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Discussion

The missing hydrogen ion, part-2: Where the evidence leads to

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ABSTRACT

The purpose of this manuscript was to present the evidence for why cells do not produce metabolic acids. In addition, evidence that opposes common viewpoints and arguments used to support the cellular production of lactic acid (HLa) or liver keto-acids have been provided. Organic chemistry reveals that many molecules involved in cellular energy catabolism contain functional groups classified as acids. The two main acidic functional groups of these molecules susceptible to $\sim H^+$ release are the carboxyl and phosphoryl structures, though the biochemistry and organic chemistry of molecules having these structures reveal they are produced in a non-acidic ionic (negatively charged) structure, thereby preventing pH dependent $\sim H^+$ release. Added evidence from the industrial production of HLa further reveals that lactate (La^-) is produced followed by an acidification step that converts La^- to HLa due to pH dependent $\sim H^+$ association. Interestingly, there is a plentiful list of other molecules that are classified as acids and compared to HLa have similar values for their H^+ dissociation constant (pK_d). For many metabolic conditions, the cumulative turnover of these molecules is far higher than for La^-. The collective evidence documents the non-empirical basis for the construct of the cellular production of HLa, or any other metabolic acid.

1. Introduction

Part-1 of this three-part series provided background information to build the foundation of a detailed, evidence-based understanding of acids and bases, and the roles of hydrogen ions (H^+) in specific chemical reactions.[1] The initial content was based on a historical account of the increasing knowledge of acid-base chemistry, and how this development was negatively influenced by a poor interpretation of the organic chemistry of chemical reactions and the roles of H^+ in these reactions. Furthermore, the scientific views of acids were largely fuelled by inference and not evidence-based deduction.

The purpose of this second instalment is to expand the evidence-based understanding of the cellular source of H^+ exchange, further supported by evidence-based examples of rationale reasoning. Evidence is provided from the organic chemistry of pertinent cellular chemical reactions, revealing that cells produce the non-acidic ionic (negatively charged) bases of molecules classified as acids. Added evidence is provided that refutes common arguments used in support of the cellular production of metabolic acids, with the result of all evidence further detailing why the construct of the cellular production of metabolic acids is incorrect. Such evidence should improve the research and dissemination of such knowledge within the disciplines of sports medicine and the added medical, clinical, health and exercise and sport sciences. As with Part-1,[1] the content contained within is structured by a combination of Question, Common View, Evidence, and Answer.

2. Question 1: are molecules that contain chemical acid functional groups produced as acids within cells?

Common View: Yes, and the best example of this is the cellular production of HLa. HLa is an end-product of the glycolytic pathway during times of high rates of cellular energy demand and/or conditions of insufficient oxygen availability (hypoxia or anoxia [anaerobiosis]). As the pK_d of HLa approximates 3.67 (will vary slightly with different ionic strength and temperature),[2] at pH = 7 approximately 99.9% of all HLa molecules dissociate to $La^- + H^+$. This H^+ dissociation contributes to the cellular and systemic acidosis of increased HLa production causing a lactic acidosis.

Evidence: The LDH reaction catalyses the interconversion of pyruvate to lactate (La^-) or vice-versa. All chemical reactions have the capability for proceeding in either direction, though each have unique natural

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Abbreviations

H^+	Hydrogen ion = a hydrogen atom that is missing its single electron
~	Fractional
$\sim H^+_e$	Fractional H^+ exchange
HLa	Lactic acid
LDH	Lactate dehydrogenase
β-HB	β-hydroxybutyrate
ACA	Acetoacetate
K_d	Dissociation constant
p	Negative $\log_{10}$
pK_d	Negative $\log_{10}$ of the K_d
ΔG°	Standard free energy change (kJ·Mol^{-1})
NADH	Reduced structure of nicotinamide adenine dinucleotide.
NAD^+	Oxidized structure of nicotinamide adenine dinucleotide.
ATP	Adenosine triphosphate
ADP	Adenosine diphosphate
H_2O	Water
G_6P	Glucose-6-phosphate

properties for favouring one direction over another for specific substrate and production concentrations, pH and temperature. In all cells, the LDH reaction favours lactate production, with a standard free energy change (ΔG°; negative is release and reveals the direction of the reaction) of −25.1 kJ·Mol^{-1}.[3] The details of the LDH reaction are provided in Fig. 1.

If attention is focussed on the direction of the molecular reduction of pyruvate (gaining of electrons) to La^-, you can see from the chemical structures presented by Robergs et al.[1] and Fig 2 that all substrates and products have a carboxyl functional group in an ionized state; the acid functional group does not have a H^+ that can be released (dissociation) as indicated by the negative charge on the single bond oxygen atom. Thus, at the cellular pH of skeletal muscle (pH = 7, though differs slightly between different tissues), the pK_d of the acid functional group will determine the $\sim H^+$ association with the negative charge of the ionic form of the acid functional group (see below). The source of these H^+ is from the ionization activity of water (see Question 6), which of course is also influenced by the added reactions that are occurring that involve $\sim H^+_e$.

The added pH dependent $\sim H^+_e$ on metabolites produced as ions is important and previously overlooked in acid-base and organic chemistry, as well as cellular biochemistry. Such organic and acid-base chemistry evidence documents and quantifies the extent of metabolic H^+ removal during the production of lactate. Robergs[4] quantified such $\sim H^+_e$ of La^- production to be largely pH independent across the skeletal muscle physiological pH range (6.0–7.0) at 1.004 to 1.000 4, respectively (positive values indicate H^+ consumption), which added to the earlier research of Kushmerick et al.[5] and Vinnakota et al.[6]

Do not be confused by the expression of $\sim H^+$ association or dissociation. What this refers to is the fractional proportion of molecules present in the acid vs. base (ionized) structure.[1] Fig. 2,4 Avogadro's constant[3] is presented in Equation (1).

$$\text{Avogadro's constant} = 6.022\ 1 \times 10^{23}\ \text{molecules·Mol}^{-1}\ \text{of a substance} \quad \text{Equation 1}$$

For lactate at pH = 7 and a concentration of 1 mmol·L^{-1} muscle water, the number of molecules of La^- can be calculated (Equation (2)).

$$6.022\ 1 \times 10^{23} \times 0.001 = 6.022\ 1 \times 10^{20}\text{·L}^{-1}\ \text{molecules of La}^- \quad \text{Equation 2}$$

As a typical muscle cell has a volume of ~0.7 mL where water represents 73% of this volume[7,8] (will vary slightly based on fibre type and vary more with hypertrophic adaptation), that actual number of La^- and HLa molecules in a muscle cell can be calculated.

$$0.7 \times 0.000\ 1 \times 6.022\ 1 \times 10^{20} = 3.077\ 3 \times 10^{17}\ \text{molecules (La}^- + \text{HLa)} \quad \text{Equation 3}$$

Using data from Robergs,[4] such cellular La^- (ionic structure) at pH = 7 amounts to 99.95% of the total.

During extreme intense exercise, muscle La^- can increase in concentration nearing 40 mmol·L^{-1} muscle water and muscle pH could be as low as 6.0.[9] This would mean that the cell now contains a larger number of La^- molecules as calculated in Equation (4).

$$40 \times 3.077\ 3 \times 10^{17} = 1.230\ 9 \times 10^{19}\ \text{molecules} \quad \text{Equation 4}$$

For this intense exercise example and slightly lowered pH, 99.53% of the molecules are La^-. The small tally of HLa present (though still a large absolute number based on Avogadro's constant) is based on pH dependent H^+ association and as such does not refer to any possibility for H^+ release.

Answer: No, cells do not produce metabolic acids from chemical reactions. Cells produce ionized forms of molecules that have historically been categorized as acids based on their pure chemical (not biological) structures (Figs. 1 and 2). The production of these molecules in biological systems (e.g., cells) in their ionized structure is expected through an understanding and application of the principles of organic chemistry, cellular biochemistry, bioenergetics, and thermodynamics.

3. Question 2: despite the presence of prior metabolites that have carboxylic acid functional groups, why is lactate production viewed to be the sole source of H^+ release that contributes to metabolic acidosis during intense exercise?

Common View: During high rates of production, HLa can accumulate in cells and thereby release a H^+, forming lactate, and cause cellular and systemic acidosis, contributing to the onset of fatigue during exercise.

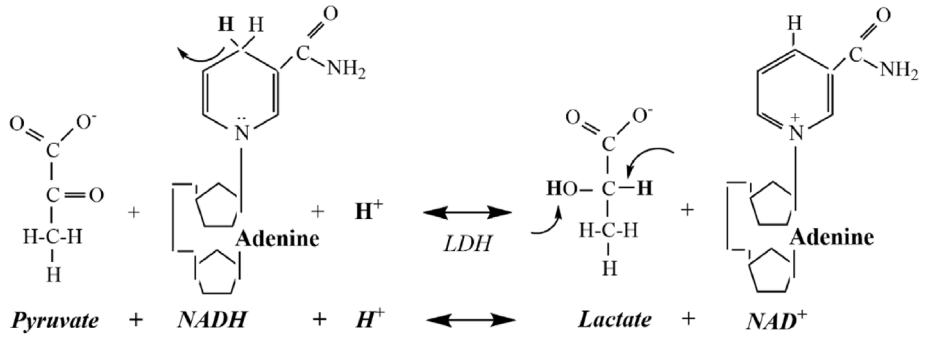

Fig. 1. The chemical structures of the substrates and products of the lactate dehydrogenase (LDH) reaction. NADH = reduced structure of nicotinamide adenine dinucleotide; NAD^+ = oxidized structure of nicotinamide adenine dinucleotide.

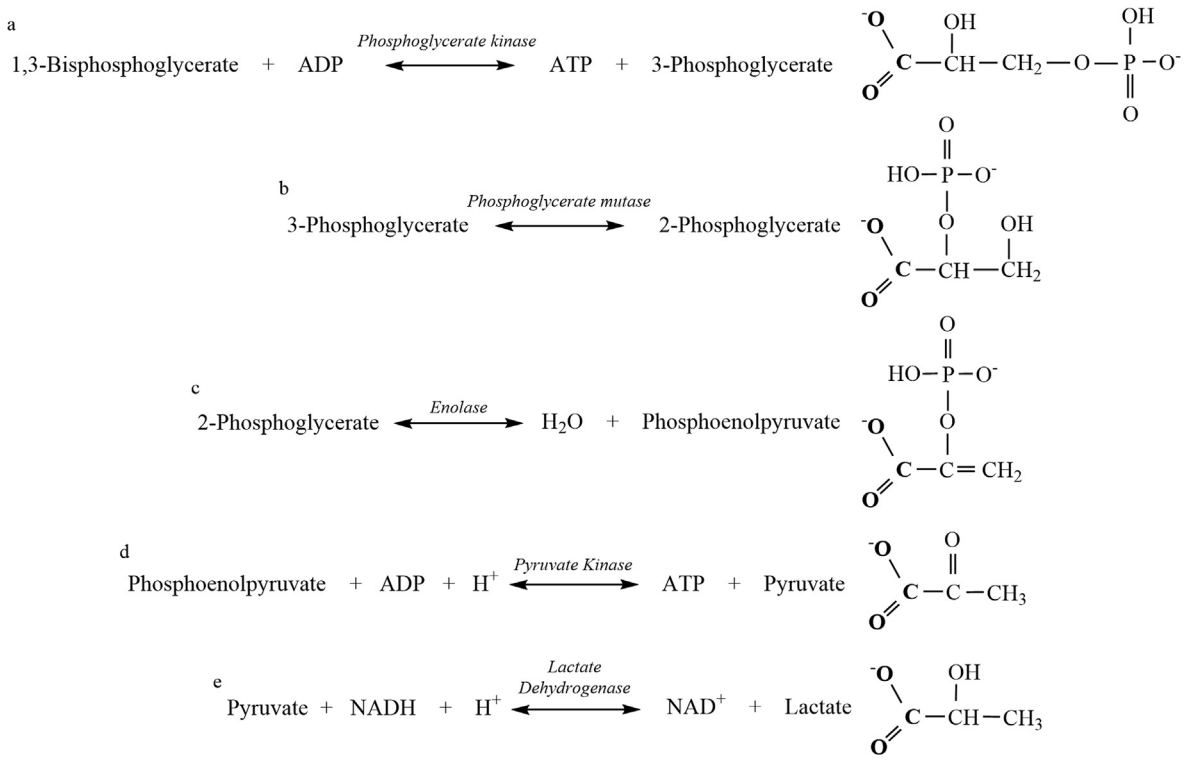

Fig. 2. The final 5 reactions of phase-II of glycolysis (a–d), ending in lactate production (e). Note the structures of the metabolites that are produced. Although these metabolites are categorized as carboxylic acids, they are all produced with a terminal ionized carboxyl group (bold functional group). For all main products, the chemical structures are provided to show the transition in the atomic composition and structure, ending in lactate production. ATP = adenosine triphosphate; ADP = adenosine diphosphate; H_2O = water.

Such conditions occur during intense exercise, as well as in disease, and collectively are referred to as lactic acidosis.[10,11]

Evidence: As documented in the evidence and answer to Question 1, no metabolite is produced as a metabolic acid, La^- production consumes not releases $\sim H^+$, and the initial metabolite of glycolysis that contains a carboxyl functional group is 3-phosphoglycerate (Fig. 2).

Even if the approach presented in the 'Current View' is entertained, the argument is further shown to be questionable because as revealed in Figure 2, 3-phosphoglycerate is produced in a phosphate transfer reaction, leaving an ionized carboxyl group. Each of the following four reactions cause covalent modifications resulting in slightly different products where this functional group remains unaltered and constantly exposed to the cellular pH. La^- production is the final cytosolic reaction based on the molecular reduction of pyruvate, which consumes a $\sim H^+$ and regenerates the oxidized structure of cytosolic nicotinamide adenine dinucleotide (NAD^+). This regeneration of cytosolic NAD^+ is essential for phase-II of glycolysis to function and thereby aid the cell to sustain glycolytic ATP regeneration during conditions of high ATP demand (e.g., intense muscle contraction) and/or decreased oxygen availability (hypoxia).

La^- accumulates because it can be produced at a faster rate than it can be transported from the cell. However, this has nothing to do with any propensity for the release of a H^+ because as previously documented, there was never a H^+ on the carboxylic acid functional group to begin with (La^- is not produced as HLa). It is also important to realize that the flux of substrate through the end reactions of glycolysis to pyruvate all occur at greater rates to La^- production due to the added fate of pyruvate entry into mitochondria. If metabolic acids did exist, the H^+ release from the reactions of glycolysis leading to pyruvate production would cause far greater H^+ release than if HLa was produced and was viewed to be the sole source of the $\sim H^+$ release.

Answer: Cells do not produce HLa, or any other acidic form of carboxylic acid type molecules in glycolysis. Whether a molecule accumulates in a cell or not has nothing to do with $\sim H^+_e$, which would occur near instantaneously for a molecule with an ionizable group vulnerable to the cellular pH. For all cellular metabolites with phosphoryl or carboxylic acid functional groups, such H^+ exchange would favour association not dissociation due to their production as charged bases not acids.

4. Question 3: does the industrial production of HLa prove that cells must produce HLa?

Common View: Acids are produced on a large scale based on a mix of chemical production, or biological production via fungi or bacteria. The fact that acid molecules are produced from biological tissues proves that cells can produce acids.

Evidence: If you own a swimming pool, or jacuzzi, you know that the pool store attendant can sell you a container of hydrochloric acid. If you need to clean rust off metal, or other surfaces, you can purchase a bottle of phosphoric acid from a hardware store. Acids are also used in food industries, with acetic acid (vinegar) being the most common acid for food taste and preservation purposes. Other common acids within the food industry are HLa and citric acid. Such food technology purposes, among many others, reveal some of the diverse industrial applications of acids.

A wealth of scientific detail exists for how commercial acids are produced, but this does not mean that cells can produce acids. A review of the pertinent processes involved in the industrial production of acids reveals key steps documenting that the process produces the acid ionized base. This solution is then acidified to cause H^+ association to the ionic form to then produce the acid structure. The acid structure is then isolated via such processes as distillation. For example, Komesu et al.[12] detailed the industrial production of HLa via each of chemical synthesis, bacterial formation, or microbial fermentation. In each biological method, there is a phase of acidification required to convert La^- to HLa.

In short, both biological methods produce La^-, and by acidification through the final addition of a strong acid solution, HLa is formed from pH dependent H^+ association and purified by distillation.

Abedi and Hashemi[13] provided a similar account of the industrial production of HLa, and these authors also revealed key evidence of how the process produces La^-. When HLa is produced via bacterial anaerobic glycolysis, the solution pH is required to be kept close to seven for the bacteria to remain metabolically active. To do this, lime is added to the solution, and the final product formed is calcium lactate. Addition of sulphuric acid is required to lower the final pH and covert the calcium lactate to HLa and calcium sulphate. More importantly, microbial production of HLa can be done at pH = 3 or lower. Given the pK_d of HLa approximates 3.67 (ionic strength and temperature dependent), this acidification allows final production of HLa (lactate is chemically produced and immediately becomes protonated due to the low pH of the medium). However, note the non-physiological pH state of the production medium and the reality of the metabolic biochemistry (presented incorrectly by these authors) causing La^- production.

Answer: It is the acidification post-La^- production (at non-physiological pH ranges) that results in the industrial production of HLa. As such, the industrial production of acids via multiple biological methods supports the cellular production of ionized acid bases, not metabolic acids.

5. Question 4: what molecules previously labelled as metabolic acids are produced in the body?

Common View: Despite there being numerous metabolites produced by cells that are defined as acids, only HLa production (from numerous tissues) and liver ketone body production (β-HB and ACA) cause changes in cellular and systemic pH.

Evidence: Cells, depending on their tissue location and function, can produce a variety of molecules that are traditionally labelled as acids. There are fatty acids, ketone bodies expressed as acids (β-HB and ACA), amino acids, deoxyribonucleic acids, ribonucleic acids, carboxylic acids of phase 2 of glycolysis, tricarboxylic acids of the Kreb's Cycle, etc.[1] However, as addressed above, only the production of HLa during exercise, or ketone bodies in the liver during compromised carbohydrate nutrition (ketoacidosis), have been interpreted to influence the pH of body fluids.

Fundamental principles of organic chemistry and acid-base chemistry that have been established for more than 40 years, as has been explained prior, provides the evidence against the cellular production of metabolic acids. Furthermore, even if we were to ignore this evidence, best estimates of the so-called 'acid load' of these different molecules reveals that if HLa production did occur in the body, especially during intense exercise at its highest rate of production (10 $mmol \cdot L^{-1} \cdot min^{-1}$ in skeletal muscle for a maximal duration of ~3 min and an active prime muscle mass of 10 kg), the rate of H^+ release would be similar to many other metabolic acid molecules. For example, such rates would be not that different to the free fatty acid (FFA) turnover from lipolysis during prolonged exercise,[14,15] or the release of amino acids during glucose restriction or prolonged exercise.[16] Most of these other 'acids' have similarly low pK_d values as HLa[1] and their total turnover (FFA and amino acids) could be even larger given their biochemical relevance across hours and even multiple days. Consequently, there are no rational, evidence-based scientific reasons for why HLa has been the sole target for the cause of acidosis throughout the historical development of the disciplines of cellular biochemistry, muscle physiology and exercise physiology.

Answer: The diverse type and large number of 'acid molecules' produced in the even more highly diverse cells and tissues of the body further reveals the illogical foundation of the lactic acidosis construct. There is nothing mysteriously special about the carboxylic acid function group of HLa that makes it the cause of cellular or systemic acidosis during intense exercise or disease, just as there are no acidic ketone bodies produced in the liver. To accept these interpretations is more belief than science, which of course means that such acceptance is not informed by science at all. The fact that chemicals are named as acids has nothing to do with their formation or presence in biological solutions as acids. Rather, these terms are reflective of historical precedent of molecular nomenclature and dichotomy within chemistry. There is no cellular production of metabolic acids via chemical reactions. The relatively small fraction of metabolic acids that might be formed in cells results from the pH dependent H^+ association to the acid molecules produced in their ionized state.

6. Question 5: does it matter where the hydrogen ions (H^+) come from during the cellular catabolism of glucose given that there is an approximate net release of 2 H^+ during the oxidation of glucose to 2 La^-; the net result remains 2 La^- + 2 H^+ = 2 HLa?

Common View: No. The net (summary) result of cellular metabolism is all that is important. For every two La^- produced there is close to two hydrogen ions (H^+) released during cellular catabolism, and for all intent and purpose, this is equivalent to two HLa molecules.[17–19] This view is also reinforced by the knowledge of the monocarboxylate transporter that co-transports one lactate + one H^+ from cells to the interstitial space,[19] which essentially is the removal of HLa from the cell.

Evidence: The common view answer above is based on a few core assumptions. 1) The glycolytic pathway is a closed system ending in La^- production, thereby allowing H^+ release from glycolytic reactions to be conceptually coupled to La^- production. 2) Due to assumption 1, the glycolytic La^- to H^+ release ratio of 1.0 (2:2) is a constant during cellular metabolism. 3) The only transport mechanism for cells to remove H^+ uses La^- as a co-transporter. 4) Two different molecular events that have no chemical or location connections can be combined if they share a final cellular transport mechanism, thereby labelling molecules as acids simply because they involve H^+ transport.

A logical way to address each of these assumptions is to provide evidence that refutes them.

6.1. Evidence opposing assumption 1: the glycolytic pathway is both anaplerotic and cataplerotic

The glycolytic pathway is presented in Fig. 3, along with known added reactions that either add or remove metabolites from the pathway. While the presence of key enzymes to support many of the side-reactions to glycolysis predominate in the liver, numerous reactions also occur in skeletal muscle that have amino acids enter or exit pathways. Glycolysis is not a closed pathway; like so many other metabolic pathways in cells, glycolysis is anaplerotic (other metabolites, from within the cell or transported from other cells, are converted to glycolytic intermediates) and cataplerotic (glycolytic intermediates are transported out of the cell or converted to other molecules that are transported out of the cell to other tissues of the body).

The reactions of glycolysis are random events in the cytosol of a cell. Most of the enzymes are dispersed in solution and the Brownian motion chaos of cell life combines with simple diffusion to assist substrate binding to enzymes and the rapid catalysis of the reactions. The linear arrangement of the glycolytic reactions in diagrams is simply an educational simplification that documents a means to summarize the eventual conversion of the initial substrates (glycogen or glucose-6-phosphate [G6P]) to final products (pyruvate or La^-). There is no intended assumption that the integral of the initial substrate turnover is converted to solely the final product. Once G6P is converted to fructose-6-phosphate (F6P) it is not committed to La^- production, and by extension, the H^+ exchange that occurs during glycolysis is not directly linked to La^- production. Furthermore, as explained below, there is considerable evidence to show that net H^+ release during intense exercise is far greater than cellular lactate production.

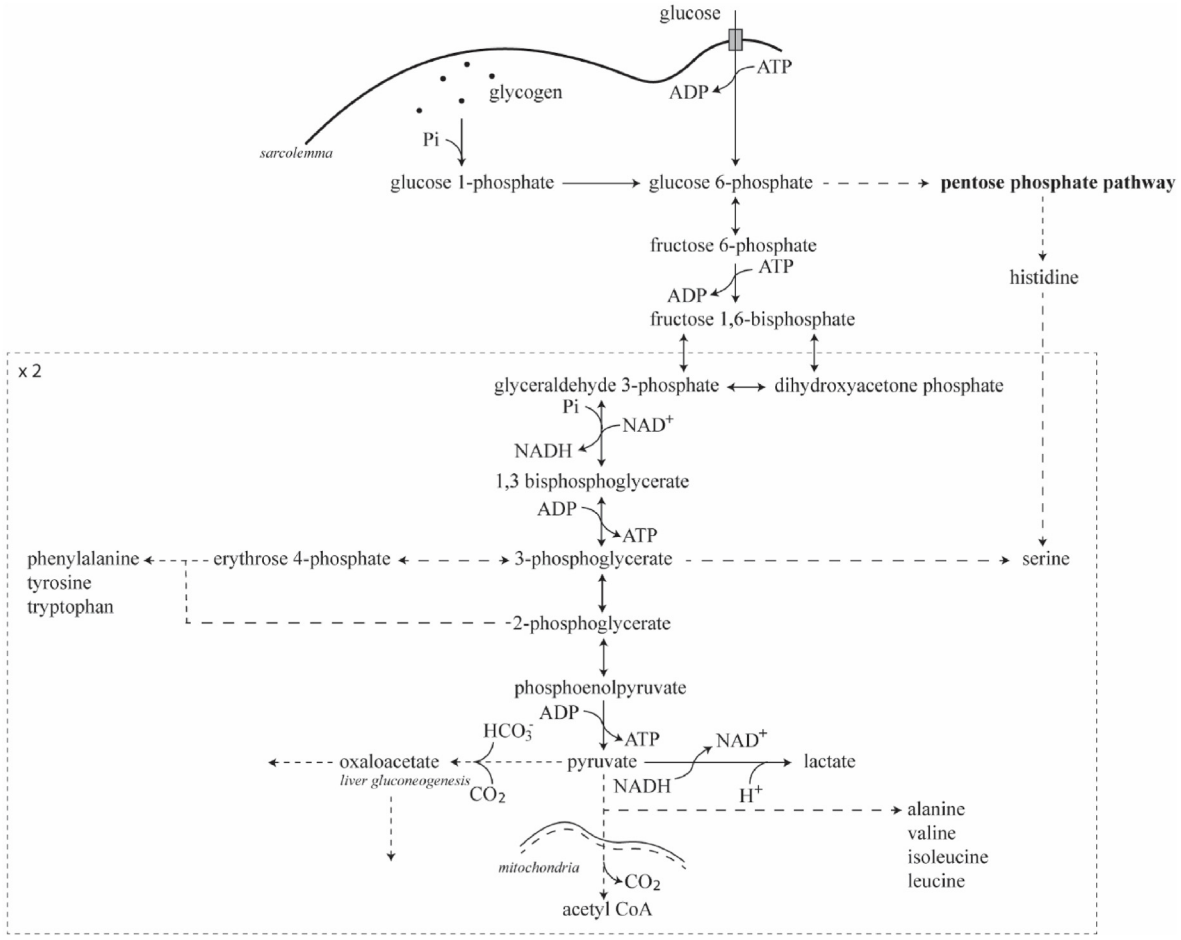

Fig. 3. A simplified depiction of the reactions of glycolysis, showing the inclusion and removal of intermediates and therefore the anaplerotic and cataplerotic nature of glycolysis. Note that tissues differ in their enzymology for supporting these 'side' reactions to glycolysis. For clarity not all substrates and products are presented for all reactions, and that for reactions from glyceraldehyde 3-phosphate through to lactate, acetyl CoA or alternate products, there is a need to double the reactions (× 2) to balance atoms.

6.2. *Evidence opposing assumption 2: lactate production reduces the H^+ exchange of glycolysis*

La^- production directly consumes a H^+, independent of H^+ exchange, and this is shown by the organic chemistry of the chemical reaction (Figs. 1 and 2)[3,20] in addition to the known computed H^+ exchange of the total reaction.[4,21,22] To then argue that you can link lactate production to H^+ release from totally different biochemical events is incorrect. Similarly, it would be incorrect to claim that because net H^+ release increases in skeletal muscle during intense exercise coincident with a decrease in cellular pH and an increase in inorganic phosphate (Pi), the acidosis is caused by the production of Pi or the degradation of glycogen.

The stoichiometry argument of cellular La^- and H^+ release is also conceptually illogical. If cells become acidic and La^- production consumes a H^+, then, by definition, there is H^+ release from somewhere not numerically accounted for by La^- production. Furthermore, if based on an overly simplistic summation of H^+, the H^+ release from other reactions of glycolysis is almost totally accounted for by the H^+ consumption of La^- production. For example, Robergs[4] quantified that the net H^+ release from the conversion of glycogen to pyruvate vs. La^- at pH = 7.0 approximates −2.01 and −0.01, respectively. Furthermore, Juel et al.[23] computed close to a two-fold greater release of H^+ than La^- from contracting skeletal muscle. While other researchers have attempted to quantify cellular H^+ handling,[5,6,18] such work did not yield estimates of net H^+ release to La^- production. Robergs[21,22] modelled non-mitochondrial energy catabolism in contracting skeletal muscle and based on covalent modifications causing H^+ release, combined with pH dependent H^+ association and dissociation, computed a net H^+ release to La^- production ratio of 4.3:1.

Added logic would mandate that as there is close to 1 H^+ consumed for every La^- molecule produced, for acidosis to occur there must be a higher net H^+ release from the combined reactions of ATP hydrolysis and glycolysis than there is La^- production. La^- production further benefits the cell by regenerating cytosolic NAD^+ and assisting H^+ transport from the cell. As such, there must be alternate explanations for cellular acidosis than those linked to the lactic acidosis construct, and such H^+ totals must far exceed the integral of the production of La^-.

6.3. *Evidence opposing assumption 3: multiple H^+ Co-transport Efflux systems in cells*

The role of La^- in H^+ transport out of cells via the mono-carboxylate transport proteins is often explained as proof of the continued relevance of HLa to cellular metabolism. However, research evidence is clear in the presence of numerous co-transport mechanisms in cell membranes, which means there are more H^+ transported than La^-. Robergs et al.[24] provided a summary of this evidence, which was originally detailed in Kaplan.[24]

If there is evidence-based proof that cells do not produce HLa and that there is no stoichiometry to H^+ and La^- release from cells, what are the arguments based on that attempt to oppose the evidence? Scientists who are antagonistic to this evidence must, at best, have misunderstandings

based on incorrect learning of cellular metabolism. As will be explained in Part-3 of this series, this is not the fault of these scientists. Considerable incorrect teaching has occurred across most disciplines throughout the historical development of science. The prior lactate to H^+ ratio argument is a classic example of this. Another is the Lohman reaction, which is not a reaction at all but a combining (false coupling) of ATP hydrolysis and the creatine kinase reaction inside cells that provides an oversimplistic view of phosphate transfer during cellular energy catabolism. In either case, the arguments are scientifically questionable. Once again, Part-3 deals with explanations for why errors occur in science and provides strategies to minimize them.

6.4. Evidence opposing assumption 4: ignoring axioms leads to repeated errors in understanding

You cannot argue for the truth of an observation when the observation and interpretation of it are not based on sound scientific principles. In science, irrefutable principles or facts are referred to as axioms. Scientists can lead themselves into non-evidence-based dilemmas (pseudoscience) if they ignore axioms and interpret concepts that are inconsistent with axioms as the truth. Often this is done unintentionally simply because a scientist may be adhering to a concept that they were taught or was accepted by the mainstream scientists and practitioners of their discipline.

The extended arguments of the lactic acidosis construct presented in the Common View and initial paragraph of the Evidence sections of this question item are examples of such a disconnect between axioms (organic and computational chemistry) and prevailing understandings of cellular acid-base and metabolic biochemistry (over-simplistic associations that are interpreted as cause-and-effect). For example, the organic chemistry of the LDH reaction, the production of all acid labelled metabolites as ionic bases, and the mathematical foundations of the computational chemistry of H^+ exchange are all axioms as they are all based on physics and mathematics. This means that if you want to argue against an axiom, without any empirical evidence, then you must deviate from core principles and practices of science.

Answer: There is no scientific evidence or rationale to support the stoichiometric relationship between glycolytic H^+ release and La^- production, or the interpretation that the monocarboxylate transporter involving the cotransport of $La^- + H^+$ equates to proof of cellular HLa production. Cells release more H^+ than they produce La^-, glycolysis is not a closed pathway, and the $La^- + H^+$ monocarboxylate co-transport protein is not the only means of H^+ transport out of cells. Each of these features are inconsistent with the view of the cellular production of HLa, but consistent with the organic chemistry and computational acid-base chemistry axioms of all chemical reactions in biological systems that reveal that cells do not produce metabolic acids.

7. Question 6: if lactic acid production does not occur, then where do the hydrogen ions (H^+) come from?

Common View: Two common answers for this question are offered. The first answer to the question is that the question is irrelevant because the H^+ come from HLa. The second answer is based on the physico-chemical account of H^+ exchange as explained by Stewart.[25] This physico-chemical approach has assumed that the H^+ exchange during chemical reactions has no influence on the pH of biological solutions. Instead, the alteration of the charge distribution between compartments electrochemically increases the $[H^+]$ in biological fluids to assist in the balance of charge.

Evidence: While there is sound chemical rationale for how alterations of charge in biological solutions can alter pH,[25] this presentation is by no means an evidence-based explanation for large perturbations in the pH of biological solutions, such as incurred during intense exercise or severe diabetic ketosis, or that this is the only explanation for cellular and systemic changes in acid-base balance. Furthermore, this theory provides no account of where the H^+ comes from, or the chemical principles that drive this exchange. See Robergs[21] for added commentary on physico-chemical explanations of acidosis.

Prior evidence presented reveals the clear chemical determinants for changes in the pH of biological solutions through the covalent removal or attachment of H^+. In addition to this, pH dependent $\sim H^+e$ occurs and depending on the pK_d of the metabolite will induce minor H^+ dissociation or association. Interestingly, altered charge of biological solutions, which in turn is reflective of changes in ionic strength, will also alter the pK_d of metabolites, which in turn will alter the extent of pH dependent H^+ dissociation or association.[2,4–6]

The organic chemistry of the reactions supporting energy transfer in contracting skeletal muscle reveal that the source of the H^+ is likely to be a combination of water and the release of H^+ after covalent modification of substrates to products, with hydrolysis reactions being a main contributor as exemplified through ATPase reactions, and the HMG-CoA synthase reaction in liver ketogenesis. As stated previously, evidence for this source of the H^+ of acidosis was originally proposed by Gevers.[26,27]

Interestingly, Green and Bishop[28] provided a concise commentary on this issue for diabetic ketosis and commented that H^+ release occurs from the added reactions that accompany ketosis, with particular emphasis on the reactions of lipolysis and fatty acid β-oxidation. Robergs et al.[29] provided evidence-based proof of the source of H^+ during ketosis from computational chemistry for the $\sim H^+$ exchange of the reactions of ketogenesis and ketolysis. While ketogenesis does release $\sim H^+$, the amount is minor and the source of the H^+ is hydrolysis and not H^+ dissociation. However, there is a need for more research on such accounts of hepatic biochemistry and the expanded influence of added chemical reactions in the liver as well as other tissues of the body that coincide with this condition.

Answer: Regardless of the cause of the H^+ exchange (chemical reactions or changes in ionic charge of biological solutions), the source of the H^+ are water and/or a combination of the covalent release or attachment of H^+, further exacerbated by the pH dependent dissociation or association of ionizable groups within the molecules. Scientists and academic professionals across the medical, clinical, health and exercise and sport sciences are urged to modify their teaching and research inquiry to recognise the evidence-base presented in these articles, and to nurture future research inquiry that can challenge and quantify the concept of the cellular exchange of H^+ devoid of the production of metabolic acids.[30]

8. Conclusions

The historical development of our understanding of acids was presented by Robergs et al.[1] in Part-1 of this series, and in this Part-2 of the series, the scientific evidence-base for the cellular production of the ionized bases of acid molecules was presented along with added evidence that refutes current alternate theories or explanations of acidosis. All scientific evidence reveals that cells produce ionized bases of molecules classified as acids, which means these molecules are incapable for releasing H^+ through pH dependent dissociation. To accept and reinforce an opposing construct, where a few molecules, out of hundreds that are classified as acids and produced by cells, account for all the acidosis of exercise or perturbations of cellular lipid and carbohydrate catabolism is not based on scientific evidence.

The questions that now remain are anticipated to be more about why such misunderstandings have existed for so long. Such questions and answers will be presented in Part-3 of this series.

Submission statement

All authors have read and agree with the manuscript content. In addition, while this manuscript is being reviewed for this journal, the manuscript will not be submitted elsewhere for review and publication.

Authors' contributions

Robert Robergs: Writing – review & editing, Writing – original draft, Resources, Formal analysis, Conceptualization. **Bridgette O'Malley:** Writing – review & editing, Writing – original draft. **Sam Torrens:** Writing – review & editing, Writing – original draft. **Jason Siegler:** Writing – review & editing, Writing – original draft.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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