

Biochemistry of exercise-induced metabolic acidosis

Robert A. Robergs,¹ Farzenah Ghiasvand,¹ and Daryl Parker²

¹Exercise Physiology Laboratories, Exercise Science Program, Department of Physical Performance and Development, The University of New Mexico, Albuquerque, New Mexico 87131; and ²Exercise Science Program, California State University-Sacramento, Sacramento, California 95819

Robergs, Robert A., Farzenah Ghiasvand, and Daryl Parker. Biochemistry of exercise-induced metabolic acidosis. *Am J Physiol Regul Integr Comp Physiol* 287: R502–R516, 2004; 10.1152/ajpregu.00114.2004.—The development of acidosis during intense exercise has traditionally been explained by the increased 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

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 pK_a (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 (H⁺) and leaving the final product to be the acid salt lactate. This process has been termed lactic acidosis (27). According to this presentation, if and when there is a rapid increase in the production of lactic acid, the free H⁺ can be buffered by bicarbonate causing the nonmetabolic production

of carbon dioxide (CO₂). In turn, the developing acidosis and the raised blood CO₂ content stimulate an increased rate of ventilation causing the temporal relationship between the lactate and ventilatory thresholds (25, 32, 44, 53).

This review supports the previous work of numerous scientists that have criticized the concept of lactic acidosis and presented alternative explanations of the biochemistry of metabolic acidosis (4, 7, 10, 11, 16, 34, 55–57, 60, 61, 63). The lactic acidosis explanation of metabolic acidosis is not supported by fundamental biochemistry, has no research base of support, and remains a negative trait of all clinical, basic, and applied science fields and professions that still accept this construct. Nevertheless, statements that imply that “lactic acid” or a “lactic acidosis” causes metabolic acidosis can still be found in the current literature (1, 2, 13, 19, 22, 48, 51–53, 59, 62), and remains an explanation for metabolic acidosis in current textbooks of biochemistry, exercise physiology, and acid-base physiology. Clearly, academics, researchers, and students of the basic and applied sciences, including the medical specialties, need to reassess their understanding of the biochemistry of metabolic acidosis.

Address for reprint requests and other correspondence: R. A. Robergs, Exercise Science Program, Dept. of Physical Performance and Development, Johnson Center, Rm. B143, The Univ. of New Mexico, Albuquerque, NM 87131-1258 (E-mail: rrobergs@unm.edu).

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 acidosis 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.

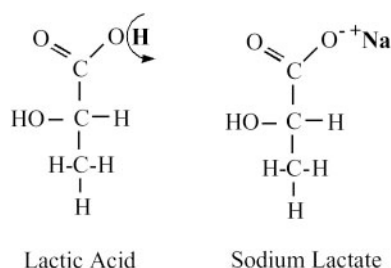


Fig. 1. Chemical structures of lactic acid and the sodium salt of lactate. When the proton of the carboxylic acid functional group ($-\text{COOH}$) of lactic acid dissociates ($\text{COO}^{-} + \text{H}^{+}$), a cation ionically interacts with the negatively charged oxygen atom of the carboxyl group,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

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

Property	Value
Chemical formula	$\text{CH}_3\text{-CHOH-COOH}$
Molecular wt (g/mol)	89.0
Solubility	Water, ethanol, ethyl ether
pKa (37°C)	3.87
Heat of combustion	321 kcal/mol

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 polylactate 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

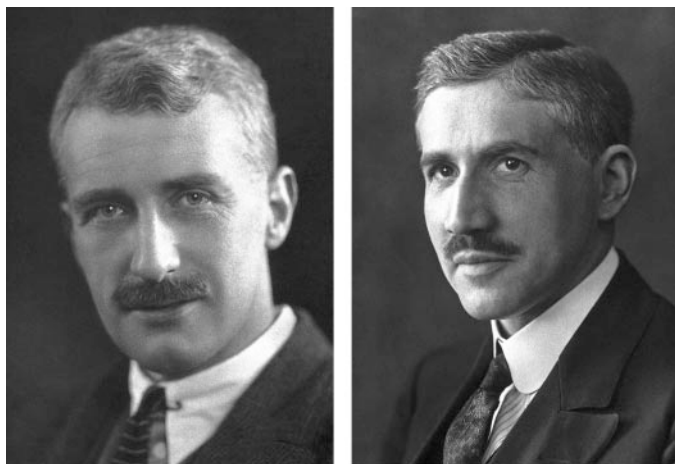


Fig. 2. Archibald V. Hill (*left*) and Otto Meyerhof (*right*). Figures borrowed with permission of the Nobel Foundation.

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 known maximal rate of oxygen consumption (14, 29).

The work of Hill and Meyerhoff cemented the acceptance of lactic acid production and acidosis into the mind-set of biochemists and physiologists. Hill documented and explained the logic for muscle to have an immediate and powerful source for energy production to fuel rapid and intense muscle contractions, and Meyerhoff revealed the biochemistry for how such a source resulted in lactic acid production. There was insufficient knowledge of acid-base chemistry at that time to comprehend the ionization of molecules other than traditional acids and there was also insufficient knowledge of mitochondrial respiration to recognize the roles of mitochondria in altering cellular proton balance. The wealth of research, even for that time, on the production of lactic acid during fermentation and its presence in numerous animal tissues established the connection between anaerobiosis, lactic acid production, and acidosis. Such an accepted connection was assumed as cause-and-effect in the applied work of Hill and basic science work of Meyerhoff. Furthermore, it is easy to comprehend how the Nobel prize quality of the work of Hill and Meyerhoff was proof enough to the scientific world at that time for the interpretation that lactate production and acidosis were cause-and-effect.

The unquestioned acceptance of a lactic acidosis is a hallmark of almost all of the basic and applied science research of muscle metabolism since the 1920s. For example, Margaria et al. (32) demonstrated that the lactic acid concentration in the blood is concomitant with changes in blood pH. A more recent classic example of this research and interpretation is that of Sahlin et al. (42). These researchers measured muscle pH, lactate, and pyruvate during exercise and recovery from different intensities of exhaustive exercise. Plots of the sum of lactate and pyruvate to muscle pH revealed a strikingly linear

relationship between the two variables (Fig. 3). Furthermore, such linearity was maintained despite the different intensities of exercise and exercise vs. recovery conditions. Our illustration and analysis of Sahlin's results, using combined exercise and recovery data, revealed the following statistics: $r = 0.912$; $S_{y \cdot x} = 0.083$ pH units.

Certainly, the linear relationship between muscle pH and the sum of lactate and pyruvate, which at this time were still interpreted as metabolic acids, was strong indirect evidence for a cause-effect relationship between lactate and pyruvate production and acidosis. More recent studies also accepted a cause-effect interpretation between decreases in blood or muscle pH with increases in "lactic acid" production (1, 2, 13, 19, 49–53, 59).

THE CONSTRUCT OF LACTIC ACID AND LACTIC ACIDOSIS

The previous brief historical evaluation of the research of acidosis, lactic acid, and lactate reveals that no experimental evidence has ever been shown to reveal a cause-effect relationship between lactate production and acidosis. Past research on this topic that is used to support the lactic acidosis concept is entirely based on correlations, which at best remains indirect evidence. Despite the efforts of academics to teach students that results from correlation do not imply cause and effect, it seems that on the topic of lactic acidosis, the world'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

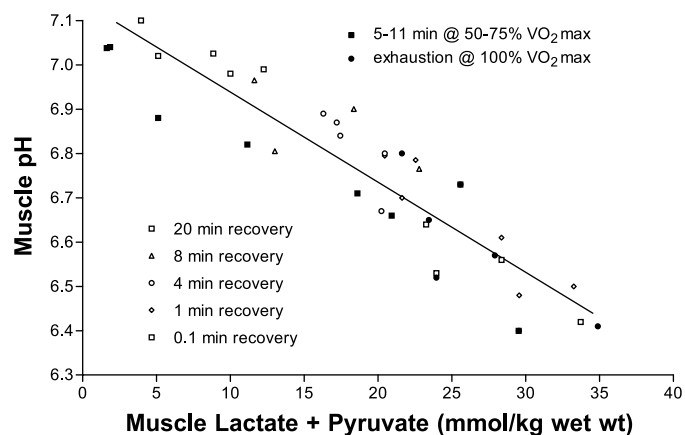


Fig. 3. An original figure redrawn from data from Sahlin et al. (42) (Figs. 1 and 2, p. 46), showing the linear relationship between the sum of muscle lactate and pyruvate vs. muscle pH. Data are combined from different exercise intensities and different durations of recovery after exercise to exhaustion (see original figure legends).

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 glycolysis 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.

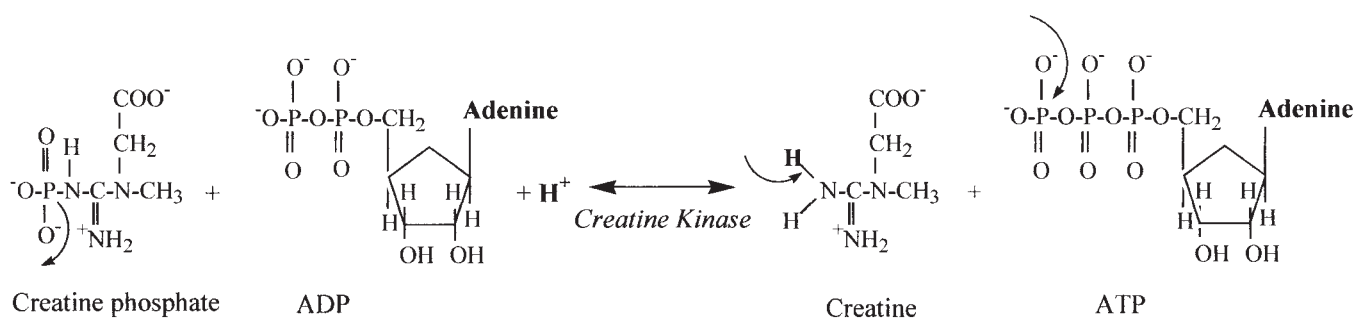


Fig. 4. Chemical structures of the substrates and products of the creatine kinase reaction. A proton is required to complete the structure of creatine after the phosphate is removed from creatine phosphate to ADP, forming ATP.

Table 2. The reactions of glycolysis balanced for charge, protons, and water

#	Reaction	Enzyme	H ⁺ Source	
			Glu	Gly
<i>G6P from glycogen</i>				
	Glycogen _n + Pi ²⁻ → Glycogen _{n-1} + Glucose 1-phosphate	Phosphorylase		
	Glucose 1-phosphate → Glucose 6-phosphate	Phosphoglucomutase		
<i>G6P from glucose</i>				
	Glucose + MgATP ²⁻ → Glucose 6-phosphate ²⁻ + MgADP ⁻ + H ⁺	Hexokinase	1	
<i>Glycolysis</i>				
1	Glucose 6-phosphate ²⁻ → fructose 6-phosphate ²⁻	Glucose-6-phosphate isomerase		
2	Fructose 6-phosphate ²⁻ + MgATP ²⁻ → fructose 1,6-bisphosphate ⁴⁻ + MgADP ⁻ + H ⁺	6-Phosphofructokinase	1	1
3	Fructose 1,6-bisphosphate ⁴⁻ → Dihydroxyacetone phosphate + Glyceraldehyde 3-phosphate ²⁻	Aldolase		
4	Dihydroxyacetone phosphate → Glyceraldehyde 3-phosphate ²⁻	Triose Phosphate Isomerase		
5	2 Glyceraldehyde 3-phosphate ²⁻ + 2NAD ⁺ + 2Pi ²⁻ → 2 1,3-bisphosphoglycerate ⁴⁻ + 2 NADH + 2 H ⁺	Glyceraldehyde-3-Phosphate dehydrogenase	2	2
6	2 1,3-bisphosphoglycerate ⁴⁻ + 2 MgADP ⁻ → 2 3-phosphoglycerate ³⁻ + 2 MgATP ²⁻	Phosphoglycerate kinase		
7	2 3-phosphoglycerate ⁴⁻ → 2 2-phosphoglycerate ⁴⁻	Phosphoglycerate mutase		
8	2 2-phosphoglycerate ³⁻ → 2 phosphoenolpyruvate ³⁻ + 2H ₂ O	Phosphopyruvate hydratase		
9	2 phosphoenolpyruvate ³⁻ + 2 MgADP ⁻ + 2 H ⁺ → 2 pyruvate ⁻ + 2 MgATP ²⁻	Pyruvate kinase	-2	-2
		Net protons per 2 pyruvate	2	1

Proton 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 presents the pK_a 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.

Presenting chemical structures for the substrates and products of the phosphoglycerate kinase reaction is important for demonstrating that glycolysis does not produce metabolic acids that release protons (Fig. 5).

The phosphoglycerate kinase reaction involves a phosphate transfer from carbon 1 of 1,3-bisphosphoglycerate. The removal of this phosphate group leaves a negatively charged (ionized) carboxylic acid functional group. This functional group remains the same for 2-phosphoglycerate, phosphoenolpyruvate, and pyruvate. This fundamental biochemistry is clear evidence for the error in the concept of a lactic acidosis, as well as the production of metabolic acids in glycolysis. In reality, there is never a proton to be dissociated from any glycolytic acid intermediate (Table 3).

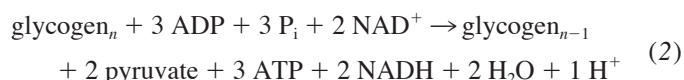
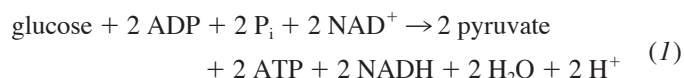
Table 2 reveals that glycolysis releases protons. The proton release from glycolysis is associated with the hydrolysis of ATP in the hexokinase and phosphofructokinase reactions, as well as the oxidation of glyceraldehyde 3-phosphate in the glyceraldehyde 3-phosphate dehydrogenase reaction. The chemical structures for these reactions are presented in Figs. 6, 7, and 8.

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

Carboxylic Acid Intermediates of Glycolysis	pK _a
3-Phosphoglycerate	3.42
2-Phosphoglycerate	3.42
Phosphoenolpyruvate	3.50
Pyruvate	2.50
Lactate	3.87

Data from Ref. 4a.

Tables 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:



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 alkalizing 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

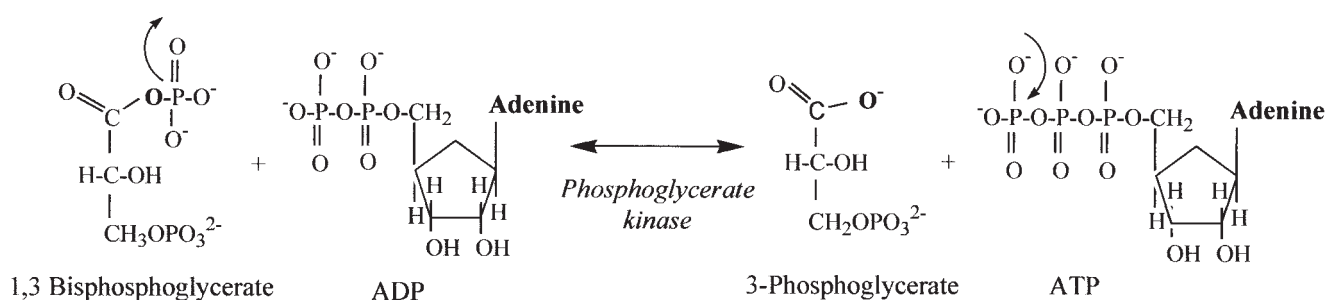
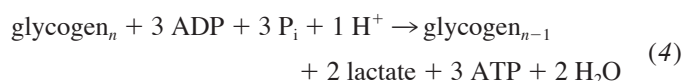
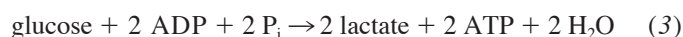


Fig. 5. Substrates and products of the phosphoglycerate kinase reaction. Product 3-phosphoglycerate is the first "carboxylic acid" formed in glycolysis. Phosphate transfer of this reaction reveals that a proton was never present to be released to the cytosol and alter cellular proton exchange and pH. As such, 3-phosphoglycerate and all of the remaining glycolytic "carboxylic acid" intermediates do not function as acids as they never have a proton that can be released into solution. Arrows pointing away from a bond represent bond/group removal. Arrows pointing to a bond represent addition of an atom/group.

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).



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 regen-

eration 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 $\text{H}_2\text{PO}_4^{-1}$. Consequently, as P_i increases during intense exercise, the proton buffering capacity of P_i is quantified by

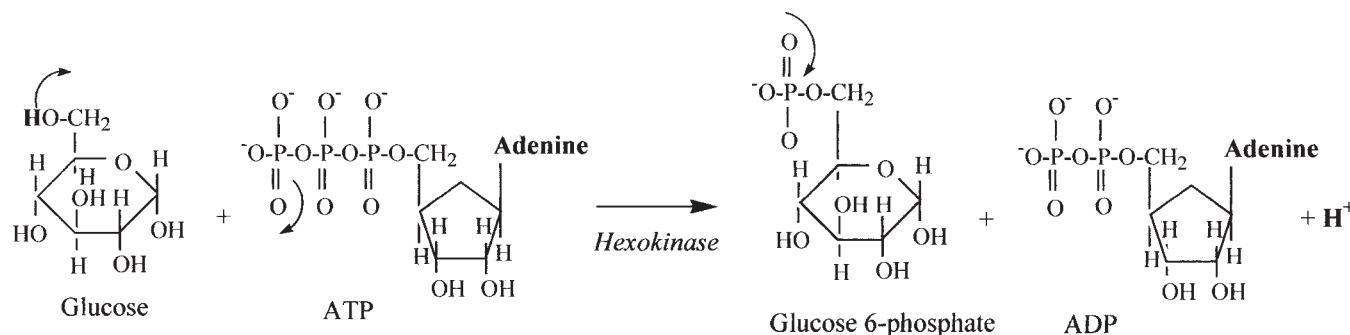


Fig. 6. Substrates and products of the hexokinase reaction. Proton release from this reaction comes from the hydroxyl group of the 6th carbon of glucose. Arrows pointing away from a bond represent bond/group removal. Arrows pointing to a bond represent addition of an atom/group.

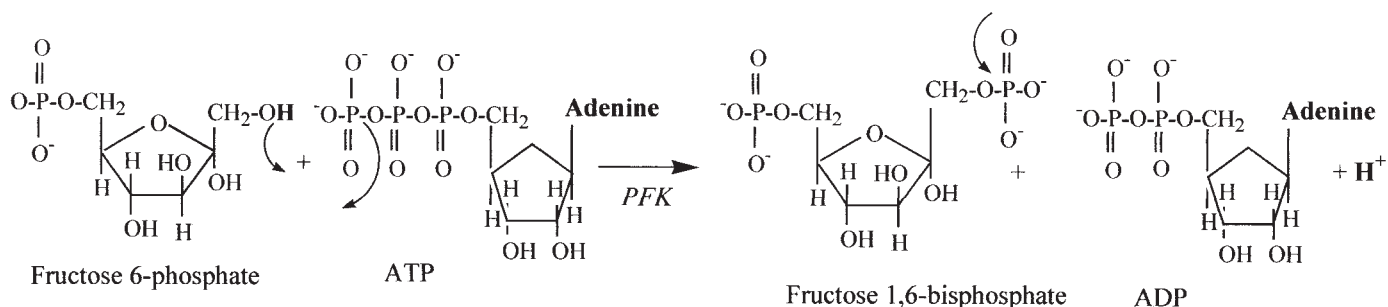


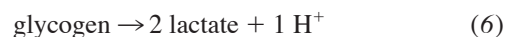
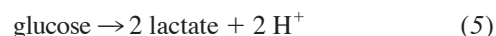
Fig. 7. Substrates and products of the phosphofructokinase (PFK) reaction. Proton release from this reaction comes from the hydroxyl group of the 6th carbon of fructose 6-phosphate. Arrows pointing away from a bond represent bond/group removal. Arrows pointing to a bond represent addition of an atom/group.

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.



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.

NADH + H⁺ as a source of H⁺. Additional 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

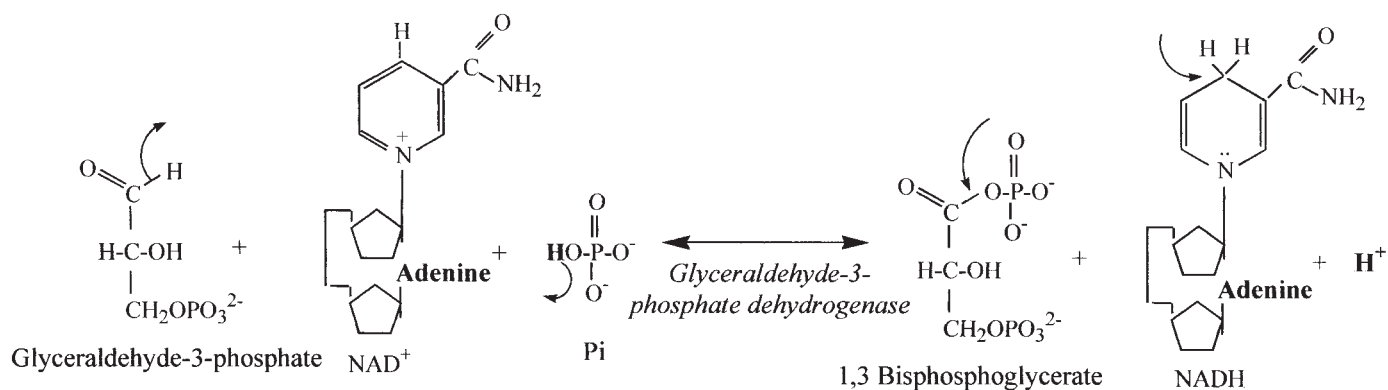


Fig. 8. Substrates and products of the glyceraldehyde 3-phosphate dehydrogenase reaction. Two electrons and a proton are used to reduce NAD^+ to NADH . The remaining proton, which in this depiction is accounted for by the proton release from free inorganic phosphate, is released into solution. Arrows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

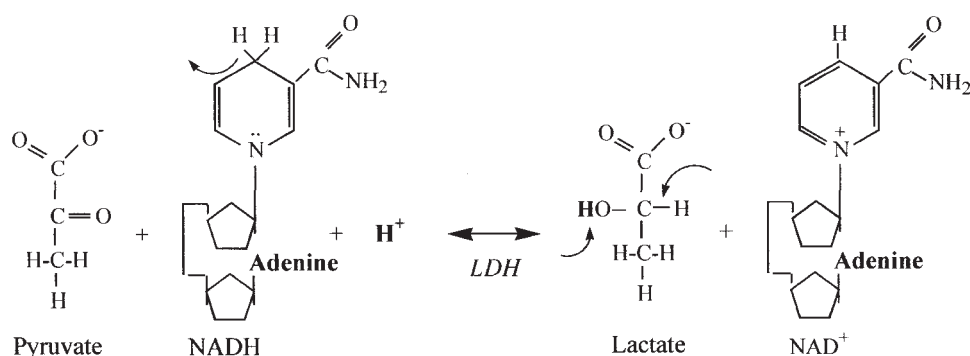


Fig. 9. Substrates and products of the lactate dehydrogenase (LDH) reaction. Two electrons and a proton are removed from NADH and a proton is consumed from solution to reduce pyruvate to lactate. Arrows pointing away from a bond represent bond/group removal. Arrows pointing to a bond represent addition of an atom/group.

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

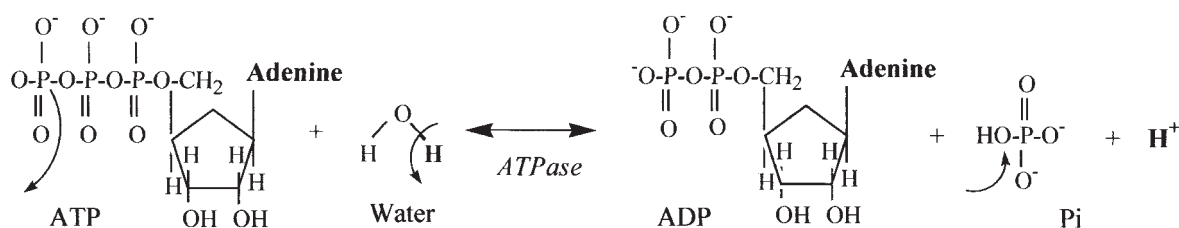
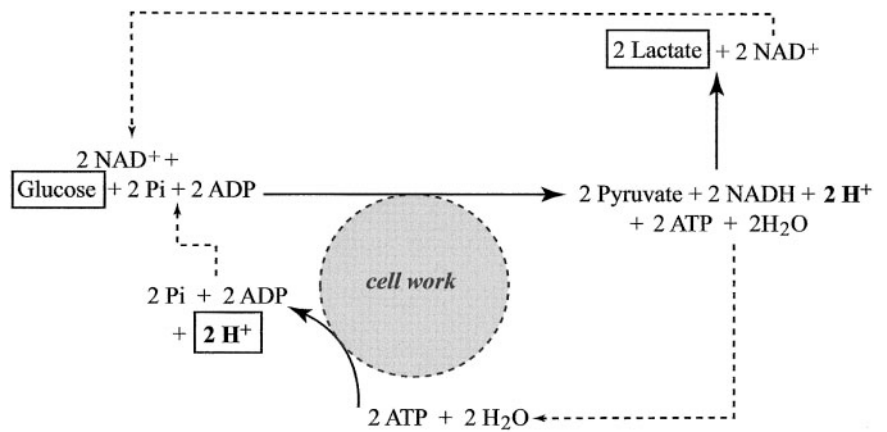


Fig. 10. Substrates and products of the ATPase reaction. This reaction is referred to as a hydrolysis reaction (ATP hydrolysis) due to the involvement of a water molecule. An oxygen atom, 2 electrons, and a proton from the water molecule are required to complete the free inorganic phosphate product of the reaction. The remaining proton from the water molecule 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. 11. Glycolytic regeneration of ATP coupled to ATP hydrolysis as would be the case during skeletal muscle contraction with no ATP contribution from mitochondrial respiration. Source of the protons that can accumulate in the cytosol is ATP hydrolysis. Balance of these reactions leaves the molecules highlighted by rectangles (glucose \rightarrow 2 lactate + 2 H⁺; Eq. 5).



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-

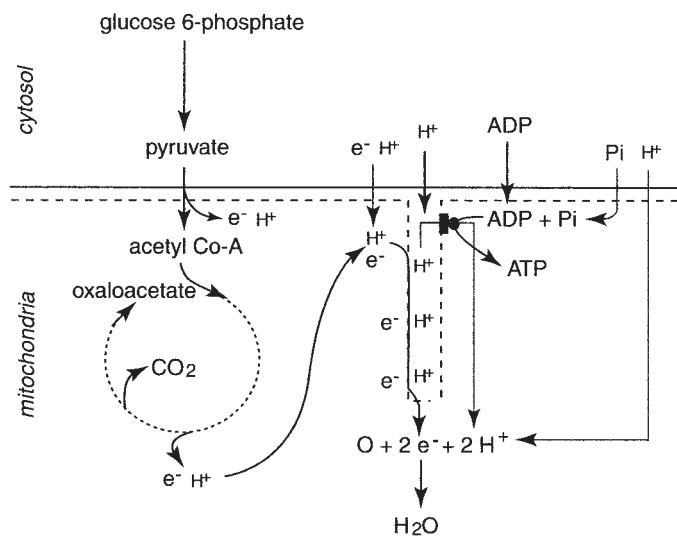


Fig. 12. A summary of the main reactions of mitochondrial respiration that support ATP regeneration. Note that each of cytosolic ADP, Pi, electrons (e⁻), and protons (H⁺) can enter the mitochondria (whether directly or indirectly) and function as substrates for oxidative phosphorylation.

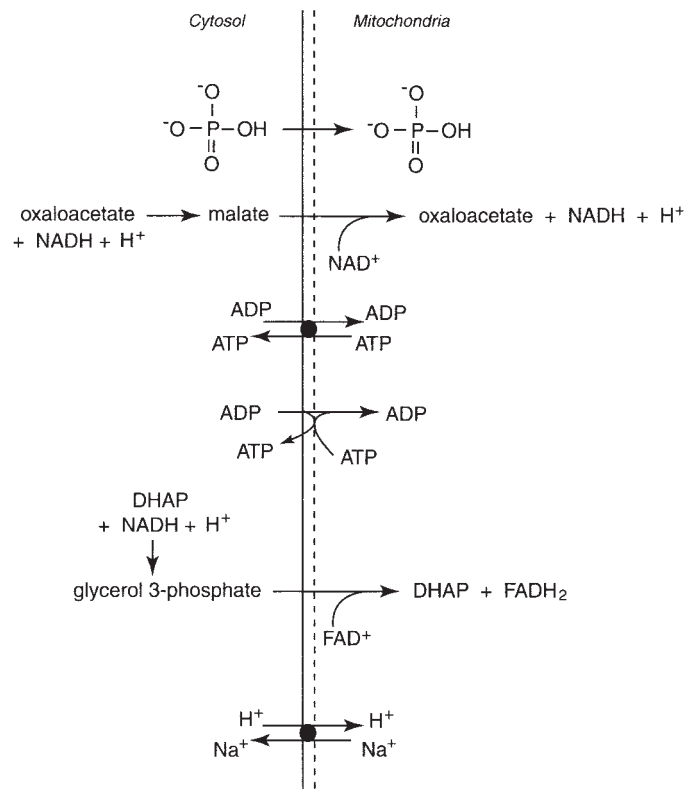


Fig. 13. Examples of the cytosolic metabolites that can diffuse or be transported into the mitochondria. Note that protons (H⁺) can enter the mitochondria indirectly via the malate-aspartate and glycerol-phosphate shuttles or directly via several cation exchange mechanisms. Note that complete reactions are not presented to simplify the diagram.

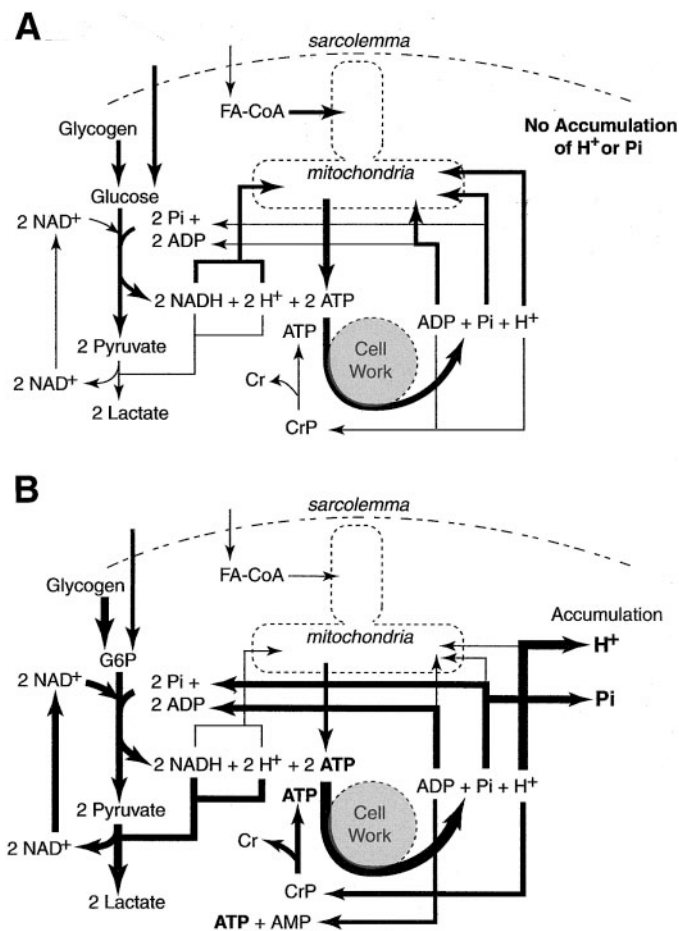


Fig. 14. Two diagrams representing energy metabolism in skeletal muscle during two different exercise intensities. *A*: steady state at $\sim 60\% \dot{V}O_{2\max}$. Note that macronutrients are a mix of blood glucose, muscle glycogen, blood free fatty acids, and intramuscular lipid. Blood free fatty acids and intramuscular lipolysis eventually yield the activated fatty acid molecules (FA-CoA). Pyruvate, NADH, and protons produced from substrate flux through glycolysis are predominantly consumed by the mitochondria as substrates for mitochondrial respiration. The same is true for the products of ATP hydrolysis (ADP, P_i , H^+). Such a metabolic scenario can be said to be pH neutral to the muscle cells. *B*: short-term intense exercise at $\sim 110\% \dot{V}O_{2\max}$, causing volitional fatigue in ~ 2 – 3 min. Size of the arrows approximate relative dependence/involvement of that reaction and the predominant fate of the products. Note that P_i is also a substrate of glycogenolysis. In this scenario, cellular ATP hydrolysis is occurring at a rate that cannot be 100% supported by mitochondrial respiration. Thus there is increased reliance on using cellular ADP for ATP regeneration from glycolysis and creatine phosphate. For every ADP that is used in glycolysis and the creatine kinase reaction under these cellular conditions, a P_i and proton is released into the cytosol. However, the magnitude of proton release is greater than for P_i due to the need to recycle P_i as a substrate in glycolysis and glycogenolysis. As explained in the text, the final accumulation of protons is a balance between the reactions that consume and release protons, cell buffering, and proton transport out of the cell. This diagram also clearly shows that the biochemical cause of proton accumulation is not lactate production but ATP hydrolysis.

sis resulting from glycolysis is primarily due to ATP hydrolysis by myosin ATPase that yields ADP, P_i , and H^+ . According to these authors, only ADP and P_i are recycled via glycolysis to produce ATP, leaving H^+ behind to accumulate within the cytosol. In other words, the glycolytic generation of ATP and ATPase-catalyzed hydrolysis of ATP are, to some extent, coupled (Fig. 11). Busa and Nuccitelli (4) also commented on this topic in an invited opinion in 1984. These authors essen-

tially reaffirmed the writings of Gevers (10, 11) and Hochachka and Mommsen (16). As stated by Busa and Nuccitelli (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 ^{31}P -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 used in the electron transport chain (ETC) to produce an H^+ gradient across the inner mitochondrial membrane that facilitates ATP synthesis via the F_0F_1 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^+ \leftrightarrow lactate $^-$ + NAD $^+$). Thus lactate formation

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

Energy System	Maximal Rate of ATP regeneration, mmol ATP \cdot s $^{-1}$ \cdot kg wet wt $^{-1}$
<i>Cytosolic</i>	
Phosphagen	2.4
Glycolytic	1.3
<i>Mitochondrial respiration</i>	
CHO oxidation	0.7
Fat oxidation	0.3

Adapted from Sahlin (46).

Table 5. Causes of acidosis and proton buffering in skeletal muscle

Causes of Proton Release	H ⁺ Buffering and Removal		
	Blood and ventilatory buffering	Intracellular H ⁺ buffering	H ⁺ removal
Glycolysis	H ⁺ + HCO ₃ ⁻	Proteins Amino acids	Mitochondrial transport
	H ₂ CO ₃	CrP hydrolysis	Lactate ⁻ /H ⁺ Symport
ATP hydrolysis		Lactate production	Sarcolemmal Na ⁺ /H ⁺ exchange
	H ₂ O + CO ₂	IMP formation HCO ₃ ⁻ P _i	HCO ₃ ⁻ /Cl ⁻ Exchange SID

HCO₃, bicarbonate; H₂CO₃, carbonic acid; CrP, creatine phosphate; SID, strong ion difference

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, HCO₃⁻, 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 (HCO₃⁻/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.

THE STOICHIOMETRY OF LACTATE PRODUCTION AND PROTON ACCUMULATION IN CONTRACTING SKELETAL MUSCLE

Apart from biochemical facts, the most compelling additional evidence for the acidosis of nonmitochondrial ATP turnover in contracting skeletal muscle comes from a compilation of research that enables calculations of the components of proton release, buffering, and removal presented in Table 5.

Juel et al. (19) quantified lactate and proton release from contracting skeletal muscle during one-legged knee extensor exercise. The data of Fig. 15 were obtained from data of lactate and proton release during incremental exercise to fatigue and drawn as this original presentation. Clearly, muscle proton release was greater than lactate release, with the difference increasing with increases in exercise intensity with an almost twofold greater proton to lactate release at exhaustion.

When concerned with intracellular nonmitochondrial ATP turnover and proton and lactate balance, the best exercise and research model to use is the quadriceps occluded blood flow model (50), as this minimizes the effect of blood flow on proton and lactate removal from the active muscle. However, the highest calculations for nonmitochondrial ATP turnover (370 mmol/kg dry wt) come from Bangsbo et al. (1), who quantified muscle metabolites and blood lactate efflux from the quadriceps muscles during one-legged dynamic knee extensor exercise to exhaustion. Bangsbo and colleagues (1, 2) argued that when totally accounting for all muscle lactate production (accumulation, removal, and oxidation), a more valid estimate of nonmitochondrial ATP turnover (ATP-NM) is obtained. The generally accepted equation (2) for this calculation is presented in Eq. 7.

$$\text{ATP-NM} = (\Delta\text{CrP} + \Delta\text{ATP}) \cdot (1.5 \cdot \Delta\text{La}) \quad (7)$$

When combining data from multiple studies (1, 33, 50, 51), with the data from Spriet and colleagues (50, 51) used for muscle metabolite accumulation during intense exercise to fatigue and the data from Bangsbo et al. (1) for ATP-NM, the data totally support the biochemically proven nonmitochondrial turnover origin of exercise-induced skeletal muscle metabolic acidosis. For example, Fig. 16 represents the biochemical balance of protons if you assume that lactate production causes acidosis. The published data reveal that the muscle buffer capacity (structural and metabolic) is almost double that

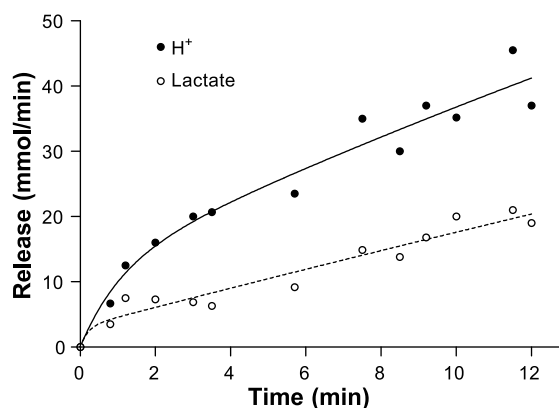


Fig. 15. Data for proton and lactate removal from contracting skeletal muscle. Figure is an original presentation of data from Juel et al. (19).

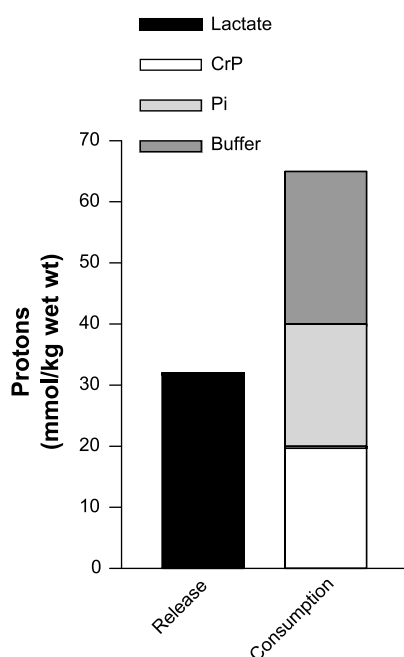


Fig. 16. Comparison between the theoretical proton release from lactate production to the known skeletal muscle buffer capacity (structural and metabolic). For example, if lactate production released protons, then the magnitude of the 2 columns of data should equal each other. Data for muscle lactate, CrP and P_i from Spriet et al. (49, 50). Data for muscle buffer capacity (by titration) from Sahlin (38) at 42 slykes for a muscle pH decrease from 7.0 to 6.4.

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_i . 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 is not just a theoretical explanation 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 ($\text{glucose} \rightarrow 2 \text{ lactate} + 2 \text{ H}^+$). 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

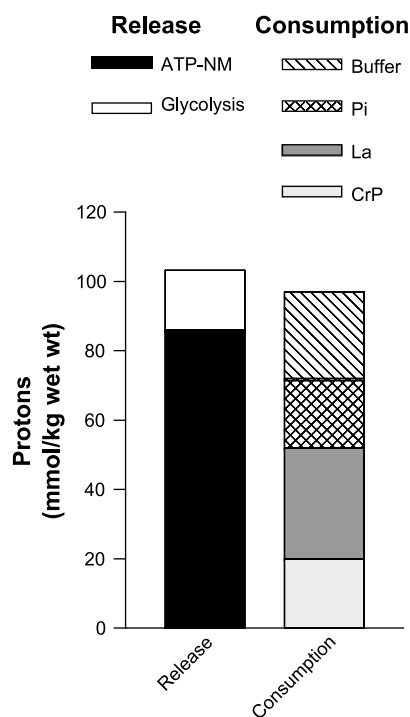


Fig. 17. Balance between intramuscular proton release and consumption based on fundamental biochemistry, as explained in the text. Data for nonmitochondrial ATP turnover (ATP-NM) from Bangsbo et al. (1) at 370 mmol/kg dry wt. Data for glycolysis from Spriet et al. (50) at 73.8 mmol glucosyl units/kg dry wt. Data for muscle lactate, CrP, P_i , and buffer capacity as for Fig. 16.

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 dichloroacetate 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 by stimulating mitochondrial respiration. For a given ATP demand, any effort to decrease lactate production without increasing mitochondrial respiration will worsen metabolic acidosis.

Quantifying buffering: the unit of slyke. The buffer capacity or value of a solution was first quantified and defined by van Slyke in 1922 (24, 38). This initial definition was based on the amount of free H^+ or OH^- added to cause one unit change in pH ($\Delta H^+/\Delta pH$). Typically, the reference mass of muscle is 1 kg. In 1955 it was recommended that Slyke's name be used as the unit expression for the buffering capacity (β) of a tissue when quantified by the $\Delta H^+/\Delta pH$ ratio, and the unit of slyke has been used to quantify proton buffering ever since.

Traditionally, the buffer capacity of skeletal muscle is measured *in vitro* and is influenced by the structural constituents of skeletal muscle. Consequently, the buffer capacity does not include the proton removal from skeletal muscle metabolism or the transfer of protons from the cytosol to the mitochondria or out of the cell. Sahlin (38) referred to these two components of cell proton buffering as structural and metabolic, with the combination representing the total *in vivo* proton buffering capacity.

Unfortunately, when attempting to quantify the skeletal muscle buffer capacity *in vivo* the determination of protons added to a cell is difficult. To make this process easier, researchers have often assumed the source of the protons to be the production of metabolic acids: namely lactic and pyruvic acid. Obviously, this has been incorrect. The result has been an incorrect estimation of proton buffering, with such values varying from 60 to 80 slykes (24, 38, 49, 50). When based solely on lactate production corrected for muscle water [Spriet et al. (50) used 3.3 l/kg dry wt], a value of 74.5 slykes is calculated, which clearly shows the bias of this unit in assuming lactate production contributes most of the proton release

during muscle catabolism. As is clear from this review, such an approach to quantify skeletal muscle proton buffering is invalid and its use should never have been associated with the unit slyke.

On the basis of the content of this review, the best estimate of a muscle buffer capacity, which of course will vary with the degree and quality of exercise training of a given individual, is 208 slykes. This value is obtained from the data of Fig. 17, where 2–3 min of intense exercise to volitional exhaustion is associated with a pH decrease from 7.0 to 6.4, and the release/production of 125 mmol H^+ /kg muscle ($125/0.6 = 208$). The total muscle buffer capacity is the sum of the components of structural and metabolic buffering. The data of Fig. 17 provide an estimate of metabolic buffering to be 120 ($72/0.6$) slykes, with 88 slykes remaining as structural buffering. Interestingly, our estimate of structural buffering is similar to that reported by Sahlin (38). Past research estimates of the muscle buffer capacity from assuming lactate and pyruvate production estimate proton release are gross underestimations of the true muscle buffer capacity, as they do not account for all proton release (nonmitochondrial ATP hydrolysis), and in assuming lactate production is a source of protons rather than a consumer of protons, they underestimate metabolic 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 NAD^+ 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 indirect 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 $\{[(\Delta \text{lactate} + \Delta \text{pyruvate})/\Delta pH] \sim (\Delta H^+/\Delta pH)\}$ 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 lactic acidosis. These individuals and their scholarship (4, 7, 10, 11, 16, 34, 55–57, 60, 61, 63) collectively represent the original source of inspiration for writing this review. Such inspiration, combined with the largely ignored message that acidosis is not caused by lactate production, fueled our desire to write a comprehensive and critical review on such an important component of basic, applied, and clinical physiology and muscle metabolism.

REFERENCES

- Bangsbo J, Gollnick PD, Graham TE, Juel C, Kiens B, Mizuno M, and Saltin B. Anaerobic energy production and O₂ deficit-debt relationship during exhaustive exercise in humans. *J Physiol* 422: 539–559, 1990.
- Bangsbo J. Quantification of anaerobic energy production during intense exercise. *Med Sci Sports Exerc* 30: 47–52, 1998.
- Bernardi P. Mitochondrial transport of cations: channels, exchangers, and permeability transition. *Physiol Rev* 79: 1127–1155, 1999.
- Busa WB and Nuccitelli R. Metabolic regulation via intracellular pH. *Am J Physiol Regul Integr Comp Physiol* 246: R409–R438, 1984.
- Clarence DH. *The Handbook of Biochemistry and Biophysics*. Cleveland, OH: World, 1966.
- Corey HE. Stewart and beyond: new models of acid-base balance. *Kidney Int* 64: 777–787, 2003.
- Davis EJ, Bremer J, and Akerman KE. Thermodynamic aspects of translocation of reducing equivalents by mitochondria. *J Biol Chem* 255: 2277–2283, 1980.
- Dennis SC, Gevers W, and Opie LH. Protons in ischemia: where do they come from; where do they go to? *J Mol Cell Cardiol* 23: 1077–1086, 1991.
- Finkel KW and DuBose TD. Metabolic acidosis. In: *Acid-Base and Electrolyte Disorders: A Companion to Brenner and Rector's The Kidney*, edited by DuBose TD and Hamm LL. Philadelphia, PA: Saunders, 2002, p. 55–66.
- Fitts RH and Holloszy JO. Lactate and contractile force in frog muscle during development of fatigue and recovery. *Am J Physiol* 231: 430–433, 1976.
- Gevers W. Generation of protons by metabolic processes in heart cells. *J Mol Cell Cardiol* 9: 867–874, 1977.
- Gevers W. Generation of protons by metabolic processes other than glycolysis in muscle cells: a critical view [letter to the editor]. *J Mol Cell Cardiol* 11: 328, 1979.
- Hagberg H. Intracellular pH during ischemia in skeletal muscle: relationship to membrane potential, extracellular pH, tissue lactic acid and ATP. *Pflügers Arch* 404: 342–347, 1985.
- Harmer AR, McKenna MJ, Sutton JR, Snow RJ, Ruell PA, Booth J, Thompson MW, Mackay NA, Stathis CG, Crameri RM, Carey MF, and Eager DM. Skeletal muscle metabolic and ionic adaptations during intense exercise following sprint training in humans. *J Appl Physiol* 89: 1793–1803, 2000.
- Hill AV, Long CNH, and Lupton H. Muscular exercise, lactic acid, and the supply and utilization of oxygen. *Proc R Soc Lond B Biol Sci* 16: 84–137, 1924.
- Hill AV. Croonian lecture. *Proc R Soc Lond B Biol Sci* 100: 87, 1926.
- Hochachka PW and Mommsen TP. Protons and anaerobiosis. *Science* 219: 1391–1397, 1933.
- Holten CH, Muller A, and Rehbinder D. *Lactic Acid: Property and Chemistry of Lactic Acid and Derivatives*. Germany: Verlag Chemie, 1971.
- Howlett RA, Heigenhauser GJF, and Spriet LL. Skeletal muscle metabolism during high-intensity sprint exercise is unaffected by dichloroacetate or acetate infusion. *J Appl Physiol* 87: 1747–1751, 1999.
- Juel C, Klarskov C, Nielsen JJ, Krstrup P, Mohr M, and Bangsbo J. Effect of high intensity intermittent training on lactate and H⁺ release from human skeletal muscle. *Am J Physiol Endocrinol Metab* 286: E245–E251, 2004.
- Juel C. Lactate/proton co-transport in skeletal muscle: regulation and importance for pH homeostasis. *Acta Physiol Scand* 156: 369–374, 1996.
- Juel C. Lactate-proton cotransport in skeletal muscle. *Physiol Rev* 77: 321–358, 1977.
- Juel C. Muscle pH regulation: role of training. *Acta Physiol Scand* 162: 359–366, 1988.
- Kaplan RS. Structure and function of mitochondrial anion transport proteins. *J Membr Biol* 179: 165–183, 2001.
- Karlsson J. Lactate and phosphagen concentrations in working muscle of man. *Acta Physiol Scand Suppl* 358: 1–72, 1971.
- Katz A and Sahlin K. Regulation of lactic acid production during exercise. *J Appl Physiol* 65: 509–518, 1988.
- Kowalchuk JM, Heigenhauser GJF, Lindinger MI, Sutton JR, and Jones NL. Factors influencing hydrogen ion concentration in muscle after intense exercise. *J Appl Physiol* 65: 2080–2089, 1988.
- Laski ME and Wesson DE. Lactic acidosis. In: *Acid-Base and Electrolyte Disorders: A Companion to Brenner and Rector's The Kidney*, edited by DuBose TD and Hamm LL. Philadelphia, PA: Saunders, 2002, p. 835–108.
- Lehninger AL. *The Principles of Biochemistry* (2nd ed). New York: Worth, 1982.
- Lusk G. *The Elements of the Science of Nutrition*. Philadelphia: Saunders, 1928.
- Lindinger MI and Heigenhauser GJ. The roles of ion fluxes in skeletal muscle fatigue. *Can J Physiol Pharmacol* 69: 246–253, 1991.
- MacRae HSH and Dennis SC. Lactic acidosis as a facilitator of oxyhemoglobin dissociation during exercise. *J Appl Physiol* 78: 758–760, 1995.
- Margaria R, Edwards HT, and Dill DB. The possible mechanisms of contracting and paying the oxygen debt and the role of lactic acid in muscular contraction. *Am J Physiol* 106: 689–715, 1933.
- Medbo JO and Tabata I. Anaerobic energy release in working muscle during 30 s to 3 min of exhaustive bicycling. *J Appl Physiol* 75: 1654–1660, 1993.
- Noakes TD. Challenging beliefs: ex Africa semper aliquid novi. *Med Sci Sports Exerc* 29: 571–590, 1997.
- Raju TN. The Nobel Chronicles. 1922: Archibald Vivian Hill (1886–1977), Otto Fritz Meyerhoff (1884–1951). *Lancet* 352: 1396, 1998.
- Robergs RA. Exercise-induced metabolic acidosis: where do the protons come from? *Sportsmedicine* 5 [sportsmedicine.org/jour/0102/rar.htm, 2001].
- Roth DA and Brooks GA. Lactate transport is mediated by a membrane-bound carrier in rat skeletal muscle sarcolemmal vesicles. *Arch Biochem Biophys* 279: 377–385, 1990.
- Sahlin K. Intracellular pH and energy metabolism in skeletal muscle of man. *Acta Physiol Scand Suppl* 455: 7–50, 1978.
- Sahlin K. NADH in human skeletal muscle during short-term intense exercise. *Pflügers Arch* 403: 193–196, 1985.
- Sahlin K, Edstrom L, Sjöholm H, and Hultman E. Effects of lactic acid accumulation and ATP decrease on muscle tension and relaxation. *Am J Physiol Cell Physiol* 240: C121–C126, 1981.
- Sahlin K, Edstrom L, Sjöholm H, and Hultman E. Effects of lactic acid accumulation and ATP decrease on muscle tension and relaxation. *Am J Physiol Cell Physiol* 240: C121–C126, 1981.
- Sahlin K, Harris RC, Ny Lind B, and Hultman E. Lactate content and pH in muscle samples obtained after dynamic exercise. *Pflügers Arch* 367: 143–149, 1976.
- Sahlin K and Henriksson J. Muscle buffer capacity and lactate accumulation in skeletal muscle of trained and untrained men. *Acta Physiol Scand* 122: 331–339, 1984.
- Sahlin K, Katz A, and Henriksson J. Redox state and lactate accumulation in human skeletal muscle during dynamic exercise. *Biochem J* 245: 551–556, 1987.
- Sahlin K, Tonkonogi M, and Soderlund K. Energy supply and muscle fatigue in humans. *Acta Physiol Scand* 162: 261–266, 1998.
- Sahlin K. Metabolic changes limiting muscle performance. In: *Biochemistry of Exercise*, edited by Saltin B. Champaign, IL: Human Kinetics, 1986, vol. 16, p. 323–344.
- Shampo MA and Kyle RA. Otto Meyerhoff—Nobel Prize for studies of muscle metabolism. *Mayo Clin Proc* 74: 67, 1999.
- Smith GL, Donoso P, Bauer CJ, and Eisner DA. Relationship between intracellular pH and metabolite concentrations during metabolic inhibition in isolated ferret heart. *J Physiol* 472: 11–22, 1993.
- Spriet LL, Sodeland K, Bergstrom M, and Hultman E. Aerobic energy release in skeletal muscle during electrical stimulation in men. *J Appl Physiol* 62: 611–615, 1987.
- Spriet LL, Sodeland K, Bergstrom M, and Hultman E. Skeletal muscle glycogenolysis, glycolysis, and pH during electrical stimulation in men. *J Appl Physiol* 62: 616–621, 1987.
- Spriet LL. Anaerobic metabolism in human skeletal muscle during short-term, intense activity. *Can J Physiol Pharmacol* 70: 157–165, 1992.

52. **Stringer W, Wasserman K, Casaburi R, Porzasz J, Maehara K, and French W.** Lactic acidosis as a facilitator of oxyhemoglobin dissociation during exercise. *J Appl Physiol* 76: 1462–1467, 1994.
53. **Stringer W, Casaburi R, and Wasserman K.** Acid-base regulation during exercise and recovery in humans. *J Appl Physiol* 72: 954–961, 1992.
54. **Stryer L.** *Biochemistry* (4th ed). San Francisco: Freeman, 1995.
55. **Tafaletti JG.** Blood lactate: biochemistry, laboratory methods and clinical interpretation. *CRC Crit Rev Clin Lab Sci* 28: 253–268, 1991.
56. **Trump BD, Mergner WJ, Kahng MW, and Saladino AJ.** Studies on the subcellular pathophysiology of ischemia. *Circulation* 53: 117–126, 1976.
57. **Vaghy PL.** Role of mitochondrial oxidative phosphorylation in the maintenance of intracellular pH. *J Mol Cell Cardiol* 11: 933–940, 1979.
58. **Webster's Ninth New Collegiate Dictionary.** Springfield: Merriam-Webster, 1984.
59. **Westerblad H, Allen DG, and Lannergren J.** Muscle fatigue: lactic acid or inorganic phosphate the major cause? *News Physiol Sci* 17: 17–21, 2002.
60. **Wilkie DR.** Generation of protons by metabolic processes other than glycolysis in muscle cells: a critical view. *J Mol Cell Cardiol* 11: 325–330, 1979.
61. **Williamson JR, Schaffer SW, Ford C, and Safer B.** Contribution of tissue acidosis to ischemic injury in the perfused rat heart. *Circulation* 53: 13–116, 1976.
62. **Wilson MC, Jackson VN, Heddle C, Price NT, Pilegaard H, Juel C, Bonen A, Montgomery I, Hutter OF, and Halestrap AP.** Lactic acid efflux from white skeletal muscle is catalyzed by the monocarboxylate transporter isoform MCT3. *J Biol Chem* 273: 15920–15926, 1998.
63. **Zilva JF.** The origin of the acidosis in hyperlactataemia. *Ann Clin Biochem* 15: 40–43, 1978.

