LETTER TO THE EDITOR

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Lactate, not Lactic Acid, is Produced by Cellular Cytosolic Energy Catabolism

In the November issue of *Physiology*, Sun et al. (12) published a review describing the role of lactate as a multi-tissue autocrine regulatory molecule influencing multiple cellular and systemic physiological functions. Such functions included transmembrane H^+ transport, enzyme regulation, downregulation of multi-tissue lipolysis, anti-inflammation, improved immune tolerance, stimulation of long-term memory, and improved wound healing including ischemic tissue injury, while deleteriously supporting cancer growth and metastasis. We acknowledge the detail and quality of the presentation of the contemporary evidence for the involvement of lactate in the regulation of these processes. Nevertheless, it is unfortunate that the authors referred to cellular lactate production as lactic acid, repeatedly associated cellular lactic acid production as a cause of acidosis, and used the term lactic acid within their title. Such repeated use, totaling 50 occurrences of "lactic acid" throughout the entirety of the manuscript, severely detracts from the scientitic quality of their work.

As we will explain, there is no such entity as lactic acid in any living cell or physiological system. Indeed, it is impossible, based on the fundamental laws of physics that underpin the disciplines of organic chemistry, metabolic biochemistry, acid-base chemistry, and physiology, for lactic acid to be produced or present in living systems where cellular and tissue pH is regulated to be between 6.0 and 7.45. Thus Sun et al. (12) repeatedly errored with every mention of the term "lactic acid" within their manuscript, reinforcing the false knowledge of an outdated construct (non-empirically supported concept assumed to be fact). Such content detracted from the quality content for what their article actually focused on: the roles of lactate in metabolic regulation across multiple tissues and regulatory systems.

We will explain the organic chemistry and metabolic biochemistry of metabolite ionization and cation association/dissociation, the H^+ exchange during glycolysis, and the lactate dehydrogenase reaction to document the reality of cellular lactate production and the separate, although coincident, development of tissue and systemic metabolic acidosis. Such commentary was presented in detail by the lead author in 2004 (9), and, for brevity, pertinent figures and tables from this prior publication will be referred to and not repeated here. It is also worth emphasizing that commentary on the false construct of a lactic acidosis dates back to the 1970s (2, 4, 14, 15), and, as such, this argument is not new, although it is bolstered by decades of advancements in our understanding of organic and computational chemistry understanding since this time.

Importance of the History of Lactic Acid and Lactate

The brief history of lactic acid discovery described by Sun et al. (12) was largely accurate. However, a key omission was the fact that the very early discovery and isolation of lactic acid (1780), followed by subsequent refinements in purification and optical isomer detection (to 1869), both occurred 100–150 years before the understanding of acid-base chemistry and physiology. For example, the work and achievements of Haldane in acid-base chemistry did not occur until the 1940s (11), but the impact of World War II (distractions in scientific achievement and dissemination) delayed the understanding and application of this work to chemistry and physiology into the 1960s, and perhaps with regard to lactic acid, to current time. These time discrepancies and delays functioned to indirectly reinforce an assumed cause-effect connection between tissue lactate production and acidosis. However, a fundamental error in science is to assume correlation is cause-and-effect, and despite more than 70 years since the work of Haldane, the error of a lactic acid-derived metabolic acidosis (lactic acidosis) continues to be reported in the basic and applied biochemical, physiological, and clinical sciences.

Organic Chemistry of ATP Hydrolysis, Ionization, Glycolysis, and Lactate Production

Sun et al. (12) explained cellular lactate production as the conversion of pyruvate to lactic acid (not true), that this was a reaction within glycolysis (not true, although there is debate as to what constitutes the true end of glycolysis, pyruvate or lactate), and that because of the low pK_a of lactic acid (pK = 3.86) (true) [although the NIST (8) reference resource has this as pK = 3.67], there was an immediate and near-complete dissociation of lactic acid to lactate and a proton (H^+) (p. 453) (not true because in living systems there is no lactic acid to begin with).

ATP hydrolysis is a chemical reaction, which in living systems is mostly coupled to another reaction via an enzyme, resulting in a phosphate transfer from ATP to another substrate, forming a product with an added phosphate group. During this process, water is involved in the chemical reaction (hence the term hydrolysis), providing a hydroxyl group for addition to the cleaved phosphate group, and releasing a H^+ (see Ref. 9, Fig. 10, p. R509). Such H^+ release is never purely unitary, since the proportion (hence a fractional numeric representation) of ATP molecules that release H^+ is dependent on the localized pH and additional concentrations of competing cations such as potassium (K^+), sodium (Na^+), calcium (Ca^{2+}), and magnesium (Mg^{2+}). Similarly, for any molecule, when exposed to physiological pH that can release H^+ during ionization or because of being ionized associate with a cation, there will be a fractional (~) H^+ exchange that either releases or consumes ~H^+. For these reasons, we present metabolites in their main cation-associated form and for this reaction (*Eq. 1*) with a variable coefficient (~) associated with the released H^+. Furthermore, such an organic and acid-base chemistry fact reveals that the H^+ activity during cellular metabolism is not a dependent entity, as proposed by Stewart (10), but a process

Table 1. *The coefficients for H^+ exchange for pH 6.0 and 7.0 for select reactions of glycolysis and lactate production*

Reaction*	~H^+ pH = 6.0	~H^+ pH = 7.0
Fructose-6-phosphate + ATP → fructose-1,6-bisphosphate + ADP + ~H^+	−0.0160	−0.7350
Glyceraldehyde-3-phosphate + NAD^+ + ~HPi → 1,3-bisphosphoglycerate + NADH + ~H^+	−1.5798	−0.7603
phosphoenolpyruvate + ADP + ~H^+ → pyruvate + ATP	0.3516	0.8830
pyruvate + NADH + ~H^+ → lactate + NAD^+	1.004	1.0004

*For simplicity, other than H^+ and NAD^+, molecules are presented without charges. Positive values refer to consumption; negative values refer to release. Based on data from Vinnakota (13), Kushmerick (6), and Robergs (unpublished observations).

where chemical reactions directly influence H^+ activity.

$$Mg^{+2}\text{-}ATP^{-2} + H_2O \rightarrow Mg^{2+}\text{-}ADP^{-1} + HPi^{-2} + \sim H^+ \quad (1)$$

We do not have the scope in this rebuttal to detail the computational chemistry of the dissociation of metabolite-cation complexes, although it is sufficient to state here that the compilation of research of such constants across all ionizable molecules and their competing cations is compiled in the NIST (8). The more complex computation required to account for competing cations is the difficult step in this process of quantification and accountability of the origins of the multiple sources of H^+ during metabolic acidosis (6, 9, 13).

For glycolysis, there are nine reactions, commencing with the 6-carbon substrate glucose-6-phosphate (G6P) and ending with two 3-carbon pyruvate molecules (see Ref. 9, Table 2, p. R506). The first carboxylic functional group intermediate of glycolysis is produced in the sixth reaction where 1,3-bisphosphoglycerate is converted to 3-phosphoglycerate (see Ref. 9, Fig. 5, p. R507). This is a phosphate transfer reaction, adding the phosphate to ADP forming ATP, with the co-production of 3-phosphoglycerate having an ionized (unprotonated) carboxylic functional group at carbon-3. This is key to understanding the H^+ load of glycolysis and H^+ metabolic buffering from lactate production. Each glycolytic intermediate following this reaction remains in an ionic form. There is never a glycolytic production of a carboxylic acid since they are all carboxylic ions. This remains true for obvious acid-base reasons from 3-phosphoglycerate to 2-phosphoglycerate to pyruvate and then to lactate. There is no metabolic production of lactic acid or any preceding glycolytic carboxylic ion metabolite, and, as previously explained, lactate production consumes, not releases, ~H^+ (see Table 1; also see Ref. 9, Fig. 9, p. R509).

Table 1 presents the coefficients for ~H^+ exchange for pH 6.0 and 7.0 (based on computations accounting for the multiple competing cations of K^+, Na^+, Ca^{2+}, and Mg^{2+}) summed across all the ionizable intermediates for select reactions of glycolysis and lactate production. Note, as pH decreases, there is increased fractional H^+ addition to HPi for H_2Pi, which accounts for the increasing fractional H^+ release during the production of 1,3-bisphosphoglycerate (Robergs RA, unpublished observations).

From the data of Table 1, it is clear that lactate production consumes a H^+ load that is essentially stoichiometric to lactate production, regardless of pH across the cellular pH range. Conversely, as cellular pH declines, pertinent reactions of glycolysis sum to be more net ~H^+ releasing. Glycolysis is independently ~H^+ releasing, and the ~H^+ consumption of lactate production opposes this, and it is unlikely that perfect matching of H^+ exchange ever occurs, as is commonly represented in summary metabolic equations of glycolysis [−2 H^+ (release)] and lactate production [+2 H^+ (consumption)]. Indeed, as a cell becomes more acididic, there is an increasing ~H^+ release from glycolysis, whereas that for lactate remains essentially unchanged.

Clinical Use and Interpretation of Lactic Acidosis

We understand that the term "lactic acidosis" has been used in clinical research and practice for more than 100 years. With the duration of this use comes considerable engrained misunderstanding and misapplication, and to expect a rapid change from any engrained convention may be unrealistic. However, given that the terminology is wrong based on incorrect understanding of metabolic biochemistry and acid-base chemistry, that clinical practice involves treating illnesses and saving lives from premature mortality, and that correct treatment most often requires a correct understanding of the true mechanisms of disease and symptomology, one would hope that clinical professionals would prefer to base their practice on empirical truths rather than engrained convention.

It has been encouraging to see many physicians altering their view of a lactic acidosis based on revised explanations consisting of expressions of elevated blood lactate (hyperlactatemia) and an associated (or not) systemic acidosis (3, 5, 7). For example, considerable research of hyperlactatemia occurs for the condition of sepsis (3, 5, 7) and also metformin toxicity (1). For sepsis, hyperlactatemia is predictive of disease severity and premature mortality, with more than a threefold increase in mortality when hyperlactatemia is accompanied by tissue hypoperfusion (5). The prior conventional interpretation of sepsis-associated hyperlactatemia accompanied by acidosis is framed on belief in a causal connection between the disease state, altered perfusion causing a localized hypoxia, stimulation of glycolysis, and lactic acid-induced metabolic acidosis. This is false knowledge, since there is no such condition as lactic acid-induced metabolic acidosis. The increased lactate presumably occurs due to increased stimulation of energy catabolism, causing increased substrate flux through glycolysis, which will therefore also increase lactate production and/or compromise blood lactate removal. For many patients, there is no accompanied acidosis (3, 5, 7), which is consistent with the metabolic biochemistry of the combined production of lactate and the retained function of mitochondrial respiration, since a continual H^+ supply is needed as a substrate for each

aspect of energy catabolism. For patients with a systemic acidosis, there could be a localized or systemic inflammatory response that triggers altered mitochondrial function and a metabolic milieu now consistent with metabolic acidosis (3, 7). Such a scenario is more aligned with altered mitochondrial respiration (normally a H^+ sink) accompanied by increased glycolytic stimulation, the consequence of the two conditions causing increased net H^+ release and an eventual acidosis.

Cellular lactate production occurs to facilitate sustained glycolysis by regenerating cytosolic NAD^+, consuming a near stoichiometric H^+ per lactate produced (Table 1), and allowing for both lactate and H^+ efflux from the metabolically active tissue via the monocarboxylate transport proteins (12). ■

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