Tracing the lactate shuttle to the mitochondrial reticulum

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Isotope tracer infusion studies employing lactate, glucose, glycerol, and fatty acid isotope tracers were central to the deduction and demonstration of the Lactate Shuttle at the whole-body level. In concert with the ability to perform tissue metabolite concentration measurements, as well as determinations of unidirectional and net metabolite exchanges by means of arterial–venous difference (a-v) and blood flow measurements across tissue beds including skeletal muscle, the heart and the brain, lactate shuttling within organs and tissues was made evident. From an extensive body of work on men and women, resting or exercising, before or after endurance training, at sea level or high altitude, we now know that Organ–Organ, Cell–Cell, and Intracellular Lactate Shuttles operate continuously. By means of lactate shuttling, fuel-energy substrates can be exchanged between producer (driver) cells, such as those in skeletal muscle, and consumer (recipient) cells, such as those in the brain, heart, muscle, liver and kidneys. Within tissues, lactate can be exchanged between white and red fibers within a muscle bed and between astrocytes and neurons in the brain. Within cells, lactate can be exchanged between the cytosol and mitochondria and between the cytosol and peroxisomes. Lactate shuttling between driver and recipient cells depends on concentration gradients created by the mitochondrial respiratory apparatus in recipient cells for oxidative disposal of lactate.

INTRODUCTION

The results of isotope tracer studies at the whole-body and tissue levels have clarified longstanding issues concerning metabolic regulation. In particular, the role of the mitochondrial reticulum in lactate disposal has been identified. The reticulum lowers cellular [lactate] by oxidative disposal, thus acting to develop the concentration gradients necessary for lactate fluxes across organ, tissue, and cellular compartments. Similar to other solutes, gases, and entities in physics and physiology, lactate moves from areas of high to areas of low concentrations. Hence, lactate-producing cells, organs, and tissues with high lactate levels drive carbon flux to disposal (recipient) sites with low lactate levels. In this scenario, the mitochondrial reticulum, in which lactate is disposed of via oxidation, creates the driving force for shuttling lactate around the body and within organs, tissues and cells. Key features of the process involve the mitochondrial (m) Lactate Oxidation Complex (mLOC), which contains mitochondrial lactate dehydrogenase (mLDH), a monocarboxylate (lactate) transporter (mMCT), a scaffolding protein (CD147) and cytochrome oxidase (COx). However, within the context of this special issue on the use of isotopic tracers to study metabolism, the results of lactate tracer studies directly led to the discovery of the mLOC for lactate shuttling to occur in vivo. We begin with a discussion of mLDH.

Similar to the concept of an mLOC, discussions on the role of mLDH in intermediary metabolism have endured a long and winding history, perhaps a path not unusual in the history of science. Because of the fundamental importance of mLDH in physiology and metabolism, revisiting the history of discoveries is warranted at the outset of this paper. The presence of mLDH is essential to explain the role of lactate in affecting lactate flux and ultimately the regulation of energy substrate partitioning in vivo.

Background knowledge

Today, we know that some types of facultative cells can be cultured with lactate as a preferred fuel because their mitochondrial reticula consume and oxidize lactate directly without conversion to pyruvate in the cytosol. For instance, mitochondria of yeast (Saccharomyces cerevisiae) contain flavocytochrome b2, a lactate-cytochrome c oxidoreductase that couples lactate dehydrogenation to the reduction of cytochrome c. In fact, the association between cytochrome b2 and LDH in yeast can be traced to the 1940s. A similar phenomenon occurs in mammalian muscle in persons resting at the sea level, and even in men exercising at sea level or at an altitude of 4300 m. Technological limitations, such as the availability of isotope tracer methodology, the ability to catheterize veins and arteries for continual (a-v) measurements, the ability to determine tissue and systemic blood flow rates, and mass spectrometry for turnover analyses, made such data unobtainable in the 1920s. Consequently, longstanding ideas on the role of lactate in physiology originated on the basis of incomplete data on isolated, nonperfused, nonoxygenated amphibian muscles made to contract continually in ways not intended by nature. More recently, with the advent of isotope tracers and nuclear magnetic resonance (NMR) spectroscopy, we now know that contractions stimulate glycolysis in muscle independent of O2 availability and that lactate is oxidized in working skeletal and cardiac muscles.
MITOCHONDRIAL LACTATE OXIDATION (THE MIND’S EYE VIEW OF POLYPHEMUS)

Contemporary textbooks of biochemistry and physiology abound the 19th-century concepts that metabolism is either “anaerobic” (without O₂) or “aerobic” (with O₂). Glycolytic flux from glucose and glycogen is typically depicted in textbooks as progressing to pyruvate and then to the tricarboxylic acid (TCA) cycle. However, if oxygen is absent, textbooks assert that glycolysis progresses to lactate. This is a convenient motif, typically copied by one textbook author from another and then through serial editions of texts. Amazingly, some textbook authors who advanced the idea of lactate production due to oxygen limitation were biochemists who worked with cells in high-glucose-containing culture media under fully aerobic conditions of one atmosphere pressure in which the partial pressure of oxygen was at least 50% greater than in the arterial blood of individuals at sea level altitudes. Because the maintenance of cells in such preparations required daily changing of the incubation media to maintain high [glucose], low [lactate], and physiological pH, the observations should have informed the investigators that lactate was produced under fully aerobic conditions. Contemporaneously, physiologists measured lactate [L] to pyruvate [P] concentration ratios (L/P) of 10 in muscles and blood in resting mammals, including humans, and further observed the L/P to rise more than 100 during submaximal exercise. However, did any of the textbook authors ever look to determine whether isolated mitochondria oxidize lactate? For many textbook authors, the answer is “no.” However, for a few others, the answer is “yes.” Moreover, some investigators also looked for the presence of a mitochondrial monocarboxylate (lactate) transporter (mMCT) and a lactate dehydrogenase (mLDH) enzyme. Unfortunately, while some attempts failed, fortunately, other attempts to observe mitochondrial lactate oxidation and the presence of mLDH and mMCT were successful. Importantly, lactate oxidation in human muscle mitochondrial preparations was observed. Regrettably, for a time, positive results were overlooked or castigated as being “controversial” and dismissed for failing to fit with established paradigms of metabolic regulation. However, now that the MitoCarta (https://www.broadinstitute.org/mitocarta/mitocarta30-inventory-mammalian-mitochondrial-proteins-and-pathways) and Mitominer (https://mitominer.mrc-mbu.cam.ac.uk/release-4.0/begin.do) databases have been published, mLH is a recognized constituent of the mitochondrial proteome. Acknowledging that lactate, not pyruvate, is the main gluconeogenic precursor and carbohydrate energy source shared at the organismal and tissue levels upssets archaic concepts of the organization of intermediary metabolism. Nevertheless, despite the evidence, in the manner of Polyphemus, some have ignored the literature and databases on mLH and mMCT and their roles in mitochondrial lactate oxidation.

The lactate:pyruvate affair

Regrettably and inexplicably, thus far, as the use of isotope tracers to study lactate metabolism is concerned, the literature has been muddled, creating a “Lactate:Pyruvate Affair” that needed to be corrected. Specifically, there has been controversy over whether lactate tracers measure lactate (L) or pyruvate (P) turnover. Despite a series of analytical errors, the use of inappropriate tissue and animal models, a failure to consider L and P pool sizes in modeling results, inappropriate tracer and blood sampling sites, a failure to anticipate roles of heart and lung parenchyma on L ⇔ P interactions, and a failure to appreciate results from magnetic resonance spectroscopy (MRS) and immunocytochemistry, it is clear that carbon-labeled lactate tracers can be used to quantitate lactate fluxes in situ and in vivo.

The early twentieth century

In the early literature of the 20th century, there were several threads to a contemporary understanding of lactate production and oxidation during postprandial and exercise conditions. Among those threads were the findings of Fletcher and Hopkins, who showed that lactate acid disappeared when frog muscles fatigued by electrical stimulation were placed in oxygen-rich environments. Albite in another field of endeavor, cancer research, another thread was the finding of Warburg that lactate was produced by fully oxygenated cancer cells. Nonetheless, while there were data from a reputable investigator (Nobel Laureate) that lactate could be produced under fully aerobic conditions and that oxidative removal of lactate could be accomplished without reconversion to glucose, lactate and lactic acid in cell metabolism were misconstrued as waste products of oxygen-limited metabolism and fatigue agents early in the 20th century.

Most importantly, several sets of findings in the late 20th century led to the realization of oxidative lactate disposal. Among these sets of findings was evidence that isolated mitochondrial preparations could oxidize lactate in vitro and that mitochondria contained the enzymatic apparatus to oxidize lactate. Importantly, isotope tracer studies showed oxidative lactate disposal in mammals and humans in vivo. Evidence obtained on individuals studied in vivo is elegantly supported by the results of studies using MRS technology. These important sets of findings are addressed sequentially.

OVERLOOKED FINDINGS

The first investigator to evaluate mitochondrial lactate metabolism was Mario Umberto Dianzani (1925–2014), who, in 1951, published a paper, translation of which is “Distribution of lactate acid oxidase in liver and kidney cells of normal rats and rats with fatty degeneration of the liver.” A translation of a section on p. 182 is “In conclusion, the [cellular] lactate acid-oxidizing systems are localized 80 to 100% in the mitochondria.” Although Dianzani had a prolific career in hepatic toxicology and only recently retired from the Department of Experimental Medicine and Oncology, University of Turin, he apparently never followed up on his finding of mitochondrial lactate oxidation, and his seminal findings went unrecognized even by workers in the field of mitochondrial energetics.

First to report the presence of mitochondrial LDH were Nobuhisa Baba and Hari M. Sharma, who used electron microscopy (EM) to study the hearts and pectoralis muscles of rats. Decades later, their results showing mLH were confirmed by many others, including Brandt and Kline et al., Brooks...
ET, Baba and Sharma were probably the skeletal muscle and the integument are lactate producers and lactate shuttling, the heart, liver, and kidneys are consumers or original seminal findings of Baba and Sharma went unrecognized. These phenomena underpin the tracer-measured differences between the cytosol and mitochondria across tissues. From Brooks et al.13 et al., Nakae et al.76, Taylor et al.77, Lemire16, Szczesna-Kaczmarek et al.18,19, Young et al.54, and Pagliarini et al.57. Subsequently, the results were extended to human skeletal muscle mitochondria by Dubouchaud et al. in the Brooks Laboratory15 (Fig. 2). Retrospectively, based on their seminal observations of mLDH using EM, Baba and Sharma were probably the first to use the term “lactate shuttle.” Regrettably, they never followed up on their original finding of mLDH, and similar to the discovery of Dianzani, the seminal findings of Baba and Sharma went unrecognized.

Beyond Dianzani78 and those who replicated his seminal observation that mitochondria were capable of oxidizing lactate4,5,13,52,34,36,79 were the efforts of those who failed in their attempts to isolate mitochondrial preparations that contained sufficient mLDH to oxidize lactate47–49,90,81. In addition, there were others who routinely found LDH in their mitochondrial prepara-
tions but regarded their findings as an artifact and took steps to block mLDH, thus permitting the measured rate of exogenous pyruvate oxidation to rise52. The lesson to take from history is that mLDH is necessary for lactate oxidation. When mLDH is lost in the isolation of single fibers49 or mitochondrial vesicles68 or if oxamate is used to block mLDH18, the preparation will be able to oxidize pyruvate but not lactate.

The ability of the mitochondrial reticulum to respire lactate is fundamental to the operation of lactate shuttles because lactate disposal via oxidation in the reticulum decreases the cellular [lactate], thus establishing lactate concentration gradients down which lactate fluxes. Hence, it is understandable that the rates and directions of lactate flux vary with physiological conditions (e.g., rest or exercise, early or late during exercise, endurance-trained or sedentary individuals, sea level or high altitude, carbohydrate nourished). For instance, in the postprandial state, when red skeletal muscles are glucose consumers and lactate producers4, and hence drivers of lactate shuttling, the heart, liver, and kidneys are consumers or recipients of lactate shuttle. In contrast, during exercise, white skeletal muscle and the integument are lactate producers and drivers of lactate shuttling, whereas highly oxidative (red) fiber types, cardiac tissue, liver, kidneys, and brain3–8 are sites of net lactate disposal. In these and other forms of shuttling, lactate fluxes from high to low concentrations with the cellular respiratory (mitochon-
drial) apparatus, including mLDH, functioning as removal sites3,5,88,89. These phenomena underpin the tracer-measured flux rates observed under conditions of rest and exercise83,90,91.

In a previous publication, the presence of a Postprandial Lactate Shuttle was introduced. Following a carbohydrate (CHO) meal, glycogenolysis, and lactate production in noncontracting red skeletal muscle62,93 raises lactate in the systemic circulation, thus providing a substrate for hepatic and renal gluconeogenesis85,94 and an energy substrate for skeletal muscle5,91,95 and the heart,93,95,96, thereby forming a Postprandial Lactate Shuttle4.

TRACERS AND LACTATE TURNOVER DURING REST AND EXERCISE

Pioneering work in the field of lactate kinetics in exercising mammals was performed by Florent Depocas (1923–2004) and colleagues69. Using a continuous infusion of [U-14C]lactate into dogs during rest and continuous steady-state exercise, they made several key, fundamental findings regarding lactate metabolism. These findings included (1) active lactate turnover during the resting postabsorptive condition; (2) ~1/2 of lactate formed during rest is removed through oxidation; (3) the turnover rate of lactate increases during exercise compared to rest even if there is only a minor change in blood lactate concentration; (4) the fraction of lactate disposal through oxidation increases to approximately 3/4 during exercise, and (5) a minor fraction (1/10-1/4) of lactate removed is converted to glucose via the Cori Cycle during exercise. Although the fractions are subject to species and experimental variations, the essential results have been reproduced in rats15, rabbits97, dogs69,98,99, horses100, and humans1,21,35,36,70,73,91,101. Depocas’ work on lactate metabolism was just one small part of his work at the Canadian NRC, where he held wide-ranging interests in the metabolic and endocrine responses of mammals to hypothermia.

Over the span of two decades, commencing in the mid-1980s, Brooks Lab Staff Research Associates Michael A. Horning, Gretchen C. Casazza and Jill A. Fattor; Brooks’ graduate students Timothy P. White, Glenn A. Gaesser, Casey M. Donovan, William C. (Bill) Stanley, Robert S. Mazzeo, Bryan C. Bergman, Benjamin F. Miller, David A. Roth, Sang-Hoon Suh, Rajaa Hussien, and Chi-An W. Emhoff; and postdoctoral fellows, visiting scholars and collabora-
tors such as Thomas D. Fahey, Eugene E. Wolfel, Jacques Mercier, Hashimoto, Hervé Dubouchaud, Grant McCllelland and Laurent A. Messonnier made concerted efforts to describe the effect of exercise intensity and training state on whole-body and working muscle lactate oxidation and gluconeogenesis from lactate in humans. The effects of exercise intensity on whole-body lactate turnover and oxidation were initially reported by Stanley70,91,95 and Mazzeo72, who showed that tracer-measured lactate turnover and oxidation scaled to the metabolic rate during exercise and that while oxidation accounted for ~50% of lactate disposal in resting men, oxidation accounted for 75–80% of lactate disposal during continuous hard exercise.

Studies of lactate metabolism in working skeletal and cardiac muscle in men during exercise were reported by Stanley et al. in 198670,91 and Edward W. Gertz et al. in 198899,95, respectively. The advantage of using tracer infusions along with simultaneous a-v difference and blood flow measurements across working muscle beds is that lactate uptake and net release, therefore, total production (= the sum of uptake and net release), can be determined simultaneously. Furthermore, another advantage of using tracer infusions along with simultaneous a-v 13CO2 and blood flow measurements across tissue muscle beds is that tissue metabolite oxidation can be measured. In addition, working muscle turnover and oxidation rates can be compared with whole-body rates. The results showed simultaneous lactate extraction (uptake) and release (production) and oxidation by working human leg muscles, which explains most whole-body lactate turnover and oxidation rates91. Importantly, due to intramuscular lactate extraction, turnover and oxidation, net chemical balance measurements underestimate true lactate production and
Choice of lactate carbon tracers to determine lactate flux and disposal in vivo

As noted above, initial studies of lactate metabolism in vivo utilized a uniformly labeled tracer, i.e., U-14C]lactate. However, with the advent of stable, nonradioactive 13C-labeled tracers, it was ethically feasible to study flux and oxidation in human subjects. Because lactate disposal via oxidation gave rise to 13CO2, carbon-labeled lactate tracers were preferred over heavy hydrogen (i.e., deuterated, D) tracers because the oxidation product (13CO2) could be detected at the tissue level in the venous effluent of tissues such as muscle, heart, and brain and at the whole-body level by excretion in expired air. Another advantage of the 13C-lactate tracer was that it could be used in combination with a deuterated glucose tracer, e.g., [6,6-2H]glucose, a so-called “irreversible tracer”, because the D atoms are lost to body water during glycolysis. Moreover, because a percentage (=25%) of 13C atoms from lactate are reincorporated into glucose during the process of gluconeogenesis, the combination of [3-13C]lactate and [6,6-2H]glucose (i.e., D2-glucose) could be used to simultaneously study lactate and glucose flux rates, the lactate oxidation rate and the rate of gluconeogenesis from lactate.

On the issue of which isotope tracer of lactate to use, the most common is [3-13C]lactate, which yields information about total lactate disposal as well as the components of oxidation and gluconeogenesis. Earlier use of uniformly labeled compounds to determine metabolite disposal rates was abandoned because of the extensive recycling and exchange of labeled lactate carbon atoms. Of the alternatives, 13C from [1-13C]lactate is lost as 13CO2 in the pyruvate dehydrogenase reaction, as pyruvate is converted to acetyl-CoA upon entry into the TCA cycle. This decarboxylation step shows entry into the TCA cycle, but perhaps not complete oxidation of the lactate molecule. For that, [3-13C]lactate is the preferred tracer to demonstrate lactate oxidation downstream in the TCA cycle by the actions of isocitrate and alpha-ketoglutaric dehydrogenases.

Finally, with the use of carbon-labeled tracers to determine the oxidative disposal of lactate, it needs to be acknowledged that because metabolically produced 13CO2 enters the body's bicarbonate-CO2 pool, a separate experiment under identical conditions needs to be conducted. At rest, the bicarbonate-CO2...
Lactate Oxidation Complex (mLOC) in humans, the correction factor for CO2 retention is large (≈50%)120. The large correction factor for estimating isotopic dilution and retention is daunting for investigators to make because it is so large. Fortunately, however, during exercise, the necessity of a bicarbonate correction factor disappears because the bicarbonate-CO2 pool turns over rapidly with no measurable 13CO2 retention or diversion of tracer to other, unmeasured metabolite pools121,122. In addition, some investigators have explored the use of so-called “acetate correction factors”123. However, the latter approach is not recommended because acetate is not bicarbonate and, most importantly, because the C1 and C2 of acetate behave differently, leaving an investigator uncertain about what the appropriate correction factor is120.

THE LACTATE SHUTTLE AND MEMBRANE LACTATE TRANSPORT AND LACTATE/PYRUVATE EXCHANGE

With knowledge of glucose and lactate fluxes and oxidation rates in resting and exercising rats124,125, in 1984, George A. Brooks took a unique approach to explain phenomena related to lactate responses to exercise when the “Lactate Shuttle Hypothesis” was articulated125. A key element of the hypothesis was that “the shuttling of lactate through the interstitium and vasculature provides a significant carbon source for oxidation and gluconeogenesis during rest and exercise”. As such, the lactate shuttle hypothesis represents a model of how the formation and distribution of lactate represents a central means by which the coordination of intermediary metabolism in diverse tissues and different cells within tissues can be accomplished. The initial hypothesis was developed from the results of original isotope tracer studies conducted on laboratory rats in the Brooks Laboratory along with numerous other studies, many of which were cited in the previous section. Thus, the working hypothesis was developed that much of the glycolytic flux during exercise passed through lactate.

According to the Cell–Cell Lactate Shuttle hypothesis, lactate is a metabolic intermediate rather than an end product126–129. Lactate is continuously formed in and released from diverse tissues, such as skeletal muscle, skin, and erythrocytes, and also serves as an energy source in highly oxidative tissues, such as the heart, and is a gluconeogenic precursor for the liver and kidneys. Lactate exchanges among these tissues appear to occur under various conditions, ranging from postprandial rest to sustained postabsorptive exercise128,129.

If lactate does serve as a key metabolic intermediate that shuttles into and out of tissues at high rates, particularly during exercise, then transmembrane movement is critical. For many years, lactate was assumed to move across membranes by simple diffusion. However, by 1980, facilitated protein carrier-mediated transport of lactate across erythrocyte membranes was well documented130,131. It was not until 1990 that the characteristics of sarcolemmal membrane lactate transport were described132–134. The study of cell membrane lactate transport proteins took another major leap in 1994. When looking for the mevalonate (Mev) transporter gene in Chinese hamster ovary cells, Christine Kim Garcia in the Goldstein and Brown laboratories cloned and sequenced a monocarboxylate transporter that she and her colleagues termed the monocarboxylate transporter (MCT)135. In 1985, Goldstein and Brown shared the Nobel Prize for “their discoveries concerning the regulation of cholesterol metabolism”136. The newly discovered MCT was abundant in erythrocytes, the heart, and the basolateral intestinal epithelium. MCT was also detectable in oxidative muscle fiber types but not in the liver. With an interest in describing a role for MCT isoforms in the Cori Cycle, Garcia et al.137 subsequently described the isolation of a second isoform (MCT2), in effect renaming the first transporter discovered.
knowledge, in 1998, Brooks extended the Cell–Cell Lactate Shuttle concept to include an intracellular component, the “Intracellular Lactate Shuttle.” As already noted, several of the observations, such as the presence of mLDH and mitochondrial lactate oxidation, were similar to the vision of Polyphemus—without depth perception, swamped by the mediocrity of authority, buried and forgotten in the literature for a time but later resurrected.

**COMPARTMENTATION ISSUES AND CONTROVERSIES—THE INTRACELLULAR LACTATE SHUTTLE**

While there is growing agreement on some aspects of the Cell–Cell Lactate Shuttle, within exercise physiology and other fields, such as neurobiology and cancer research, some aspects of the Intracellular Lactate Shuttle remain controversial. In essence, disagreement exists over where the first step in lactate oxidation (i.e., conversion to pyruvate) occurs. This is why a discussion over whether mitochondria contain LDH persists. If so, identifying where LDH would reside so that it is able to oxidize lactate (vide supra) is important. However, the results of light and electron microscopy studies conducted to date show that mLDH resides within the mitochondrial reticulum.

**Electron microscopy**

Prior to the MitoCarta and MitoMiner databases based on proteomics, those who looked for muscle mLDH located it in tissues. Studying rat heart, Baba and Sharma used methods to protect mLDH during tissue fixation for electron microscopy. Reaction products of mLDH seen in the mitochondria were “primarily located within the cristae” (Fig. 1) (their Fig. 11). In addition, they reported similar results for rat pectoralis Types I and II fibers. Similarly, using an antibody to LDH and gold particle secondary labeling, Brooks et al. also showed LDH located on the inner mitochondrial membranes of rat muscle, heart, and liver.

Moreover, they used agarose gel electrophoresis to separate LDH isoforms in the cytosol and mitochondria isolated from rat liver and hearts. LDH isoenzyme patterns differed between the cytosol and mitochondria in both tissues (Fig. 2).

**Immunocytochemistry**

In the Brooks Laboratory, Takeshi Hashimoto used confocal laser scanning microscopy, immunoprecipitation (IP), and cell fractionation techniques followed by western blotting to verify the presence of the purported Mitochondrial Lactate Oxidation Complex (mLOC) in cultured myocytes as well as in thin sections of adult rat tissues. The colocalization of MCT1, mitochondrial cytochrome oxidase (COx) and LDH in the mitochondria of adult rat skeletal muscle and astrocytes was visualized by means of combinations of primary and fluorescently labeled secondary antibodies plus MitoTracker Red and dual-wavelength scanning confocal microscopy (Fig. 3). Subsequently, similar techniques allowed the investigators to show the colocalization of mLOC components in cultured L6 (rat muscle-derived) cells (Fig. 4). Again, those results were confirmed by two independent techniques—IP and Western blotting of isolated cell fractions. The results of IP efforts are shown in Fig. 5, and a pictorial representation of how the mLOC is organized on the inner mitochondrial membrane with projection into the intermembrane space is constructed from the results of immunocytochemistry and immunoprecipitation studies (Fig. 6).

Both morphometry and the susceptibility to loss during mitochondrial isolation suggest that LDH resides in the intermembrane space or, similar to cytochrome c, is loosely fixed to the inner membrane. On the other hand, if it is acknowledged that cardiac and red skeletal muscle take up and oxidize lactate, while the presence of mLDH and an mLOC are denied, then opponents of the Intracellular Lactate Oxidation Complex must rely on cytosolic oxidation of lactate to pyruvate. Cytosolic oxidation of

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**Fig. 5** Results of efforts to deduce the organization of the mLOC using immunoprecipitation (IP) technology. In the upper panel, representative immunoblots (IB) are shown using anti-COX, NADH-DH, LDH, or nlgG as precipitating antibodies (IPs). COX, NADH-DH, LDH, and nlgG were immunoprecipitated from mitochondrial fractions of L6 cells resuspended in a suspension medium without detergent. COX IP proteins were probed with MCT1, CD147, and LDH antibodies. MCT1, CD147, and LDH were coprecipitated with NADH-DH, whereas COX coprecipitated with anti-NADH-dh. LDH IP proteins were probed with MCT1 and COX antibodies. Both MCT1 and COX coprecipitated with LDH. No protein coprecipitated with nlgG from mitochondrial fractions of L6 cells resuspended in medium without detergent (negative control). In the lower panel, the degree of coprecipitation evaluated by comparing signals in the IP and the lysate is shown. The results were categorized into four levels: ++ +++, 80% or more precipitated; ++ +, ~50% precipitated; +, 20% or less precipitated; -, no precipitation. IB immunoblot, IP immunoprecipitation. From Hashimoto et al. 138.

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Independent of Garcia et al. 135,137, Price et al. 142,143 identified another MCT isoform (now known as MCT4) in 1998. In terms of physiology, Halestrap and colleagues continued to hold traditional, Hill-Meyerhof views of an association between lactate transporter expression and oxidative metabolism even though their data showed a high correlation between MCT1 expression and mitochondrial markers, which was subsequently replicated by Dubouchaud et al. 15. However, with a different concept based on the knowledge that lactate was formed and oxidized continuously within muscle and heart tissue in vivo, Brooks and colleagues first observed the presence of LDH in rat liver, cardiac and skeletal muscle mitochondria. Subsequently, they also showed MCT1 to be in mitochondria of the same tissues, thus allowing mitochondria to directly oxidize lactate. With such...
lactate is unlikely and contrary to the evidence available from Peter Hochachka and colleagues, who studied glycolysis in a variety of mammalian species in the David Wasserman laboratory, who studied glucose and lactate metabolism in the hind limb muscles of running dogs. By means of tracer glucose, they showed intramuscular lactate production from glucose to occur simultaneously with net muscle lactate uptake. Clearly, glycolysis progressed to lactate production in the working muscles of running dogs, whereas lactate oxidation occurred in a compartment where it was oxidized, the mitochondrial reticulum.

Mitochondrial monocarboxylate uptake mMCTs, mPCs or Both?

Analogous to the controversial history of mLDH discovery was the discovery of the presence of mitochondrial monocarboxylate transporters (mMCTs) and cultured L6 myocytes by Takeshi Hashimoto and colleagues. These were visualized in adult rat skeletal muscle and cultured L6 myocytes by Takeshi Hashimoto and colleagues. For a time, the isoform monocarboxylate transporter 1 (i.e., MCT1) was the only lactate/pyruvate transporter known to be expressed in the mammalian mitochondrial proteome. Subsequently, the mitochondrial pyruvate transporter (mPC) was identified. Unfortunately, at present, little effort has been expended to evaluate functional or morphometric relationships between mMCTs and mPCs, particularly in mammalian skeletal muscle. Indeed, we unfortunately answer questions about mMCT and mPC colocalization and functionality and the role of mPCs in mitochondrial lactate oxidation.

Role of the mitochondrial lactate oxidation complex (mLOC) in lactate/pyruvate metabolism

Discovery of the mLOC (Fig. 5) and its role in Cell--Cell and Intracellular lactate shuttles may cause some traditional thinkers to assert something such as "the diversion of glycolytic flux from pyruvate to lactate via cytosolic LDH (cLDH) with a return to pyruvate via mLDH still means that pyruvate is the major mitochondrial energy substrate". Such an assertion would hold at the unicellular organismic level. However, to briefly reiterate from above, lactate shuttles are based on lactate concentration differences between producer and recipient cells connected through the interstitium within a tissue bed or the vasculature at the organ system level. In the shuttling of carbohydrate carbon energy substrates, lactate concentrations are orders of magnitude greater than those of pyruvate and alanine. Hence, corporal energy substrate distribution is accomplished with lactate, not pyruvate, as the vehicle of mass-energy transfer.

The mitochondrial lactate oxidation complex (mLOC) in cancer

As noted above, in the Brooks laboratory, Takeshi Hashimoto et al. showed the presence of the mLOC in mammalian skeletal muscle, muscle cell lines, neurons, and primary neuronal cell cultures. In addition, his work revealed the presence of a lactate-sensitive transcription network in cultured myocytes. Having been part of the team previously working on muscle and brain, both postmitotic tissues, Rajaa Hussien in the Brooks laboratory commenced work on breast cancer cells that display active mitosis. Initial efforts were on known mLOC proteins, including monocarboxylate transporters (MCT1, MCT2, and MCT4), a scaffold glycoprotein (CD147), lactate dehydrogenase (LDH isoforms A and B), and cytochrome oxidase (COx). The results were consistent with their primary hypothesis that mLOC expression and assembly are dysregulated in breast cancer. Hussien and Brooks tested their primary hypothesis on the breast cell lines MCF-7, MDA-MB-231 and HMEC-184; the former two are transformed breast cell lines that are cancerous, while HMEC-184 is a nontransformed primary breast cell line that was used as a control. Protein expression of MCTs, CD147 and LDHs was determined by immunoblot analysis of whole-cell extracts from the three cell lines grown under standard conditions. The expression of the seven proteins in the two transformed cell lines was compared with that in the primary untransformed cell line HMEC-184. Relative to HMEC-184, in the transformed cells, some
LACTATE SIGNALING

The effects of increases in cellular work and subsequent lactate production on redox, reactive oxygen species (ROS), allosteric binding, and histone lactylation have been recently reviewed. Briefly, lactate affects metabolic signaling via diverse mechanisms that include changing the L/P and NADH/NAD⁺ ratios and thus cell redox; activating sirtuins via the enzyme NAM phosphoribosyl transferase (Nampt); stimulating transforming growth factor beta (TGF-beta) secretion from adipose tissue; regulating gene expression by the lactylation of 28 lysine residues on histones and inhibiting lipolysis by activating Hydroxycarboxylic Acid Receptor-1 (HCAR-1) operating through cyclic AMP (cAMP) and cAMP response element-binding (CREB) pathways. With regard to gene regulation via histone lactylation, it should be noted that it was discovered using tracer methodology ([U-13C]lactate and [U-13C]glucose) and a cancer cell line (MCF-7). As noted previously, the purported effects of lactate signaling via HCAR-1, TGF-beta, Nampt, and the lactylation of histones observed in rodent muscle and brain and cell line models await validation in humans. The latter statement is appropriate because of the growing interest in evaluating the role of lactation in promoting carcinogenesis in a variety of cell types, including those of the lungs. Regrettably, thinking in the field is limited by reliance on outmoded O₂ debt (i.e., O₂-limited) ideas of metabolic regulation.

LACTATE AND MITOCHONDRIAL BIOGENESIS

Endurance exercise has long been recognized to stimulate mitochondrial biogenesis, and hence, the following question arose: 'Could the lactatemia of exercise stimulate mitochondrial biogenesis?' In an attempt to address the question, Hashimoto and colleagues incubated L6 cells in elevated sodium lactate added to buffered, high-glucose media and screened the cells for redox, reactive oxygen species (ROS), allosteric binding, and histone lactylation. Lactate increased ROS production and upregulated 673 genes, many known to be responsive to exercise training via ROS and Ca²⁺ stimulation. The induction of genes encoding components of the mLOC was confirmed by a polymerase chain reaction and electrophoretic mobility shift assay (PCR and EMSA, respectively). Among the many effects, lactate increased the mRNA and protein expression of MCT1 and COX. Increases in COX expression coincided with increases in peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC1α) expression and the DNA binding activity of nuclear respiratory factor (NRF)-2. Based upon their results, Hashimoto et al. concluded that the presence of a lactate signaling cascade involves ROS production and converges on transcription factors affecting mitochondrial biogenesis.

The presence of a lactate-sensitive transcription factor network and its putative effects on muscle mitochondrial biogenesis in exercising humans have yet to be evaluated. To date, Genders et al. in the David Bishop Laboratory have confirmed elements of the work of Hashimoto et al.; specifically, they have advanced the field to show that low or high pH can suppress the effect of lactate on mLOC protein expression, transcription, translation, and turnover in breast cancer need elucidation.

proteins (MCT2, LDH, and CD147) were upregulated. Remarkably, the expression of MCT1 was low in MCF-7 cells and undetectable in MDA-MB-231 cells. A notable novel discovery from their study was the reciprocal relationship between the MCT1 and CD147 protein levels. The data from breast cancer cell lines led to the hypothesis (as yet incompletely explored) that the dysregulation of the coordinated expression of these two proteins and their respective genes contributes to the carcinogenic process in breast cancer.

To probe for the presence of mLOC proteins in cancer cells, Hussien and Brooks also examined the subcellular localization of MCTs and LDH isoforms in the three cell lines studied. Confocal laser scanning microscopy and related immunohistochemical techniques showed the presence of MCTs, LDH isoforms, and COX. LDH isoforms MCT2 and MCT4 were colocalized with the mitochondrial protein marker COX, but distinct from the situation in muscle, MCT1 was not mitochondrial and was localized exclusively in the plasma membrane. The localization of MCT and LDH isoforms in the two cancer cell lines (MCF-7 and MDA-MB-231) and the normal cell line (HMEC-184) was the same. MCT2, MCT4, and LDH were localized in mitochondria in addition to their localization in the plasma membrane and cytosol, whereas MCT1 was mainly localized in the plasma membrane (Fig. 8). Their data showed that in a breast cancer cell line, changes in the expression of lactate shuttle proteins occur. The reasons for these changes in mLOC protein expression, transcription, translation, and turnover in breast cancer need elucidation.
lactate on histone deacetylase (HDAC) activity and protein kinase B (aka, Ak strain transforming, Akt) signaling in L6 cells. Studies on the possibility of exercise above the lactate threshold, where there is a disproportionate increase in muscle lactate production, activating a signaling cascade leading to human skeletal muscle mitochondrial biogenesis are underway (David Bishop personal communication).

THE LACTATE STORY COMES FULL CIRCLE
Considering the above text on intact individuals in vivo, tissues, and organs in situ, and cells in vitro, as well as the results on cancer cells to date, it seems that the study of lactate metabolism has come full circle. From the perception of a lowly waste product formed as the result of a lack of oxygen, as embodied in the shuttle hypothesis, lactate is now realized to have three essential metabolic functions: an energy source, a gluconeogenic precursor, and a signaling molecule. Originally conceived of within the context of exercise physiology and supporting short- and long-term adaptive processes, some investigators propose using lactate esters and salts to sustain athletes in prolonged hard exercise bouts170 and to provide nutritive support to humans following traumatic brain injury104. A clinical trial is underway to evaluate the efficacy of lactate supplementation to support the injured brain. Nevertheless, as lamented by See and Bellomo172, in intensive care units where the blood lactate level is a biomarker for the severity of sepsis, a lack of knowledge on the sites and reasons for lactatemia hampers treatment. Fortunately, progress has been made in understanding the role of lactatemia in the etiology of diabetic ketoacidosis173. Similarly, the role of dysregulated lactate metabolism is advancing, with investigators giving thought to destroy cancer cells by blocking lactate shuttling140,141,151,174,175.

SUMMARY
A chronology of significant events and important discoveries in the field of lactate metabolism is given in Table 1. As shown, in the latter part of the 20th century, the use of isotope tracer technologies enabled significant contributions to a revolution in understanding the diverse roles of lactate in the regulation and integration of intermediary metabolism in humans and other mammals in health and disease.

Isotope tracer technologies were central to the deduction and demonstration of the Lactate Shuttle at the whole-body level. In concert with the ability to perform tissue metabolite concentration measurements, as well as determinations of unidirectional and net metabolite exchanges by means of a-v difference and blood flow measurements across tissue beds including muscle and the brain, lactate shuttling within organs and tissues was
Table 1. Historical timeline of lactate metabolism thought and discoveries.

1907–1924: The Lactic Acid Era. Archibald Vivian (A.V.) Hill and the Cambridge School of Physiologists believed that the "processes of muscle contraction are due to the liberation of lactic acid from some precursor". Otto Meyerhof quantifies the relationship between glycogen and lactic acid formation in isolated, nonperfused frog hemichorpus preparations. A.V. Hill develops the O2 Debt concept in human studies. August Krogh and Johannes Lindhard provide evidence supporting Zuntz's assertion that both fat and carbohydrate are substrates for energy during exercise in humans, but those studies were outside the prevailing theory of the Cambridge School of A.V. Hill and associates.

1925: Contrary to the oxygen-limited, O2 Debt Ideas of the Cambridge school, contemporaneously in Germany, Otto Warburg describes aerobic glycolysis in tumor cells; subsequently the phenomenon became known as the "Warburg Effect". Later, it became obvious that lactate production occurs in the soma of healthy individuals while resting or exercising or after carbohydrate nutrition as well as in the gut microbiome.

1929: Carl Ferdinand Cori and Gerty Theresa Cori propose and describe a metabolic pathway in which L-lactate produced by anaerobic glycolysis in the muscles moves to the liver where it is oxidized to pyruvate to glucose, which then returns to the muscles and is metabolized. This was the first expression of a Lactate Shuttle mechanism involving the vascular exchange of precursor from a driver cell or tissue (muscle) and receipt by a recipient cell or tissue (liver), and return.

1933: Rodolfo Margaria, Harold T. Edwards and David Bruce Dill of the Harvard Fatigue Laboratory apply the new knowledge of the phosphagens to O2 Debt theory in humans and segment the phenomenon into "lactacid" and "alactacid" components.

1936: Ole Bang challenged the established lactacid O2 debt concepts. But, his results are ignored, WW II intervenes and oxygen debt ideas became entrenched.

1937, 1938: Data of Hans Krebs and William Johnson indicate that the carbohydrate derivative to enter the Tricarboxylic Acid Cycle (TCA) is pyruvate. The role of mitochondrial lactate dehydrogenase (mLDH) is unknown and unsuspected.

1940: After the elucidation of the glycolytic pathway (known as the Embden–Meyerhof–Parnas Pathway) that assumed pyruvate to be its end product under aerobic conditions, together with conclusions of Krebs and associates that pyruvate is the molecule that enters the TCA, most of the research into energy metabolism would continue unabated using the basic paradigm established by the principal players of the first half of the 20th century.

1951: Mario Umberto Dianzani shows that rat liver mitochondria oxidize lactate.

1955 & 1956: Douglas S. Drury, Arne N. Wick and Toshiko Morita use 14C-lactate tracers to show extra-hepatic oxidative disposal of lactate in rabbits.

1964: Karlman Wasserman and Malcolm B. McIlroy assume the Meyerhof-Hill O2-Debt theory of oxygen-limited lactate production and coin the term "anaerobic threshold," which purported to describe the "onset of anaerobic metabolism during exercise".

1966–1968: Wendell Stainsby and Hugh Welch demonstrate the transient nature of muscle lactic acid output in canine muscle in situ and present evidence that argues for the O2 independence of lactic acid formation during muscle contractions. They observe that lactate produced in the muscle was used in the TCA cycle, whereas formerly it was thought that lactate was not metabolized in the muscle. This phenomenon is to be observed in exercising humans; in 1998, Brooks and colleagues observe the same phenomenon in humans and name it the "Stainsby Effect".

1968: Franz Jobiss and Wendell Stainsby demonstrate oxidation of Complex 1 in the mitochondrial electron transport chain of dog gastrocnemius muscles contracting in situ at an intensity sufficient to produce and release lactate.

1969: Florent Depocas and colleagues use radioactive tracers to study lactate turnover and oxidation in resting and exercising dogs. Fundamental discoveries of Depocas et al. showing active lactate turnover in resting and exercising individuals have been replicated on numerous species, including humans.

1970: Albert Lehninger publishes the textbook Biochemistry. Concepts of glycolysis and fermentation are confused. Ideas that glycolysis leading to pyruvate under aerobic conditions and that lactate is produced due to oxygen lack were enshrined in the latter part of the 20th century. Only recently has a contemporary understanding of distinctions between fermentation and glycolysis, and the role of oxygen, or rather the lack thereof, been refuted.

1973: Richard Connett, Tom Gayeski, and Carl Honig observe lactate production in canine muscle in situ when intramuscular PO2 is apparently insufficient to produce and release lactate.

1974: Casey M. Donovan and George A. Brooks use primed continuous infusions of [U-14C]glucose and -lactate tracers, indirect calorimetry, and two-dimensional chromatography to trace the paths of lactate and glucose disposal during recovery from exhausting exercise. Again, they find little incorporation of lactate-derived carbon into glycogen but major disposal as 14CO2. In mammals, oxidation, not reconversion to glycogen, is the major fate of lactate after exercise.

1975: Glenn A. Gaesser and George A. Brooks use bolus injections of [U-14C]glucose and -lactate tracers, indirect calorimetry, and two-dimensional chromatography to determine the flux, oxidation, and conversion rates of lactate to glucose in endurance-trained and untrained rats during exercise. Training results in the classic finding of lowered arterial [lactate] that is due to increased clearance via oxidation and gluconeogenesis. This seminal study on lab rats and the resulting paper was subsequently reproduced using stable, nonradioactive tracers on human subjects.

1983: Karlman Wasserman and Malcolm B. McIlroy assume the Meyerhof-Hill O2-Debt theory of oxygen-limited lactate production and coin the term "anaerobic threshold," which purported to describe the "onset of anaerobic metabolism during exercise".

1984: Casey M. Donovan and George A. Brooks use primed continuous infusions of [U-14C]glucose and -lactate tracers, indirect calorimetry, and two-dimensional chromatography to trace the paths of lactate and glucose disposal during recovery from exhausting exercise. Again, they find little incorporation of lactate-derived carbon into glycogen but major disposal as 14CO2. In mammals, oxidation, not reconversion to glycogen, is the major fate of lactate after exercise.

1985: George A. Brooks and associates give 14C-lactate to rats after exhausting exercise and find, contrary to classic "O2 Debt" theory, little incorporation of lactate into glycogen but major disposal as 14CO2. This is the first challenge to the classic Hill-Meyerhof concept of a 1/5 – 4/5 lactate-to-lactate conversion to glycogen ratio, which is converse of what happens in a mammalian system after exercise.

1986: Albert Lehninger publishes the textbook Biochemistry. Concepts of glycolysis and fermentation are confused. Ideas that glycolysis leading to pyruvate under aerobic conditions and that lactate is produced due to oxygen lack were enshrined in the latter part of the 20th century. Only recently has a contemporary understanding of distinctions between fermentation and glycolysis, and the role of oxygen, or rather the lack thereof, been refuted.

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1988: European Commission for Research into European Medicine (2022) 54:1332 – 1347.
Table 1. continued

1988: Peter W. Watt and Peter A. MacLennan describe the characteristics of muscle lactate exchange kinetics, pH dependence, and competitive inhibition and saturation of lactate exchange in perfused rat skeletal muscle preparations.

1988: For the first time, Avital Schurr and colleagues described the ability of lactate to support synaptic function of hippocampal neurons in vitro as the sole source of energy substrate.

1990: David Roth and George Brooks describe the characteristics of sarcolemmal lactic acid transport. Concentration and pH dependence, competitive inhibition, and saturation kinetics were demonstrated, as predicted from Watt and MacLennan.

1990: Szczesna-Kaczmarek demonstrates L-Lactate oxidation by skeletal muscle mitochondria.

1991: George Brooks proposes the Intracellular Lactate Shuttle based on measured muscle lactate exchange and oxidation in vivo.

1992: Szczesna-Kaczmarek demonstrates control of mitochondrial L-Lactate oxidation by LDH.

1994: Christine Kim Garcia, Michael S. Brown, Joseph L. Goldstein, and colleagues sequence and clone the gene encoding for a muscle cell membrane monocarboxylate transport protein (MCT).

1995: Garcia and colleagues identify a second isoform, MCT2, located mainly in the liver.

1997: Avital Schurr and associates demonstrated that brain lactate, not glucose, fuels the recovery of synaptic function from hypoxia upon reoxygenation in vitro.

1998: Russ Richardson, Peter Wagner, and colleagues use magnetic resonance spectroscopy (MRS) to show lactate production and net release from fully aerobic, working human skeletal muscle.

1998: Andrew Halestrap and colleagues clone and sequence four new MCT isoforms and describe tissue variability in MCT isoform expression.

1998: An Astrocyte-Neuron Lactate Shuttle was proposed by Pierre Magistretti, Luc Pellerin, and colleagues.

1999: Paul Molé and colleagues use MRS to confirm results of Richardson et al. showing lactate production and net release from fully aerobic, working human skeletal muscle.

1999: Henriette Pilegaard, Andrew Halestrap and Carsten Juel show MCT1 and MCT4 distribution in human skeletal muscle.

1999: Robert A. Robergs and colleagues illustrate that lactate anions, not lactic acid (pKa = 3.86), are formed during exercise.

2000: Andrew Halestrap and colleagues in the Brooks Lab confirm the presence of mLDH and mMCT1 in human skeletal muscle mitochondria.

2000 & 2001: Bryan Bergman, Eugene Wolfel, Gail Butterfield, Gretchen Casaza, Michael Horning, Hervé Dubouchaud, George Brooks, and colleagues show that endurance training improves lactate clearance by intramuscular oxidation and gluconeogenesis in humans.

2000: Henriette Pilegaard, Andrew Halestrap and Carsten Juel show MCT1 and MCT4 distribution in human skeletal muscle.

2001: Avital Schurr et al. showed that blockade of lactate transport via MCT1 exacerbates neuronal damage in a rat model of cerebral ischemia.

2002: Recognition of lactate as a signaling molecule, a lactormone.

2002: Benjamin Miller, George Brooks, and colleagues use exogenous lactate infusion ("Lactate Clamp") and stable isotope tracer technology to test lactate clearance mechanisms and show preferential lactate over glucose oxidation in exercising men.

2002: Daniela Valenti, Lidia De Bari, Anna Atlante, and Salvatore Passarella publish "L-Lactate transport into rat heart mitochondria and reconstruction of the L-lactate/pyruvate shuttle.

2003: Paul Molé and colleagues sequence the gene encoding for a muscle cell membrane monocarboxylate transport protein (MCT).

2003: Diarmuid Smith and colleagues demonstrate that lactate is a preferred fuel for human brain metabolism in vivo.

2004: Robert A. Robergs and colleagues illustrate that lactate anions, not lactic acid (pKa = 3.86), are formed during exercise.

2004: C. Eric Butz and Grant McClelland confirm the presence of mLDH and mMCT1 in skeletal muscle mitochondria.

2006: Takeshi Hashimoto and George Brooks and colleagues provide evidence of a mitochondrial lactate oxidation Complex (mLOC) comprised minimally of mMCT1, basigin (CD147), and cytochrome oxidase (COx) in rodent skeletal muscle.

2006: Avital Schurr hypothesizes that lactate is the ultimate cerebral oxidative energy substrate.

2007: Takeshi Hashimoto and George Brooks and colleagues provide evidence of gene regulation by lactate. In L6 (rat muscle-derived) cells, the upregulated genes include those encoding for MCT1, mitochondrial proteins, proteins involved in ROS generation and quenching, and calcium response elements.

2007: Schurr and Payne show that lactate, not pyruvate, is the end product of glycolysis in neurons under aerobic conditions.

2007: John T. (Jack) Azevedo and colleagues demonstrate preferential lactate oxidation over glucose when co-ingested during continuous exercise.

2008: Takeshi Hashimoto and George Brooks and colleagues provide evidence of a mitochondrial lactate oxidation Complex (mLOC) comprised minimally of mMCT1, basigin (CD147), and cytochrome oxidase (COx) in rodent skeletal muscle.

2008: Pierre Sonveaux and colleagues find MCTs and cell–cell lactate shuttling to be prevalent among tumor cells. They used siRNAs and MCT pharmacological to kill cancer cells in vitro and in mice in vivo, thus making MCTs targets for cancer treatment.

2009: Garret van Hall, Niels Secher, and colleagues show cerebral lactate uptake and oxidation in exercising humans.

2010: Kashan Ahmed and colleagues elucidate a lactate-mediated autocrine response through an orphan G protein (GPR81), now known as hydroxyacytylcarboxylic acid receptor-1 (HCAR-1), demonstrating the ability of lactate to downregulate levels of AMP levels and therein having an antilipolytic effect.

2010: Lidia de Bari, Daniela Valenti, Anna Atlante, and Salvatore Passarella provide evidence of the presence of an intermembrane lactate oxidation that generates H2O2 sufficient to activate the known ROS response elements signaling mitochondrial adaptation and other adaptations to exercise. This work provides a mechanism by which lactate generation in muscle exercise participates in the feedback loop by which lactate generation in exercise leads to adaptations facilitating high rates of lactate disposal in exercise.
evident. We now know that Organ–Organ, Cell–Cell, and Intracellular Lactate Shuttles operate continuously, even after eating when a Postprandial Lactate Shuttle is evident. By means of lactate shuttling, fuel-energy substrate exchange occurs between tissues and organs such as muscle and the brain; muscle and the heart; muscle and the liver, kidneys, and gut; and muscle and other tissues. A muscle-like role of the interorgan is also highly suspected but unexplored. A Corporal-Microbiome Lactate and other tissues. A muscle-like role of the integument is also of lactate shuttling, fuel-energy substrate exchange occurs stored in diverse tissues. Isotope tracers and related technologies carbohydrates are absorbed, circulated, and taken up and used or to recognize a Postprandial Lactate Shuttle by which dietary realm of exercise physiology and biochemistry, it is now possible otherwise affect energy substrate partitioning by mass action, cell precursor; and lactate is a signaling molecule. Lactate signals that there are at least three general functions of lactate shuttling: mitochondria and between the cytosol and peroxisomes. Within cells, lactate can be exchanged between the cytosol and astrocytes and neurons in the brain. Within cells, lactate can be exchanged between the cytosol and mitochondria and between the cytosol and peroxisomes. By means of isotope tracers and related methodologies, we know that there are at least three general functions of lactate shuttling: lactate is an energy source preferred over glucose in the heart, red skeletal muscle, and brain; lactate is the major gluconeogenic precursor; and lactate is a signaling molecule. Lactate signals otherwise affect energy substrate partitioning by mass action, cell redox, the lactylation of histones, and covalent binding to HCAR-1 and signaling via CREB-dependent pathways. Discovered in the realm of exercise physiology and biochemistry, it is now possible to recognize a Postprandial Lactate Shuttle by which dietary carbohydrates are absorbed, circulated, and taken up and used or stored in diverse tissues. Isotope tracers and related technologies have made multiple fundamental discoveries possible in the field of metabolic regulation.

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GAB wrote the initial draft and edited the text depending on feedback from the coauthors. Coauthors provided technical and conceptual feedback in editing of the final manuscript. All coauthors read and approved the final manuscript version.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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