle P_iO_2 remained above the level of O_2-limited mitochondrial function. Furthermore, even in conditions of very high [La^-] after exercise or seizures, once the stimulus is removed, [La^-] rapidly returns to normal levels within a few hours. In the trauma setting, [La^-] may remain elevated for several days in patients who ultimately have poor clinical outcomes, suggesting some underlying metabolic derangement. While it is beyond the scope of this review, this persistent [La^-] elevation may be due to the underlying stress response of trauma or critical care, as catecholamines stimulate ß-adrenergic receptors on skeletal muscle, liberating skeletal muscle glycogen as La^- (Clutter et al. 1980; Stainsby et al. 1987). This response has previously been described in burn patients (Gore et al. 1991), and numerous lines of animal and human studies support this hypothesis as the mechanism for elevated La^- in many trauma and critical care patients (Irving 1968; Daniel et al. 1976a, b, 1978; Benedict and Grahame-Smith 1978; Liddell et al. 1979; James et al. 1996, 1999a, b; Luchette et al. 2002; Garcia-Alvarez et al. 2014).

Given La^-'s now well-supported role as both fuel and signaling molecule, it is worth noting that its prolonged elevation with trauma or critical illness may simply represent the body in a state of prolonged stress, and the La^- elevation a result of prolonged catecholamine elevation in an effort to mount a "fight or flight" response. Nonetheless, prolonged elevated [La^-] remains a useful clinical marker across multiple disciplines, and it is always prudent to ensure adequate oxygenation. Venkatesan et al. (Venkatesan et al. 2015) demonstrated that hip fracture patients with [La^-] > 3.0 mM on arrival have four times the 30-day mortality as those with [La^-] < 3.0 mM on arrival. Claridge et al. (Claridge et al. 2000) followed [La^-] for 24 h after admission to the ICU for trauma; those who had [La^-] that remained > 2.5 mM at 12 h had six times the mortality and four times the infection

rate of those who normalized before 12 h. Multiple examples of this are found in the clinical literature, and it remains a good predictor of how a patient might do, despite the gross mechanistic misinterpretation by many clinicians. Finally, studies that simply alter the [La^-] do not change the poor prognosis of these patients [e.g., with DCA to activate PDH complex and drive increased formation of acetyl CoA (Stacpoole et al. 1992)], further highlighting the indirect nature of the relationship between [La^-] and clinical outcomes. It is apparent that the mechanisms underlying an elevated [La^-] must be the problem.

In opposition to the negative consequences associated with elevated [La^-] in many critical care and trauma situations, there is growing evidence that deliberate induction of hyperlactatemia may be beneficial in some conditions. As summarized by Ichai et al. (2014), La^- infusion has elicited improved myocardial function in patients with septic shock, after cardiac surgery, and with acute heart failure (Nalos et al. 2014). Positive results have also been found for La^- infusion in traumatic brain injury (Ichai et al. 2013, 2014; Brooks and Martin 2014; Quintard et al. 2016). In addition, La^- has been proposed as a salvage fuel in the use of severe hypoglycemia as a potential therapy in the treatment of tumors exhibiting the Warburg effect (Nijsten and van Dam 2009) as discussed above.

Yes, Virginia, there is a lactic acidosis

While lactic acid has long been known as a strong biological acid (pKa = 3.86), hypotheses centering on its formation and eff in vivo seem to have clouded the picture. Following an earlier proposal by Hochachka and Mommsen (1983), Robergs et al. (2004, 2005, 2006; Robergs 2008) have asserted that there is no lactic acidosis during exercise (or any other condition perhaps). This assertion is proffered on the basis of a detailed accounting of the reactions of glycolysis, concluding that (1) La^- is the biochemical end-product of glycolysis, not HLa, (2) the LDH reaction from pyruvate to La^- "consumes protons" (H^+), and (3) the acidosis typically seen in conjunction with increasing [La^-] is due to the hydrolysis of ATP that was not produced by oxidative phosphorylation inside mitochondria; i.e., non-mitochondrial ATP. However, disregarding the question of whether or not La^- is the only physicochemical contributor to the acidosis of exercise (it likely is not) (Böning and Maassen 2008a, b; Lindinger and Heigenhauser 2008a, b; Gladden 2008c), there are signifi errors in Robergs' claims. First, the argument that the major source of protons is the hydrolysis of non-mitochondrially produced (glycolytic) ATP hinges on a decreasing concentration of ATP. In fact, ATP concentration changes relatively little under most conditions. Studies of phosphagen concentration in skeletal muscle during exercise date back to those of Hultman and colleagues (Hultman et al. 1967; Gollnick and Hermansen 1973) who showed that while phosphocreatine concentration declined linearly with increases in exercise intensity, changes in [ATP] were comparatively minor. Second, even though it is agreed that La^-, not HLa, is produced by glycolysis (Jones 1980; Robergs et al. 2004), La^- cannot exist in isolation. La^- must immediately be in equilibrium with the same pH as all other acids in solution; this is the isohydric principle.

So, whether HLa is viewed as (1) a strong biological acid which is almost entirely dissociated at usual muscle and blood pH values, or (2) a signifi contributor to the strong ion difference [SID; (Stewart 1981; Kellum and Elbers 2009)], both constructs entail an acidifying effect of La^- accumulation. Aside from basic principles, Marcinek et al. (2010) have found that there is close relationship between pH decline and La^- accumulation with constant [ATP] in direct experimental testing. Specifically, they (Marcinek et al. 2010) measured pH and [ATP] in ischemic mouse muscle using ^{31}P-MRS, and intracellular [La^-] chemically. Even with no change in [ATP], their calculated H^+ generation agreed closely with the increase in [La^-] over the course of the ischemic period.

Certainly one could argue that *if* pyruvate instead of La^- accumulated during exercise or some other glycolytic stimulus, then the [H^+] would increase more, because pyruvic acid is a stronger acid than HLa. However, it is La^- rather than pyruvate that increases dramatically because of an equilibrium constant for the LDH reaction that is strongly in favor of La^- (Lambeth and Kushmerick 2002). In fact, the La^- to pyruvate ratio ranges from about 10:1 at rest to values as high as 159:1 in skeletal muscle immediately following exhaustive dynamic exercise (Sahlin et al. 1976; Rogatzki et al. 2015). In conclusion, increases in [La^-] do have an acidifying effect.

Conclusion

Prebble (2010) has documented that it was research focused on muscle metabolism by a group of biochemists that led to discovery of the glycolytic pathway while also laying the foundation for discovery of the origins of aerobic phosphorylation. Most of these biochemists had the common influence of the Otto Meyerhof laboratory (Prebble 2010). Of course, the quest for an understanding of La^- production and removal in muscle was an important linchpin of the glycolytic research. Allchin (2002) asserts, and we agree, that understanding cellular metabolism/bioenergetics is a great achievement of the twentieth century that has not been acclaimed to the same extent as advancements in evolution, genetics, and molecular biology (Prebble 2010), but should be. Within this wondrous web of cellular

bioenergetics, Scheele's little three carbon molecule seems to grow in importance. Should this be surprising? No. For most vertebrates, and certainly mammals, there are two ATP-generating systems, glycolysis and oxidative phosphorylation. Glycolysis does not require O_2 and has La^- as its end-product, because the equilibrium of the LDH reaction is markedly in favor of La^-. La^-, then, is an immediate reservoir supplying pyruvate to mitochondria for complete oxidative combustion. Therefore, La^- stands at the intersection of anaerobic and aerobic metabolism of carbohydrate fuel. La^- may also coordinate cytosolic redox state among tissues throughout the body. It is of little wonder, then, that we find La^- involved throughout intermediary metabolism, including both global extraorgan exchange and intra-tissue exchange.

Compliance with ethical standards

Conflict of interest There are no conflicts of interest.

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