Point:Counterpoint: Muscle lactate and H\(^+\) production do/do not have a 1:1 association in skeletal muscle

**Point: Muscle lactate and H\(^+\) production do have a 1:1 association in skeletal muscle**

Muscle cells produce lactate and become acidic (9) during anaerobic glycolysis and during intense aerobic glycolysis, giving rise to net lactic acid production. The identity of the mechanisms involved and the size of their contributions to acidosis in muscle during exercise were debated extensively in 2003 (3, 7, 8, 12, 14, 15) and 2008 (2, 11) and now again. One reason for these continued disputes is the lack of quantitative analyses of the proton loads generated by biochemical reaction fluxes and proton transport fluxes that result in the observed cellular pH transients. Here we argue that our kinetic model, based on analysis of the time courses of phosphate metabolites and pH in intact muscle, provides definitive answers. All reactions produce or take up H\(^+\) as described below; only the lactate dehydrogenase reaction produces lactate\(^-\). Thus lactic acid is the result of a network of reactions advancing in an aqueous solution with all substrates and products in rapid equilibrium with free cations within the cell.

Reactions that generate lactate and H\(^+\). Breakdown of glucose or glycogen to lactate anion through the reactions of glycolysis occurs without generating significant H\(^+\) (see Fig. 1 and discussion below). Hence other reaction(s) must generate H\(^+\) in substantial amounts to change intracellular pH in the face of high buffer capacity. Our analysis (17) demonstrates that only cellular ATP hydrolysis (ATPase) flux generates sufficient H\(^+\) to acidify the cytoplasm during anoxia. The H\(^+\) and lactate generated separately remain nearly completely dissociated with <0.1% in the form of undissociated lactic acid because its pKa \(\approx 4\) and cellular pH \(\approx 7\).

Lactate and H\(^+\) ratio from glycolysis and ATP hydrolysis in the steady state. Gevers (5) treated biochemical reactants as multiple cation bound species and calculated that in the steady state the breakdown of glucose alone to synthesize two ATP and two lactate molecules per glucose molecule generated no proton load, but the hydrolysis of the generated ATP molecules produced one proton per ATP at physiological pH and ionic conditions. (Proton load is defined as the net number of free hydrogen ions generated.) Hochachka and Mommsen (6) showed that the net proton load generated by glycolysis coupled to ATPase resulted in the generation of two protons and two lactate molecules per glucose unit independent of the pH. The muscle cell pH, in turn, is determined by proton transport across the sarcolemmam, proton buffering, and the net metabolic proton flux, which is given by the algebraic sum of the products of proton stoichiometry and the fluxes through biochemical reactions. During a steady state the proton stoichiometric coefficients may be added and then multiplied with the steady-state flux to obtain the metabolic proton flux. However, during metabolic transients, the proton stoichiometries of reactions may not be summed as, simply, due to dynamic changes in proton binding to biochemical reactants. To establish a causal relationship between biochemical reaction fluxes and pH transients, the time courses of biochemical reactants and the consequent metabolic proton loads could be computed by applying known enzyme kinetics and physical chemistry of multiple cation equilibria.

Computing pH time courses due to biochemical reaction and transport processes. Vinnakota and colleagues (16, 18) computed pH time courses due to biochemical reaction fluxes by treating biochemical reactants as a sum of cation bound species in a dynamic computational model. The following equations summarize our approach for muscle cell cytoplasm assuming relatively constant free magnesium and potassium ion concentrations:

\[
\frac{d[H^+]}{dt} = J_{\text{transport}} - \sum_k \Delta N_{H^+} k \frac{\Delta N_{H^+}}{[H^+]} - \sum_k C_k \frac{\Delta N_{H^+} k}{[H^+]},
\]

where \(\beta\) is the buffer capacity of the muscle cell cytoplasm.

The rate of change of proton concentration is given by the following differential equation:

\[
\frac{dpH}{dt} = \frac{\text{Proton consumption flux}}{\beta},
\]

Fig. 1. Simulations of proton consumption flux in selected components and reactions during 60 min of anoxia in extensor digitorum longus (EDL) redrawn from Fig. 9 (17). Units on the ordinate are M/min where positive quantities represent H\(^+\) uptake and negative ones H\(^+\) production. Units on the abscissa are elapsed time in minutes during the experiment with anoxia starting from 22 min. Ordinate shows the proton consumption fluxes in the creatine kinase (CK), the ATP hydrolysis reactions (ATPase), proton consumption flux due to the transport of lactate into extracellular space mediated by the monocarboxylate transporter (MCT), the sum of all proton consumption fluxes through the reactions of glycolysis pathway leading to the formation of lactate (Glycogenolysis), and the sum total of all proton consumption fluxes computed in the sarcoplasm (Total). The inset shows a plot of total lactate on the ordinate and the proton load on the abscissa during 60 min of anoxia in the EDL (data from Fig. 10 in Ref. 17). A second set of axes within the plot mark the area where the metabolic proton load is acidifying and a line with slope one is plotted within these axes (solid red) for comparison with the lactate vs. protonload curve.
flux through the \( k \)th biochemical reaction, \( C_i \) is the concentration of the \( i \)th biochemical reactant including histidine related proton buffers, and \( \Delta N_{H}^{+}/\Delta[H^{+}] \) is the partial derivative of the average proton binding \( N_{H}^{+} \) (defined as the fraction of the biochemical reactant bound to protons) of the \( i \)th biochemical reactant. The time courses of biochemical reactant concentrations \( C_i \) are simultaneously computed by solving ordinary differential equations that describe the rate of change of each \( C_i \) as the algebraic sum of fluxes generating and consuming the total biochemical reactant.

Defining \( \text{pH} = -\log_{10}(\text{[H}^{+}\text{])} \), we obtain:

\[
\frac{d\text{pH}}{dt} = \frac{1}{2.303[\text{H}^{+}]} \left( -\frac{d[\text{H}^{+}]}{dt} \right) = \frac{1}{2.303[\text{H}^{+}]} \left( -J_{\text{transport}} + \sum_{i=1}^{N} \Delta N_{H}^{+} \phi_{i} \right)
\]  

(3)

The buffer capacity due to both histidine related proton buffers and biochemical reactants is given by:

\[
\beta = \frac{1}{2.303[\text{H}^{+}]} \left( 1 + \sum_{i} C_i \frac{\partial N_{H}^{+}}{\partial [\text{H}^{+}]} \right).
\]  

(4)

Buffering by the \( \text{CO}_2/\text{HCO}_3^- \) may be defined as an additional proton flux while accounting for the \( \text{CO}_2 \) generation and hydration and the transport of \( \text{CO}_2 \) and \( \text{HCO}_3^- \) across various physiological spaces. See Vinnakota and colleagues (16, 17) for a detailed derivation of Eq. 2 and Alberty (1) for a broad overview on multiple cation equilibria in biochemical thermodynamics.

**Lactate and \( H^+ \) ratio from glycolysis and ATP hydrolysis during transient anoxia.** We applied the approach summarized in Eqs. 1–4 to analyze the time courses of phosphocreatine, inorganic phosphate, and \( \text{pH} \) measured using NMR in superfused mouse extensor digitorum longus (EDL) and soleus muscles and of total lactate in the buffer outflow following transient resting anoxic perturbation of various durations (17). A detailed picture of proton consumption fluxes from our analysis in mouse EDL during anoxia in Fig. 1 (redrawn from Fig. 9 in Ref. 17) shows that 1) the net \( H^+ \) production of the 13 reactions in the glycolytenolysis and glycolytic pathway sums to zero; 2) ATPase is the major source of \( H^+ \) production and; 3) the creatine kinase reaction is an important source of \( H^+ \) uptake as PCR concentration declines during a transient metabolic stress due to anoxia. This is a key result of the analysis and simulations relevant to the question of the source(s) of proton production and uptake, which confirms the essential validity of simpler analyses (5, 6, 10). During the early phase of anoxia (~15 min) the large magnitude of proton consumption flux through creatine kinase relative to other proton fluxes results in a net alkalization. The inset in Fig. 1 (redrawn from Fig. 10 in Ref. 17) further shows that the lactate generation computed by integrating the flux through the lactate dehydrogenase reaction and proton load computed by integrating the total metabolic proton flux \( \int_{0}^{t} \sum_{i=1}^{N} -\Delta N_{H}^{+} \phi_{i} \, dt \), have a ratio of 1.06 during anoxia after the first 15 min. In addition, recent work demonstrated a 1:1 relationship (within experimental error) between proton load calculated from PCR, Pi, and \( \text{pH} \) changes measured using \( ^{31}\text{P}-\text{NMR} \) and chemical measurements of lactate during ischemia in mouse muscle in vivo (13). In summary, evidence from experimental data and theoretical studies shows that lactate and protons generated from different biochemical reactions bear a near 1:1 relationship during anaerobic glycolysis coupled to ATP hydrolysis. This 1:1 relationship between lactate generation and metabolic \( H^+ \) generation may be disrupted when ATP hydrolysis flux is uncoupled from glycolysis.

**What remains to be learned?** During intense muscle contractions, the transition from the initial alkalinizing state to the acidic state can be expected to be much faster with a 1:1 relationship between lactate and proton generation during the acidic state, but model analysis of experiments of this type have not been published yet. Additionally, the contribution of mitochondrial oxidative phosphorylation, mitochondrial transport of metabolites and transfer of reducing equivalents, glycolysis and ATPase, and sarclemmal proton transport to the observed \( \text{pH} \) transients during aerobic muscle contractions have not been worked out. The analysis of oxidative glycolysis and the resulting \( \text{pH} \) transients during aerobic muscle contractions requires a quantitative model of the dynamics of glycolysis coupled to oxidative phosphorylation. Such models could be constructed by integrating existing validated models of glycolysis (17), mitochondrial oxidative phosphorylation and the Krebs cycle (19), and sarclemmal proton transport mechanisms (4) with a suitable model of muscle perfusion.

REFERENCES

COUNTERPOINT: MUSCLE LACTATE AND H⁺ PRODUCTION DO NOT HAVE A 1:1 ASSOCIATION IN SKELETAL MUSCLE

When applying rationalism and empiricism to the topic of lactate production and metabolic acidosis, it is important to once again acknowledge and understand the organic chemistry of the reactions of muscle nonmitochondrial energy catabolism that involve ATP hydrolysis, consumption or release of inorganic phosphate (HPi⁻), or hydrogen ion (proton; H⁺) exchange, as shown below.

Creatine kinase (CK): CrP + ADP⁻² + αH⁺ ↔ Cr + ATP⁻³

AMP Deaminase (AMPDase): AMP + αH⁺ ↔ IMP + NH₄⁺

ATPase: ATP⁻³ + H₂O ↔ ADP⁻² + HPi⁻² + αH⁺

Phosphorylase (Phos): glycogen(n) + HPi⁻² ↔ glycogen(n-1) + G₁P

Hexokinase (HK): glucose + ATP⁻³ ↔ G₃P + ADP⁻² + αH⁺

Phosphofructokinase (PFK): F₆P + ATP⁻³ ↔ F₁,₆P + ADP⁻² + αH⁺

Glyceraldehyde-3-phosphate dehydrogenase (G₃PDH): G₃P + HPi⁻² + NAD⁺ ↔ 1,3BPG + NADH + αH⁺

Pyruvate kinase (PK): PEP + ADP⁻² + αH⁺ ↔ Pyr + ATP⁻³

Lactate dehydrogenase (LDH): Pyr + NADH + αH⁺ ↔ La⁻ + NAD⁺

(Note that for simplicity the prefix α is used for the H⁺ coefficient of each reaction, although α is different for each reaction and is also pH dependent. Pertinent charged molecules are presented in their predominant form at pH = 7.0.)

All of these reactions occur with structural independence of each other, although the sequential reactions of glycolysis do influence each other based on substrate and product concentration determinants to enzyme kinetics and bioenergetics. Nevertheless, the relative contributions of each reaction to cellular ATP turnover is as yet impossible to measure directly with acceptable precision and temporal resolution, although we know that there is an increasing glycolytic preponderance and a decreasing phosphagen system contribution as intense exercise duration increases beyond ~15 s (1, 6, 12–14). With regard to the LDH reaction, the H⁺ stoichiometry varies little across the cellular pH range: being +0.9938 and +0.9987 (positive = H⁺ consumption) for pH conditions from 7.0 to 6.0, respectively (3, 15).

It is important to clarify the difference between the absolute (gross) H⁺ release of muscle contraction as opposed to net H⁺ release. Gross H⁺ release must be the sum of the reactions that release H⁺, which includes the ATPase, HK, PFK, and G₃PDH reactions, modified by strong ion electrochemical forces. This H⁺ release is metabolically buffered by reactions that consume H⁺, which as previously described are the CK, AMPDase, PK, and LDH reactions. Gross H⁺ release minus the H⁺ consumption from chemical reactions (metabolic buffering) represents net H⁺ release that requires structural (e.g., amino acids) and bicarbonate buffering (5). Based on Eq. 11 from Marcinek et al. (5) (δH⁺ = δpHｃₜot + γ·δpCr, p. 1481), it appears they were computing the gross H⁺ release.

The details of the organic chemistry of non-mitochondrial energy catabolism provide tremendous insight to understanding gross H⁺ release. For example, a stoichiometric approach to understanding H⁺ balance during catabolism would require summation of the pH-dependent and reaction-specific H⁺ coefficients of each of the ATPase, HK, PFK, and G₃PDH reactions. As it is difficult to accurately quantify the kinetics of these reactions in vivo, the fact that acidosis does develop during intense exercise means that gross H⁺ release must exceed the sum of H⁺ metabolic buffering and the tissue H⁺ buffer capacity. It is therefore logical to assess H⁺ release based on estimates of H⁺ metabolic buffering. Given that the LDH reaction shows close to a 1.0 H⁺ metabolic buffering per lactate produced, we must commence an accounting of a metabolic H⁺ buffering with 1.0 H⁺ per lactate. Of course, the CK and PK reactions are also H⁺ consuming. There is wide variation in the tissue buffer capacity of skeletal muscle, with typical values approximating 90 mmol·kg⁻¹·pH⁻¹ (Slykes) (8, 11). For routine intense exercise to volitional fatigue in 2 to 3 min, muscle pH can decrease to ~6.4, which represents 54 mmol/kg H⁺. When the added pH-dependent H⁺ metabolic buffering from the LDH, CK, and PK reactions are added to tissue buffering, it would be reasonable to hypothesize an H⁺ load of intense muscle contraction to approximate in excess of 100 mmol/kg; a value close to 3 H⁺:La⁻. Surely such a rationale and empirical foundation should occur for the development of research hypotheses for scientific research within acid-base physiology/biochemistry.

Criticisms of the Marcinek et al. Manuscript

The manuscript by Marcinek et al. (5) adds confusion and errors of science to the topic of the biochemistry of exercise-induced metabolic acidosis. The following content is an at-