Comments on Point:Counterpoint: Muscle lactate and H⁺ production do/do not have a 1:1 association in skeletal muscle

CALCULATIONS OF ROBERGS SUPPORT THE VIEW OF VINNAKOTA AND KUSHMERICK

TO THE EDITOR: Vinnakota and Kushmerick (2) highlight experimental evidence and computer simulations showing that net H⁺ generation and lactate production occur in an ~1:1 stoichiometric ratio during anaerobic glycolysis coupled to ATP hydrolysis in muscle. Robergs (1) asserts that the stoichiometry is closer to 3 H⁺ to 1 lactate. Frankly, while I am able to follow the simple clear logic of Vinnakota and Kushmerick, I don’t fare so well with Robergs’.

Specifically, Robergs reports a value of 54 mmol of H⁺/kg of muscle generated during a particular exercise protocol. He then transforms the number 54 to 100 by accounting for “the added pH-dependent H⁺ metabolic buffering from LDH, CK, and PK reactions.” My interpretation of this calculation is that Robergs is (loosely) estimating the proton load that would occur without those reactions present. Yet those reactions and their associated reactants do occur, both in real muscle and in the calculations of Vinnakota and Kushmerick. Admittedly, here I may be invoking a straw man; but I am at a loss to invent an alternative explanation for the mysterious calculation. In any event, if my reverse-engineered explanation of Robergs’ claim is appropriate, then his estimate for what he defines as the net H⁺ generated per lactate is 1.5, a value that (given the gross uncertainty in the calculation) it is perhaps not meaningfully different from 1.

Additional points made by Robergs do not speak to the debate at hand (a discussion on semantics) or are too inflammatory (“errors of science”) to have a place in the scientific debate.

REFERENCES

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TO THE EDITOR: Biochemical reactions within living muscle cells do not occur in isolation of water, nor in isolation of all other chemical and physical reactions that contribute to changes in [H⁺] during the progress of any one reaction or series of reactions. Importantly, biochemical reactions do not consume or produce protons—the protons are already there and what changes is the association of protons with H₂O and other proton-binding molecules (1, 2). Thus many factors simultaneously determine the [H⁺], or correctly [H₃O⁺], in physiologic solutions such as sarcoplasm (3). The biochemical approaches presented in the arguments (4, 5) have measured pH and attempted to count protons “generated,” “consumed,” “buffered,” or “released” in relation to lactate accumulation. Biochemical accounting of protons under any discrete set of conditions at select points in time may provide charge balance but only incompletely describes a more complex physiochemical series of hundreds of simultaneously occurring reactions that instantaneously affect [H⁺] (3). Such non-mechanistic descriptions of changes in selected variables fails to consider the importance of water in physiochemical reactions within the cells. The physical behavior of molecules in aqueous solutions depends on physiochemical interactions with water, and one must consider the associations of reaction substrates and products with water within the constraints of physical and chemical laws (maintenance of electroneutrality, conservation of mass). Therefore, when one takes a truly integrative approach to consider the physical chemistry of the intracellular environment of muscle cells, it seems moot to describe a stoichiometry between lactate and proton “production.”

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NO EVIDENCE FOR THE COUNTERPOINT POSITION

TO THE EDITOR: While the contribution of Vinnakota and Kushmerick (6) is logical and clear, Robergs (3) presents an interpretation of lactate and H⁺ production by anaerobic metabolism (4) partly already criticized in a former Point:Counterpoint discussion (1).

Both Point and Counterpoint papers state that glycolysis per se finally produces no H⁺, but only the lactate ion Lac⁻. H⁺ is however liberated by the concomitant ATP splitting. But while H⁺ is consumed during ATP resynthesis in the pyruvate kinase reaction, this does not occur during the phosphoglycerate kinase reaction: 1,3-biphosphoglycerate³⁻ + ADP⁻ + 3-phosphoglycerate²⁻ + ATP⁺. The unconsumed H⁺ (one per 1 Lac⁻) causes acidosis. Both ions coexist and may leave the muscle fiber across monocarboxylate transporters; because of the low pK value only few combine to undissociated lactic acid.

Robergs, however, speculates about production of 3 H⁺ per Lac⁻. His main argument is that the physiochemical muscle buffer capacity amounts to 90 slykes according to Sahlin (5). But Sahlin has calculated only 38 slykes, applying this value
causes ~2 of the mysterious H⁺ to disappear. Surprisingly, Robergs continues to use a too high value as in his former review (4) despite a letter communicating this (2).

If buffering is a reversible binding of protons, Robergs’ use of the term “metabolic buffering” for irreversible reactions where H⁺ are transiently liberated and immediately tightly bound to other compounds is very misleading. Shall we in the future also rename oxygen as a buffer because it combines with H⁺ in the mitochondria?

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WHY ADD COMPLEXITY/CONFUSION TO A SIMPLE ISSUE?

TO THE EDITOR: Muscle lactate and H⁺ production do have a 1:1 association. From an organic chemical view, there is a simple/1:1 association. But which reactions are we discussing in this Point:Counterpoint (3)? Are we discussing complete glycolysis, anaerobic glycolysis, or both? Do we include pyruvate dehydrogenase, lactate dehydrogenase (LDH), and other glycolytic and non-glycolytic reactions?

Confusion-1: glycolytic ATP production has been included in the analysis (1, 3). Glycolytic ATP production cannot occur without prior ATP hydrolysis. The net change of muscle ATP content is, despite high ATP turnover, negligible and has therefore no effect on cellular acid-base balance (see Fig. 1).

Confusion-2: glycolytic reactions have been analyzed separately from each other. Although most glycolytic reactions are associated with production or consumption of H⁺ they are connected in a metabolic pathway without major net changes in glycolytic intermediates (2). Robergs (3) concludes that the LDH reaction can buffer H⁺. By examining the LDH reaction, isolated from the remaining glycolytic reactions, one could falsely come to this conclusion. However, oxidation of NADH in the LDH reaction equals glycolytic NADH production by glyceraldehyde-phosphate dehydrogenase (GAPDH). The conversion of pyruvate to lactate has therefore no influence on cellular acid-base balance (see Fig. 2).

Confusion-3: non-glycolytic processes/reactions have been included in the analysis (3). It is correct that the reactions catalyzed by AMP-deaminase and creatine kinase have implications for cellular acid-base balance. However, they are not linked to glycolysis and should not be included in this Point: Counterpoint discussion.

Cellular acid-base balance is complex, but I am afraid that the published Point:Counterpoints (3) have added more confusion.

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CONFUSION CONCERNING THE LACTATE PROTON RATIO: A PROBLEM OF DEFINITION?

TO THE EDITOR: In biochemistry, products can be defined as “compounds that are formed when a reaction goes to completion.” But which reactions are we discussing in this Point: Counterpoint? While stating that the lactate- proton ratio from anaerobic glycolysis is close to 1:1 (5), it appears that Vinnakota and Kushmerick are actually referring to the lactate-proton ratio from ATP hydrolysis (the predominate source of H⁺ in the conditions being discussed) coupled with anaerobic glycolysis. In this context, their 1:1 ratio is consistent with estimations that can be derived from skeletal muscle biopsies obtained before and after intense exercise. Using one of our studies as an example (2), lactate production (70 mmol/kg dry wt) can be shown to approximate the proton load calculated from the in vitro muscle buffer capacity (~45 mmol/kg dry wt; (1)) and PCr hydrolysis (~15 mmol/kg dry wt; (3)); the small difference can probably be attributed to additional H⁺ buffering by intracellular bicarbonate and the sodium-hydrogen exchanger (4). Robergs’ opposition to this value seems to stem from his preference to calculate the lactate proton ratio as the ratio of protons released via ATP hydrolysis compared to the lactate produced via anaerobic glycolysis. While Robergs’

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\frac{1}{2} \text{Glucose} \xrightarrow{\alpha \text{H}^+ + \text{Pi} + \text{ADP} \rightarrow \text{ATP}} \text{Lactate} + \text{H}^+ \xrightarrow{\text{ATP} \text{ase}} \text{LDH} \xrightarrow{\text{H}^+ + \text{NADH} \rightarrow \text{NAD}^+} \text{GAPDH}
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Fig. 1.

Fig. 2.
approach rightly emphasizes that ATP hydrolysis is the predominant source of the proton load, it is counterintuitive and distorts the total proton load when both reactions go to completion.

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TO THE EDITOR: Vinnakota and Kushmerick (3) report measurements and computational prediction of a near 1:1 free proton-to-lactate ratio in skeletal muscle under resting anoxic conditions. Robergs (2) disputes these results and states that the ratio is closer to 3:1. Neither protagonist gives an explanation as to why one would expect there to be a stable ratio over a range of physiological conditions.

Lactate is a product of (anaerobic) glycolysis. By contrast, protons are produced and consumed in a variety of reactions, and bind promiscuously. While there is no “structural” dependence between these reactions, they interact through common species, including protons. These interactions are described in the computational model used to infer the predominant source of protons and the 1:1 ratio.

One might then interpret this dispute as a challenge to the use of modeling for interpretation of data. The claim that their model provides “definitive answers” (3) is certainly an overstatement—the old cliche holds, that a model is only as good as the data (here the reaction species, stoichiometries, and kinetic parameters) used to make it. But the approach appears sound, including, as it should, binding constants for different phosphate moieties, cation-bound states, and so forth (1).

But why should one expect there to be a fixed relationship between free proton accumulation and lactate production? For example, protons are produced in ATP hydrolysis, which varies significantly with workload. A challenge to both authors, then, is to predict, compute, and/or measure whether and how this ratio changes with, say, exercise intensity.

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TO THE EDITOR: As Dr. Brooks described (1), a recent paper of Marcinek et al. (2) is interesting. If the 1:1 ratio of lactate and H+ concentration change is relevant to human high-intensity exercise during which significant changes in lactate and H+ concentration in recruited-skeletal muscle take place, discussions regarding relationship between lactate, H+ to fatigue shall be enhanced because estimating pH
value from biopsy sampled is quite intriguing. However, several limitations should be applicable. First of all, substantial influences of circulation on concentration of metabolites in skeletal muscle should be considered. As we reported (3), difference in lactate concentration in skeletal muscle after 2–3 min of high-intensity exhaustive exercise from resting value is ~30 mmol/kg wet muscle, while muscle glycogen degradation is ~25 mmol glucosyl units/kg wet muscle (6), suggesting that two-fifths of produced lactate is removed from skeletal muscle by circulation during the exercise. Therefore, 1:1 ratio of lactate and H+ observed in the study (2) is only relevant to anoxic condition without blood stream. Furthermore, since during such exercise, aerobic metabolism dominantly releases ATP (~60–70% of total ATP supply), an important simulation hypothesis of Marcinek et al. (2) that oxidation process does not work is not applicable to the high-intensity exhaustive exercise. Oxidation state may affect glycolysis by changing NADH and/or ATP concentrations, which may affect enzyme activity of rate limiting enzyme (presumably PFK) of glycolysis. Future research using NMR with conventional biochemical analyses should be conducted for the purpose of elucidating fatigue during high-intensity exercise.

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