Biochemistry of exercise-induced metabolic acidosis

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DURING INTENSE EXERCISE the increase in blood and muscle lactate and the coincident decrease in pH in both tissues has been traditionally explained by the production of lactic acid. Such a traditional interpretation assumes that due to the relatively low pKa (pH = 3.87) of the carboxylic acid functional group of lactic acid, there is an immediate and near total ionization of lactic acid across the range of cellular skeletal muscle pH (∼6.2–7.0) (12, 28, 40–46, 54). This interpretation is best represented by the content of numerous textbooks of exercise physiology, physiology, and biochemistry that explain acidosis by the production of lactic acid, causing the release of a proton and the formation of the acid salt sodium lactate. On the basis of this explanation, if the rate of lactate production is high enough, the cellular proton buffering capacity can be exceeded, resulting in a decrease in cellular pH. These biochemical events have been termed lactic acidosis. The lactic acidosis of exercise has been a classic explanation of the biochemistry of acidosis for more than 80 years. This belief has led to the interpretation that lactate production causes acidosis and, in turn, that increased lactate production is one of the several causes of muscle fatigue during intense exercise. This review presents clear evidence that there is no biochemical support for lactate production causing acidosis. Lactate production retards, not causes, acidosis. Similarly, there is a wealth of research evidence to show that acidosis is caused by reactions other than lactate production. Every time ATP is broken down to ADP and P_i, a proton is released. When the ATP demand of muscle contraction is met by mitochondrial respiration, there is no proton accumulation in the cell, as protons are used by the mitochondria for oxidative phosphorylation and to maintain the proton gradient in the intermembranous space. It is only when the exercise intensity increases beyond steady state that there is a need for greater reliance on ATP regeneration from glycolysis and the phosphagen system. The ATP that is supplied from these nonmitochondrial sources and is eventually used to fuel muscle contraction increases proton release and causes the acidosis of intense exercise. Lactate production increases under these cellular conditions to prevent pyruvate accumulation and supply the NAD^+ needed for phase 2 of glycolysis. Thus increased lactate production coincides with cellular acidosis and remains a good indirect marker for cell metabolic conditions that induce metabolic acidosis. If muscle did not produce lactate, acidosis and muscle fatigue would occur more quickly and exercise performance would be severely impaired.

metabolism; skeletal muscle; lactate; acid-base; lactic acidosis

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Given the basic, applied, and clinical importance of a correct understanding of the causes of acidosis, the purpose of this review is to 1) present a short history of the discovery and isolation of lactic acid and the early research that established the association between muscle lactate production and acidosis, 2) identify that lactic acid and lactic acidosis are constructs and not facts, 3) review the fundamental biochemistry of reactions in contracting skeletal muscle that alter either of H⁺ production or consumption, 4) provide the true biochemical explanation for metabolic acidosis and present and explain a model of these events, 5) provide research evidence that refutes the concept of a lactic acidosis, 6) present data comparing lactate and proton release from skeletal muscle, as well as intramuscular lactate and proton production, and 7) identify key arguments for the need to correct the way in which acidosis is explained, taught, and interpreted in academia as well as basic and applied research.

A BRIEF HISTORY OF LACTIC ACID

Due to the importance and acceptance of lactic acid in metabolic biochemistry and human physiology, a short history of lactic acid is warranted. Such a short history is not only interesting in and of itself, but also reveals and aids in understanding the early incorrect acceptance of the lactic acid concept.

Discovery and isolation. The Swedish chemist Carl Wilhelm Scheele (17) first discovered lactic acid in 1780. Scheele found lactic acid in samples of sour milk and isolated it in relatively impure conditions. The milk origin of the first discovery of lactic acid led to the acceptance of the trivial name for this molecule (“lactic,” of or relating to milk). However, the true chemical name for lactic acid is 2-hydroxypropanoic acid. The accepted trivial name for the sodium salt of lactic acid is sodium lactate (Fig. 1).

The impurity of Scheele’s original sample of lactic acid led to considerable criticism of the existence of such an acid, with alternate explanations of Scheele’s findings to be a sample of impure acetic acid. Nevertheless, by 1810 chemists had verified the presence of lactic acid in other organic tissues, such as fresh milk, ox meat, and blood (17). By 1833, pure samples of lactic acid had been prepared and the chemical formula for lactic acid was determined. The finding that lactic acid exists in multiple optical isomers (D- and L-isomers) was made in 1869 (17), with the L-isomer having biological metabolic activity. Due to the prevalence of lactic acid formation from fermentation reactions, fermentation was the main direction of early scientific inquiry into the biochemistry of lactic acid production.

Fig. 1. Chemical structures of lactic acid and the sodium salt of lactate. When the proton of the carboxylic acid functional group (–COOH) of lactic acid dissociates (COO⁻ + H⁺), a cation ionically interacts with the negatively charged oxygen atom of the carboxyl group, forming the acid salt lactate. In this example, the cation is sodium (Na⁺).

Table 1. A summary of the physical properties of lactic acid

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical formula</td>
<td>CH₃–CHOH–COOH</td>
</tr>
<tr>
<td>Molecular wt (g/mol)</td>
<td>89.0</td>
</tr>
<tr>
<td>Solubility</td>
<td>Water, ethanol, ethyl ether</td>
</tr>
<tr>
<td>pKa (°C)</td>
<td>3.87</td>
</tr>
<tr>
<td>Heat of combustion</td>
<td>321 kcal/mol</td>
</tr>
</tbody>
</table>

Note that the pKa varies with temperature and the ionic strength of the solution. Compiled from Holten et al. (17).

Physical properties. Table 1 provides a summary of the known properties of lactic acid that have relevance to its functions in cellular metabolism. Work on identifying the chemical and physical properties of lactic acid were complicated by the tendency of solutions of lactic acid to form intermolecular esters, forming poly lactate structures such as the two molecular lactoyllactic acid. Nevertheless, the discovery that lactic acid could crystallize occurred as early as 1895 (17). Subsequent work on quantifying the physical properties of lactic acid were complicated by the difficulties in purifying samples, with research of accepted accuracy for many, but not all, properties not occurring until the 1960s.

Diverse applications. The fact that lactic acid was a naturally occurring molecule, with original detection in food products, led to the possibility for its use in the food industry. Such intended application was aided by lactic acid’s solubility, mild acidic taste, and proven functions as a preservative. Not surprisingly, lactic acid has been used to acidify foods and beverages, assist in the fermentation of cabbage to sauerkraut, to preserve cucumbers, as an ingredient in the brewing and flavoring of beer, an ingredient to make cheese, as a source of calcium (calcium lactate) in baby food, and an ingredient in bread (17). Lactic acid polymers have also been used to improve the function of many polymers and resins used in the construction industry.

The origins and continued acceptance of the “lactic acidosis” concept. The presence of what has been termed a “lactic acidosis” in humans, which is an extension from the aforementioned interpretation of the production of “lactic acid” in fermentation, can be traced to the pioneering research of skeletal muscle biochemistry during exercise. Two early pioneers of this research were Otto Meyerhoff and Archibald V. Hill (Fig. 2) who in 1922 both received a Nobel prize for their work on the energetics of carbohydrate catabolism in skeletal muscle (14, 15, 35, 47). In particular, Meyerhoff elucidated most of the glycolytic pathway and demonstrated that lactic acid was produced as a side reaction to glycolysis in the absence of oxygen. Hill quantified the energy release from glucose conversion to lactic acid and proposed that glucose oxidation in times of limited oxygen availability, as well as when the energetic demands of muscle contraction exceeded that from oxidation involving oxygen, can supply a rapid and high amount of energy to fuel muscle contraction.

Hill was notably impressive in his abilities to use common sense in his scientific theories. For example, at that time, a common belief was that, “in muscle this oxygen was used during the contraction itself in some kind of explosive chemical change which induced the motion” (15). To Hill, such an explanation was inconsistent with the observation that muscles in a hypoxic environment can still contract and do so for...
several minutes. Clearly, an additional metabolic source of energy that did not rely on oxygen was available to fuel muscle contraction. Hill’s own experiments on the maximal rate of oxygen consumption during exercise (at that time thought to be limited to 4 l/min), as well as estimations of the heat release from glucose conversion to lactate and the energetics of muscle contraction, revealed that intense muscle contraction required energy exchange equivalent to approximately eight times the contraction. Hill documented and explained the lactate production and acidosis into the mind-set of biochemists and physiologists. Hill’s leading scientists and academics have and continue to make this error. As such, there is a need to define what is a fact and what is a construct. A fact is defined as “something that has actual existence; that has objective reality” (58). Conversely, when applied to the topic of research methods and design, a construct is defined as an unproven, nonfactual interpretation that has mistakenly been accepted as fact. The belief that lactate production releases a proton and causes acidosis (lactic acidosis) is a construct and, as such, needs to be corrected.

PAST CRITICISM OF THE LACTIC ACIDOSIS CONSTRUCT

Despite the common acceptance of the lactic acidosis construct, its continued promotion and broad acceptance have not gone without criticism. Examination of the literature from the several minutes. Clearly, an additional metabolic source of energy that did not rely on oxygen was available to fuel muscle contraction. Hill’s own experiments on the maximal rate of oxygen consumption during exercise (at that time thought to be limited to 4 l/min), as well as estimations of the heat release from glucose conversion to lactate and the energetics of muscle contraction, revealed that intense muscle contraction required energy exchange equivalent to approximately eight times the contraction. Hill documented and explained the lactate production and acidosis into the mind-set of biochemists and physiologists. Hill’s leading scientists and academics have and continue to make this error. As such, there is a need to define what is a fact and what is a construct. A fact is defined as “something that has actual existence; that has objective reality” (58). Conversely, when applied to the topic of research methods and design, a construct is defined as an unproven, nonfactual interpretation that has mistakenly been accepted as fact. The belief that lactate production releases a proton and causes acidosis (lactic acidosis) is a construct and, as such, needs to be corrected.

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late 1960s to the 1990s revealed that several physiologists who were attempting to explain ischemic injury of myocardial tissue by metabolic acidosis questioned the common belief that lactic acid production was the source of $H^+$ production (4, 7, 10, 11, 16, 60, 63). In fact, a number of researchers during this era agreed that ATP hydrolysis coupled with glycolysis is the main source of $H^+$ production, resulting in decreased muscle and blood pH. Taffaletti (55) also clearly stated that lactate production consumes protons and, more importantly, separated increased lactate production from proton release and acidosis during lactic acidosis. These scientists believed that “only by understanding these important biochemical facts can the clinician found his/her diagnosis and treatment on a firm, and rational basis” (63). As previously identified, it appears that this criticism has not been accepted or reexamined in detail during the last 25 years, with the cause-and-effect relationship between acidosis and the production of “lactate acid” still being accepted and published in basic science, applied physiology, and medical research. A presentation of the biochemistry of acidosis is clearly needed to thwart the continued acceptance and propagation of the lactic acidosis construct.

THE BIOCHEMISTRY OF EXERCISE-INDUCED METABOLIC ACIDOSIS

Overview. An assessment of the biochemical reactions that support muscle energy catabolism reveals that proton balance in a muscle cell can be influenced by each of the phosphagen, glycolytic, and mitochondrial respiration energy systems that function to produce cellular ATP. A review of each of these energy systems follows for the purpose of identifying the reactions involving proton release and consumption.

Phosphagen system. The cellular store of creatine phosphate provides a near immediate metabolic system to produce ATP during the onset and initial seconds of muscle contraction. Creatine phosphate is also believed to be important for the general transfer of phosphate groups from the mitochondria throughout the cytosol, and as such could also be important for all metabolic states of skeletal muscle cells. The chemical structures of the substrates and products of the creatine kinase reaction are provided in Fig. 4.

The creatine kinase reaction is alkalinizing to the cell, as a proton is consumed in this reaction. The proton is required to replace the phosphate group of creatine phosphate, completing the second amine ($NH_2$) functional group of creatine.

Figure 2 also reveals that the increasing concentration of $P_i$ during intense exercise is not the result of the creatine kinase reaction, as is often mistakenly interpreted. The accumulation of intramuscular $P_i$ results from cellular conditions characterized by a rate of ATP demand that exceeds ATP supply from mitochondrial respiration. During these conditions there is an increased reliance on cytosolic ATP turnover (nonmitochondrial). Such added ATP hydrolysis produces $P_i$ at a rate that now exceeds the rate of $P_i$ entry into the mitochondria, causing $P_i$ accumulation. More detailed content will be given to the cellular conditions associated with increasing nonmitochondrial ATP turnover, as this cellular condition causes acidosis.

Glycolysis. Glycolysis is fueled by the production of glucose-6-phosphate (G6P), which is derived from either blood glucose or muscle glycogen. Despite glycogen providing the majority of carbohydrate that fuels muscle glycolysis during intense exercise, traditional biochemical explanations of glycolysis depict the pathway commencing with glucose and consisting of 10 reactions that result in pyruvate formation. The use of glycogen as the primary substrate (glycogenolysis) differs from glycolysis in bypassing the first reaction and thus shares the remaining nine reactions. This simple distinction between the glucose and glycogen origin of glycolysis is important, for as will be shown, the proton release from glycogenolysis differs depending on whether glucose or muscle glycogen is used to form G6P and fuel glycolysis.

The reactions of glycolysis are summarized in Table 2. Close scrutiny of the contents of the table reveals the following. 1) Despite academic convention, the multiple sources of G6P production in skeletal muscle (blood glucose and endogenous glycogen) indicate that the first reaction of glycolysis is the G6P isomerase reaction, not the hexokinase reaction. As such, glycolysis consists of nine reactions when including the triose phosphate isomerase reaction. 2) For the production of 2 pyruvate, there is a net release of 2 protons when glucose is the source of G6P, and 1 proton when glycogen is the source. Using glycogen as the source of G6P, as opposed to blood glucose, is less acidifying to muscle during intense exercise. 3) Net proton release occurs in glycolysis for the reactions ending in phosphoenolpyruvate. Thus the accumulation of glycolytic intermediates before pyruvate formation during intense exercise causes greater proton release compared with the oxidation of G6P to pyruvate. 4) The first carboxylic acid intermediate of glycolysis is 3-phosphoglycerate from the phosphoglycerate kinase reaction. Subsequent glycolytic intermediates are all carboxylic acid molecules, yet these molecules are all produced as acid salts and not acids.

![Fig. 4. Chemical structures of the substrates and products of the creatine kinase reaction. A proton is required to complete the structure of creatine after the phosphate is removed from creatine phosphate to ADP, forming ATP.](https://www.ajpregu.org)
Glucose 6-phosphate 2 group remains the same for 2-phosphoglycerate, phospho-ionized carboxylic acid functional group. This functional moval of this phosphate group leaves a negatively charged transfer from carbon 1 of 1,3-bisphosphoglycerate. The release of these protons (Fig. 5).

Glycolysis

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose 6-phosphate $\rightarrow$ fructose 6-phosphate $\rightarrow$ 2</td>
<td>Glucose-6-phosphate isomerase</td>
</tr>
<tr>
<td>Fructose 6-phosphate $\rightarrow$ 1,6-bisphosphate $\rightarrow$ 2</td>
<td>6-Phosphofructokinase</td>
</tr>
<tr>
<td>Fructose 1,6-bisphosphate $\rightarrow$ Dihydroxyacetone phosphate + Glyceraldehyde 3-phosphate $\rightarrow$ 2</td>
<td>Aldolase</td>
</tr>
<tr>
<td>Dihydroxyacetone phosphate $\rightarrow$ Glyceraldehyde 3-phosphate $\rightarrow$ 2</td>
<td>Triose Phosphate Isomerase</td>
</tr>
<tr>
<td>2 Glyceraldehyde 3-phosphate $\rightarrow$ 2 NADH $+ 2 H^+$</td>
<td>Glyceraldehyde-3-Phosphate dehydrogenase</td>
</tr>
<tr>
<td>1,3-bisphosphoglycerate $\rightarrow$ 2 3-phosphoglycerate $\rightarrow$ 2 MgADP $+ 2 H^+$</td>
<td>Phosphoglycerate kinase</td>
</tr>
<tr>
<td>2-phosphoglycerate $\rightarrow$ 2 phosphoenolpyruvate $+ 2 H^+$</td>
<td>Phosphoglycerate mutase</td>
</tr>
<tr>
<td>Phosphoenolpyruvate $\rightarrow$ Pyruvate $+ 2 H^+$</td>
<td>Phosphopyruvate hydratase</td>
</tr>
</tbody>
</table>

Glycogen $\rightarrow$ glucose

Table 3 presents the pKa values for the acid intermediates of glycolysis. The term “acid intermediate” is misleading. Although these molecules are carboxylic acid structures, subsequent biochemical content will show that these molecules are formed as acid salts and, as such, neither molecule is ever in an acid form and does not function as a source of protons.

Protein source refers to the number of protons released (positive numbers) or consumed (negative numbers). Either glucose (Glu) or glycogen (Gly) fuel glycolysis. Adapted from Stryer (54).

Table 3. The $pK_a$ values of the “acid intermediates” of glycolysis and lactate

<table>
<thead>
<tr>
<th>Carboxylic Acid Intermediates of Glycolysis</th>
<th>$pK_a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-Phosphoglycerate</td>
<td>3.42</td>
</tr>
<tr>
<td>2-Phosphoglycerate</td>
<td>3.42</td>
</tr>
<tr>
<td>Phosphoenolpyruvate</td>
<td>3.50</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>2.50</td>
</tr>
<tr>
<td>Lactate</td>
<td>3.87</td>
</tr>
</tbody>
</table>

Table 1 and 2 and Figs. 6–8 reveal that the proton release from glycolysis occurs without any production of metabolic acids. The metabolic summaries of glycolysis, starting from glucose or glycogen are as follows:

\[
glucose + 2 ADP + 2 P_i + 2 NAD^+ \rightarrow 2 pyruvate + 2 ATP + 2 NADH + 2 H_2O + 2 H^+ \]  

(1)

\[
glycogen_n + 3 ADP + 3 P_i + 2 NAD^+ \rightarrow glycogen_{n-1} + 2 pyruvate + 3 ATP + 2 NADH + 2 H_2O + 1 H^+ \]  

(2)

Lactate dehydrogenase reaction. From a biochemical perspective, the cellular production of lactate is beneficial for several reasons. First, the lactate dehydrogenase (LDH) reaction also produces cytosolic NAD$^+$, thus supporting the NAD$^+$ substrate demand of the glyceraldehyde 3-phosphate dehydrogenase reaction. This in turn better maintains the cytosolic redox potential (NAD$^+$/NADH), supports continued substrate flux through phase two of glycolysis, and thereby allows continued ATP regeneration from glycolysis. Another important function of the LDH reaction is that for every pyruvate molecule catalyzed to lactate and NAD$^+$, there is a proton consumed, which makes this reaction function as a buffer against cellular proton accumulation (acidosis). The chemical structures for the LDH reaction are presented in Fig. 9.

In the LDH reaction, two electrons and a proton are removed from NADH, and an additional proton is gained from solution to support the two electron and two proton reduction of pyruvate to lactate. Consequently, the LDH reaction is alkalinizing to the cell, not acidifying, as is the basis of the lactic acidosis construct.

There are additional benefits of the LDH reaction. The lactate produced is removed from the cell by the monocarboxylate transporter (12, 20, 21, 30, 37, 62). The lactate is circulated away from the origin cell where it can be taken up and used as a substrate for metabolism in other tissues, such as other muscle cells (skeletal and cardiac), the liver, and kidney. As
the monocarboxylate transporter is also a symport for proton removal from the cell, lactate production also provides the means to assist in proton efflux from the cell. Thus lactate and a proton leave the cell stoichiometrically via this transporter mechanism. However, this does not mean that lactate production is the source of the proton. As has been presented thus far, there is no biochemical evidence for lactate production releasing a proton, and research evidence is clear in quantifying far greater proton removal than lactate removal from contracting skeletal muscle (19). Conversely, the organic chemistry of the LDH reaction clearly reveals that lactate production consumes protons. The correct physiological interpretation of these biochemical facts is that lactate production retards a developing metabolic acidosis, as well as assists in proton removal from the cell.

The coupling of glycolysis to lactate production. The end products of glycolysis and the lactate dehydrogenase reaction are provided in Eq. 3. When the pyruvate from glycolysis is converted to lactate, there is no net production of protons when starting with glucose, and a decrease in one proton and a gain of an additional ATP when starting with glycogen (Eq. 4).

\[
\text{glucose} + 2 \text{ADP} + 2 \text{P}_i \rightarrow 2 \text{lactate} + 2 \text{ATP} + 2 \text{H}_2\text{O} \quad (3)
\]

\[
\text{glycogen}_n + 3 \text{ADP} + 3 \text{P}_i + 1 \text{H}^+ \rightarrow \text{glycogen}_{n-1} + 2 \text{lactate} + 3 \text{ATP} + 2 \text{H}_2\text{O} \quad (4)
\]

This coupling is important for many cells of the body, with the red blood cell being a good example. Red blood cells are devoid of mitochondria and rely on glycolysis for ATP regeneration using glucose as the original glycolytic substrate. The two-proton yield from glycolysis is balanced by the two-proton consumption in converting two pyruvate to two lactate, and red blood cell cytosolic redox is also maintained by the NAD\(^+\) produced from the LDH reaction. For the red blood cell, lactate production is essential to prevent an acidosis and maintain cellular NAD\(^+\).

In skeletal muscle, the presence of mitochondria and the involvement of glycogen as a source of glucose 6-phosphate to fuel glycolysis alters the stoichiometry between glycolytic flux, proton release, and lactate and proton consumption. In addition, the high metabolic rate incurred during muscle contraction, and hence the high rate of ATP hydrolysis and regeneration pose unique metabolic stresses not seen in nonmuscular tissues.

ATP hydrolysis as a major source of H\(^+\). The removal of the terminal phosphate of ATP to form ADP and the concomitant release of free energy and P\(_i\) requires the involvement of water as an additional substrate. The chemical structures for this reaction are presented in Fig. 10.

The P\(_i\) produced in the ATPase reaction has the potential to buffer the free proton that is released. The three single bond oxygen atoms of P\(_i\) have the following pK values: 2.15, 6.82, and 12.38 (28, 54). Thus one oxygen atom is able to become protonated within the intracellular physiological pH range (cellular pH range ~6.1 to 7.1) converting the P\(_i\) from HPO\(_4\)\(^{2-}\) to H\(_2\)PO\(_4\)\(^{-}\). Consequently, as P\(_i\) increases during intense exercise, the proton buffering capacity of P\(_i\) is quantified by
the extent of \( P_i \) accumulation when cellular pH falls well below 6.8.

At first glance, the buffering potential of \( P_i \) decreases the importance of ATP hydrolysis as a meaningful source of proton release contributing to acidosis. However, this is not true. The increase in intracellular \( P_i \) is not proportional to, and in fact considerably less than, the accumulated total of ATP hydrolysis. During ATP hydrolysis, the ADP and \( P_i \) produced both function as substrates for glycolysis to produce ATP (Table 2, Fig. 11), leaving the free proton to accumulate when buffering and transport systems for proton efflux from the cell have been surpassed. Free \( P_i \) is also a substrate for glycogenolysis and is transported into the mitochondria as a substrate in oxidative phosphorylation. As such, \( P_i \) accumulation is not stoichiometric to ATP turnover and occurs when there is a greater rate of cytosolic ATP turnover than cellular ATP supply.

A diagrammatic model for the coupled connection between glycolysis and cytosolic ATP, ADP, and \( P_i \) turnover is presented in Fig. 11. When lactate production is added to glycolysis, and assuming that the ATP turnover from metabolism is not supported from mitochondrial respiration, the rate of proton release is equal to the rate of ATP turnover. Under these circumstances, there would be a rapid release of protons, a rapid exceeding of the cellular proton removal and buffer capacities, and the rapid onset of cellular metabolic acidosis. When combining ATP hydrolysis and glucose conversion to lactate (Eq. 3 and Fig. 10), the content of Fig. 11 and Table 2 can be summarized in Eq. 5. Equation 6 presents the summary equation of the conversion of glycogen to lactate.

\[
glucose \rightarrow 2 \text{ lactate} + 2 \text{ H}^+ \quad (5)
glycogen \rightarrow 2 \text{ lactate} + 1 \text{ H}^+ \quad (6)
\]

Obviously, Eqs. 5 and 6 appear as though lactic acid has been produced. However, as is detailed in this manuscript, to assume that such a summary equation is evidence for lactic acidosis is an interpretation based on an oversimplistic account of the biochemistry of metabolic acidosis. The content of Fig. 10 clearly shows the source of the two protons of Eq. 5 is ATP hydrolysis, not lactate production.

\[\text{NADH} + \text{H}^+ \text{ as a source of H}^+\]. Additional \( \text{H}^+ \) accumulation could arise from an accumulation of \( \text{NADH} + \text{H}^+ \) produced by the glyceraldehyde 3-phosphate dehydrogenase reaction. These products would increase during any cellular condition that caused a greater rate of substrate flux through glycolysis than the rate of electron and proton uptake by the mitochondria, or lactate production (Fig. 9).

The importance of mitochondrial respiration. Although metabolic acidosis is caused by cytosolic (nonmitochondrial) catabolism, an understanding of why and when metabolic acidosis occurs in contracting skeletal muscle is partly explained by knowing how and why mitochondrial function can be rate limiting to ATP regeneration. To view metabolic acidosis as a nonmitochondrial event is a mistake, for as will be explained, the rate-limiting function of mitochondria is an important

Fig. 8. Substrates and products of the glyceraldehyde 3-phosphate dehydrogenase reaction. Two electrons and a proton are used to reduce \( \text{NAD}^+ \) to \( \text{NADH} \). The remaining proton, which in this depiction is accounted for by the proton release from free inorganic phosphate, is released into solution. Arrows pointing away from a bond represent bond/group removal. Arrows pointing to a bond represent addition of an atom/group.

Fig. 7. Substrates and products of the phosphofructokinase (PFK) reaction. Proton release from this reaction comes from the hydroxyl group of the 6th carbon of fructose 6-phosphate. Arrows pointing away from a bond represent bond/group removal.
reason for the need to rely more on nonmitochondrial ATP turnover, which in turn causes metabolic acidosis.

A concise summary of the key metabolic events in mitochondria is presented in Fig. 12. Mitochondrial metabolism functions to release electrons and protons from substrates, produce carbon dioxide, and use electrons and protons to eventually produce ATP. The main molecules involved in these functions are acetyl CoA, NAD\(^+\), FAD\(^+\), molecular oxygen, ADP, P\(_i\), electrons, and protons. It is important to note that each of ADP, P\(_i\), and protons is transported into the mitochondria (6, 23) (Figs. 12 and 13). The protons are required for the reduction of molecular oxygen, and ADP and P\(_i\) are required for ATP regeneration. Such transport mechanisms connect cytosolic and mitochondrial metabolism. This is especially true for the transfer of P\(_i\) molecules and protons between the cytosol and mitochondria. The proton transport systems between the cytosol and mitochondria are revealing of the power of mitochondrial respiration in contributing to the control over the balance of protons within the cell during conditions of muscle contraction that rely on mitochondrial respiration for ATP turnover.

Figure 14, A and B, present two scenarios of metabolism pertinent to the study of acidosis. Figure 14A depicts the movement of carbon substrate, electrons, protons, and phosphate molecules within and between the cytosol and mitochondria during moderate intensity steady-state exercise where the rate of glycolysis and subsequent pyruvate entry into the mitochondria for complete oxidation and mitochondrial ATP regeneration meet the rate of cytosolic ATP demand. Conversely, Fig. 14B pertains to non-steady-state exercise as is typified by intense exercise to volitional fatigue within a time frame of 2–3 min. In each figure example, the magnitude of the arrows is proportionate to substrate flux through that reaction or pathway.

On the basis of the biochemistry presented thus far, Fig. 14 indicates the shift in proton flux as exercise progresses from steady state to nonsteady state. Central to the cellular proton balance is the hydrolysis of ATP required to fuel cell work, such as muscle contraction. This is clearly the main source of proton release in contracting skeletal muscle, and when the NADH and protons from cytosolic reactions are produced at rates in excess of mitochondrial capacity, cytosolic redox is aided by lactate production, which essentially accounts for the proton release from glycolysis. However, as the rate of ATP hydrolysis exceeds all other reactions, the rate of proton release eventually exceeds metabolic proton buffering by lactate production and creatine phosphate breakdown, as well as proton buffering by P\(_i\), amino acids, and proteins. In addition, once the maximal capacity of lactate/proton removal from the cell is exceeded, proton accumulation (decreasing cellular pH) results. Also, note that Fig. 14B clearly shows that the origin of the accumulating intramuscular P\(_i\) is ATP hydrolysis, not creatine phosphate breakdown, which is still mistakenly interpreted by many physiologists (59).

The additional underlying message of Fig. 14 is that the cellular mitochondrial capacity is pivotal in understanding metabolic acidosis. The mitochondrial capacity for acquiring cytosolic protons and electrons retards a dependence on glycolysis and the phosphagen system for ATP regeneration, essentially functioning as a depository for protons for use in oxidative phosphorylation. Metabolic acidosis occurs when the rate of ATP hydrolysis, and therefore the rate of ATP demand, exceeds the rate at which ATP is produced in the mitochondria. The maximal rates for ATP regeneration from the main energy systems of skeletal muscle are provided in Table 4.

A classic study that showed the importance of mitochondrial proton uptake was conducted by Vaghy (57). Vaghy examined oxidative phosphorylation in isolated rabbit heart mitochondria.
and the accompanying pH changes in the same sample. It was suggested that when oxidative phosphorylation was blocked either by inhibitors or by the absence of oxygen the proton concentration would increase considerably. The results of Vaghy’s investigation revealed that during ischemic conditions there was a decreased net proton consumption by oxidative phosphorylation and that this alteration to metabolism plays an important role in the development of the acidosis of the myocardium. In addition, Vaghy experimentally confirmed the idea that when glycolysis and ATP hydrolysis are not coupled to mitochondrial respiration, acidosis develops. However, the question of where the protons came from was not clarified by this research.

ADDITIONAL RESEARCH EVIDENCE FOR THE PROTON RELEASING AND CONSUMING REACTIONS OF CATABOLISM

Although the biochemistry of exercise-induced metabolic acidosis is unquestionable, there is considerable research support and therefore validation of nonmitochondrial ATP turnover as the cause of acidosis. For example, several researchers have denoted that the assumption that “lactic acid” is the source of H⁺ is inaccurate. Gevers (10, 11) first drew attention to the very important possibility that protons might be generated in significant quantity in muscle by metabolic processes other than the lactate dehydrogenase reaction. He suggested that the major source of protons was the turnover of ATP produced via glycolysis. This important concept, quite contrary to the general concept of that time that “lactic acid” is the end product of glycolysis, aroused little interest for six years.

It was not until 1983 that Hochachka and Mommsen (16) rediscovered and wrote an extensive review on this topic. Hochachka et al. supported Gevers’ idea that metabolic acido-
sibly reaffirmed the writings of Gevers (10, 11) and Hochachka and Mommsen (16). As stated by Busa and Nucitelli (4), “ATP hydrolysis, not lactate accumulation, is the dominant source of the intracellular acid load accompanying anaerobiosis.”

To experimentally show that lactate production does not contribute to acidosis or that decreased lactate production exacerbates acidosis, glycolysis, lactate production, and mitochondrial respiration need to be uncoupled in a controlled manner. In a 31P-nuclear magnetic resonance (NMR) study, Smith et al. (48) investigated the role of “lactic acid” production in the isolated ferret heart by using three different applications of cyanide (cyanide blocks mitochondrial respiration), cyanide plus iodoacetate (inhibits glycolysis), and cyanide plus a glucose-free solution (restricts substrate to glycolysis). The experimental results indicated that when only cyanide was applied to the heart muscle, there was a net lactate and H+ accumulation. When glycolysis was blunted (cyanide plus glucose-free solution), there was less lactate production, a greater rate of nonmitochondrial ATP hydrolysis, and increased acidosis. As expected, an increased acidosis with less lactate production was also observed when cyanide plus iodoacetate was applied to the myocardium. Therefore, the authors concluded that the increased acidosis produced by cyanide when glycolysis is completely inhibited (in the presence of iodoacetate) was due to a more rapid hydrolysis of intracellular ATP. In addition, these results showed the role of lactate production in thwarting a developing acidosis.

Later commentary by MacRae and Dennis (31) indicated that the metabolic generation of protons during heavy exercise is a consequence of the rise in glycolytic ATP turnover with increasing work rate. When ATP is resynthesized by glycolysis, rather than by oxidative phosphorylation or creatine phosphate, the protons produced by ATP hydrolysis are not reused in mitochondrial respiration. Conversely, during steady-state exercise the protons generated from glycolysis are transported into the mitochondria and used directly in water formation or in the electron transport chain (ETC) to produce an H+ gradient across the inner mitochondrial membrane that facilitates ATP synthesis via the F0F1 ATPase. Therefore, these protons are generated irrespective of lactate formation or pyruvate delivery to the mitochondria for oxidation. Consequently, an increase in cytosolic H+ concentration must also coincide with a decrease in cytosolic redox, which collectively shifts the LDH equilibrium toward lactate production (pyruvate− + NADH + H+ ⇌ lactate− + NAD+). Thus lactate formation

Table 4. The estimated maximal rates of ATP regeneration from the main energy systems in skeletal muscle

<table>
<thead>
<tr>
<th>Energy System</th>
<th>Maximal Rate of ATP regeneration, mmol ATP·s−1·kg wet wt−1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytosolic</td>
<td></td>
</tr>
<tr>
<td>Phosphagen</td>
<td>2.4</td>
</tr>
<tr>
<td>Glycolytic</td>
<td>1.3</td>
</tr>
<tr>
<td>Mitochondrial respiration</td>
<td></td>
</tr>
<tr>
<td>CHO oxidation</td>
<td>0.7</td>
</tr>
<tr>
<td>Fat oxidation</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Adapted from Sahlin (46).
and efflux from working muscles is more a consequence than a cause of acidosis.

Noakes (34) recognized and supported the opinions of Gevers (10, 11) by stating that the protons released from the hydrolysis of ATP are not needed for the resynthesis of ATP by the glycolytic pathway. He also suggested that at first some of the protons generated by high rates of glycolytic ATP breakdown are taken into the mitochondria with pyruvate. Some are used in the reduction of pyruvate to lactate and some are buffered by intracellular histidine residues and P_i. Consequently, unbuffered intracellular protons leave the cell via the sarcolemmal Na^+/H^+ exchangers and H^+ + lactate symporters and can alter blood pH, which coincides with an increase in the blood lactate concentration.

COMPONENTS OF CELLULAR PROTON PRODUCTION, BUFFERING, AND REMOVAL

The cause of metabolic acidosis is not merely proton release, but an imbalance between the rate of proton release and the rate of proton buffering and removal. As previously shown from fundamental biochemistry, proton release occurs from glycolysis and ATP hydrolysis. However, there is not an immediate decrease in cellular pH due to the capacity and multiple components of cell proton buffering and removal (Table 5). The intracellular buffering system, which includes amino acids, proteins, P_i, HCO_3^−, creatine phosphate (CrP) hydrolysis, and lactate production, binds or consumes H^+ to protect the cell against intracellular proton accumulation. Protons are also removed from the cytosol via mitochondrial transport, sarcolemmal transport (lactate−/H^+ symporters, Na^+/H^+ exchangers, and a bicarbonate-dependent exchanger (HCO_3^−/Cl^−)) (Fig. 13). Such membrane exchange systems are crucial for the influence of the strong ion difference approach at understanding acid-base regulation during metabolic acidosis (5, 26). However, when the rate of H^+ production exceeds the rate or the capacity to buffer or remove protons from skeletal muscle, metabolic acidosis ensues. It is important to note that lactate production acts as both a buffering system, by consuming H^+, and a proton remover, by transporting H^+ across the sarcolemma, to protect the cell against metabolic acidosis.
of lactate production. There is no stoichiometry to the assertion that lactate production directly releases protons and causes a lactic acidosis.

When evaluating past research for evidence in support of the nonmitochondrial ATP turnover cause of metabolic acidosis, the stoichiometry is far more impressive. These data are presented in Fig. 17. When the main consumers of protons are combined, there is a near equality between proton release (ATP-NM and glycolysis) and proton consumption. A slight discrepancy is presented here, but in reality additional proton consumption occurs via the muscle bicarbonate stores, a small proton efflux that occurs regardless of exercise models having zero blood flow, and the influence of the strong ion difference across the sarcolemma of the contracting muscle fibers (5, 12, 26). Furthermore, we presented data assuming complete ionization of key metabolites such as ATP, ADP, and P_l. This is not entirely accurate, yet adjustments based on proton balances from fractional ionization provide unnecessary complications to the proton balance, do not change the relative depiction of data, and therefore do not alter the cause of metabolic acidosis.

The data from Figs. 16 and 17 are very important as they show that nonmitochondrial ATP turnover cause of metabolic acidosis, the stoichiometry is far more impressive. These data are presented in Fig. 17. When the main consumers of protons are combined, there is a near equality between proton release (ATP-NM and glycolysis) and proton consumption. A slight discrepancy is presented here, but in reality additional proton consumption occurs via the muscle bicarbonate stores, a small proton efflux that occurs regardless of exercise models having zero blood flow, and the influence of the strong ion difference across the sarcolemma of the contracting muscle fibers (5, 12, 26). Furthermore, we presented data assuming complete ionization of key metabolites such as ATP, ADP, and P_l. This is not entirely accurate, yet adjustments based on proton balances from fractional ionization provide unnecessary complications to the proton balance, do not change the relative depiction of data, and therefore do not alter the cause of metabolic acidosis.

The data from Figs. 16 and 17 are very important as they show that nonmitochondrial ATP turnover cause of metabolic acidosis, as is argued by many due to Eq. 5. The fact is that research clearly supports the stoichiometry of the nonmitochondrial ATP turnover cause of metabolic acidosis. In so doing, research also clearly discredits the interpretation of acidosis as being caused by lactate production. Consequently, we showed that the biochemistry of metabolism supports nonmitochondrial ATP turnover as the cause of acidosis. Furthermore, we present data from experimental research that through direct measurement and indirect

computations of the stoichiometry of proton and lactate balance in contracting skeletal muscle, prove that metabolic acidosis is caused by an increased reliance on nonmitochondrial ATP turnover and not lactate production.

IS THE DIFFERENTIATION BETWEEN LACTATE PRODUCTION AND THE TRUE BIOCHEMICAL CAUSE OF ACIDOSIS REALLY THAT IMPORTANT?

This is the crucial question that all physiologists must be able to answer. There are several examples of why the correct cause of metabolic acidosis needs to be accepted, communicated in education, and used in research interpretation and publication.

Scientific validity. The most important reason to discard the lactic acidosis concept is that it is invalid. It has no biochemical justification and, to no surprise, no research support. We have been criticized for our stance on the need to change how to teach and interpret metabolic acidosis based on Eq. 5 (glucose $\rightarrow$ 2 lactate $+ 2$ $H^+$). However, this is a summary equation that does not represent cause and effect, as previously described and illustrated in Fig. 10. As such, the concept of a lactic acidosis remains evidence of 1920s academic and scientific inertia that, out of simple convenience and apathy, still remains today. We would hope that the academics and professionals from the basic and applied fields that continue to accept the lactic acidosis construct immediately change the way they teach and interpret this topic.

Education. Education is a powerful force that can induce change or reinforce error. Rather than continuing to reinforce error, educators need to recognize their power in reshaping
how students and academics alike explain and discuss all matters pertaining to metabolic acidosis and skeletal muscle proton buffering. The correct teaching of metabolic acidosis is crucial for the promotion and acceptance of the correct understanding of exercise-induced metabolic acidosis.

Sports physiology, coaching, and training. An acceptance of the true biochemistry of metabolic acidosis means that terms and descriptions used throughout sports physiology and coaching need to be changed. The terms “lactate” or “lactic acid” need to be removed from any association with the cause of acidosis or the training that is used to delay the onset of acidosis.

Research of strategies to retard exercise-induced metabolic acidosis. If it is assumed that lactate production causes acidosis, then it is a logical extension to hypothesize that reducing lactate production for a given cellular ATP demand should retard acidosis. If decreasing the rate of lactate production is accomplished by stimulating increased mitochondrial respiration [such as through dichloracetate infusion/ingestion (18)], such a strategy might also increase mitochondrial proton uptake and decrease/delay acidosis. However, as is clear from the biochemistry, for a given rate of mitochondrial respiration, decreasing lactate production will decrease proton buffering and removal from skeletal muscle and increase the rate of onset and worsen the severity of acidosis. On the basis of the biochemistry of muscle metabolism, the best way to decrease metabolic acidosis is to decrease nonmitochondrial ATP turnover during intense cycle ergometry exercise and assuming this is true, then it is logical to hypothesize that reducing lactate production will also decrease proton buffering. However, as is clear from the biochemistry, for a given rate of mitochondrial respiration, decreasing lactate production will decrease proton buffering and removal from skeletal muscle and increase the rate of onset and worsen the severity of acidosis. On the basis of the biochemistry of muscle metabolism, the best way to decrease metabolic acidosis is to decrease nonmitochondrial ATP turnover during intense cycle ergometry exercise and assuming this is true, then it is logical to hypothesize that reducing lactate production will also decrease proton buffering.

Conclusions and recommendations. There is no biochemical support for the construct of a lactic acidosis. Metabolic acidosis is caused by an increased reliance on nonmitochondrial ATP turnover. Lactate production is essential for muscle to produce cytosolic NADH to support continued ATP regeneration from glycolysis. The production of lactate also consumes two protons and, by definition, retards acidosis. Lactate also facilitates proton removal from muscle. Although muscle or blood lactate accumulation are good indicators of increased proton release and the potential for decreased cellular and blood pH, such relationships should not be interpreted as cause and effect.

The aforementioned interpretations of the biochemistry of lactate production and acidosis are also supported by research evidence. As such, research evidence also disproves the concept of a lactic acidosis. Quantifying nonmitochondrial ATP turnover during intense cycle ergometry exercise and assuming this value to be identical to proton release, reveals a near perfect stoichiometry to known components of proton consumption within contracting skeletal muscle. Conversely, research data of muscle lactate production and proton release yield a lactate-to-proton stoichiometry approximating 1:3 (33:103 mmol H+/kg wet wt; Figs. 16 and 17).

Educating students on the correct biochemical cause of acidosis is extremely important for reasons of academic credibility and scientific validity. In addition, the past incorrect interpretations of lactic acidosis have yielded questionable research applications and data interpretations, with the indirect calculation of the muscle proton buffering unit of slyke [([Δlactate + Δpyruvate]/ΔpH) ~ (ΔH+/ΔpH)] being the best example of this error.

It is strongly recommended that all educators and researchers incorporate the application of the correct cause of acidosis into their professional practice.
ACKNOWLEDGMENTS

We want to highlight our recognition of the scientists and academics that preceded our efforts on constructively criticizing the concept of a lactate acidosis. These individuals and their scholarship (4, 7, 10, 11, 16, 34, 55) have contributed significantly to our understanding of the topic. The contributions of Juel C., Gollnick PD, Graham TE, Juel C, Kiens B, Mizuno M, and Saltin B. are acknowledged. The authors would like to thank Hill AV, Long CNH, and Lupton H. for their assistance and support.

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