Is lactate production related to muscular fatigue? A pedagogical proposition using empirical facts

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Macedo DV, Lazarim FL, Silva FOC, Tessuti LS, Hohl R. Is lactate production related to muscular fatigue? A pedagogical proposition using empirical facts. Adv Physiol Educ 33: 302–307, 2009; doi:10.1152/advan.00039.2009.—The cause-effect relationship between lactic acid, acidosis, and muscle fatigue has been established in the literature. However, current experiments contradict this premise. Here, we describe an experiment developed by first-year university students planned to answer the following questions: 1) Which metabolic pathways of energy metabolism are responsible for meeting the high ATP demand during high-intensity intermittent exercise? 2) Which metabolic pathways are active during the pause, and how do they influence phosphocreatine synthesis? and 3) Is lactate production related to muscular fatigue? Along with these questions, students received a list of materials available for the experiment. In the classroom, they proposed two protocols of eight 30-m sprints at maximum speed, one protocol with pauses of 120 s and the other protocol with pauses of 20 s between sprints. Their performances were analyzed through the velocity registered by photocells. Blood lactate was analyzed before the first sprint and after the eighth sprint. Blood uric acid was analyzed before exercise and 15 and 60 min after exercises. When discussing the data, students concluded that phosphocreatine restoration is time dependent, and this fact influenced the steady level of performance in the protocol with pauses of 120 s compared with the performance decrease noted in the protocol with pauses of 20 s. As the blood lactate levels showed similar absolute increases after both exercises, the students concluded that lactate production is not related to the performance decrement. This activity allows students to integrate the understanding of muscular energy pathways and to reconsider a controversial concept with facts that challenge the universality of the hypothesis relating lactate production to muscular fatigue.

lactate; fatigue; acidosis; physical exercise; teaching

UNTIL THE 1990s, the hypothesis that skeletal muscles produced lactic acid during high-intensity exercise was quite widely accepted. That is, there was a common assumption that the dissociation of lactic acid into lactate and a proton (H⁺) is responsible for muscular acidosis during high-intensity exercise, causing muscular fatigue and performance decrement (14).

In two articles published in 2000 and 2004, Robergs (13) and Robergs et al. (14) demonstrated that anaerobic glycolysis produces lactate, but not lactic acid, during intense exercise. This is due to the formation of 3-phosphoglycerate from the reaction catalyzed by the enzyme phosphoglycerate kinase (PGK). The PGK reaction involves a simple phosphate transfer from the first carbon of 1,3-bisphosphoglycerate to ADP, forming ATP. An oxygen atom and an electron remain on the carboxyl group. This, according to Robergs’ proposal (13, 14), the ATP hydrolysis needed for muscular contraction is the main source of H⁺ and, therefore, largely responsible for intramuscular acidosis during intense exercise. It is important to point out that there has been considerable technical and conceptual debate about these issues (4, 10, 11), which is beyond the scope of the present article.

Intense exercise requires high levels of ATP, due to the contraction of fast glycolytic fiber types IIa and IIx, which are characterized by low mitochondrial oxidative capacities. Historically, researchers have assumed that the inverse relationship between the blood lactate increase, pH decrease, and/or performance reduction is a cause and effect phenomenon (14). Although correlations do not necessarily imply cause and effect relationships, the explanation that lactate formation causes acidosis (2, 15, 18) and muscular fatigue (9, 8) is present in the periodic literature and in biochemistry, physiology, and exercise physiology textbooks. Therefore, teachers have the difficult task of reconsidering the lactate/acidosis paradigm, which is also supported by a historical scientific theory.

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et al. (1) published an experiment comparing different protocols of high-intensity intermittent exercises with different pause durations (30, 60, and 120 s) between efforts for a total of 15 sprints (running at maximum speed). The authors generated evidence that, as the 15 sprints progressed, a significant decrease in velocity was observed in the protocol with pauses of 30 s. In contrast, maintenance of starting velocity was observed in the protocol with pauses of 120 s. However, both protocols presented similar increases in blood lactate concentrations. The authors (1) proposed another theory that disregarded the production of lactate as an explanation for the fatigue.

Our pedagogical proposal involves the suitable reproduction of the experiments of Balsom et al. (1) by students of biochemistry of the exercise discipline. The objective was to demonstrate that the cause-effect relationship between lactate production and muscular fatigue is a construct using a dynamic and integrated experimental process as a pedagogical tool. We also present in this work the application of this pedagogical methodology in the classroom. According to our proposal, the students are the main agents in the production of data. Students can use their results to propose their own theory to explain fatigue in high-intensity intermittent exercises through the integration of muscular metabolic pathways, allowing them to challenge the cause-effect relationship between lactate production, acidosis, and fatigue.

**EXPERIMENTAL ACTIVITY: THE PEDAGOGICAL PROPOSAL**

**Context**

This experimental activity was carried out by first-year physical education students as the conclusion to a seminar in the Basic Biochemistry discipline. This activity was applied after the students learned about all the metabolic pathways that produce ATP and lactate shuttle transporters (e.g., monocarboxylate transporters) (5). We note that anaerobic glycolysis and glycolgenolysis were taught according to the stoichiometry presented by Robergs and colleagues (13, 14). The experimental pedagogical activity was approved by the Human Experimentation Ethics Committee of the State University of Campinas.

**Objective**

The experiment was designed to confirm or reject a hypothesis based on the classic theory that establishes a causal relationship between lactate production, muscular acidosis, and fatigue, namely that “Lactate production is responsible for muscular fatigue and performance decrement during high-intensity intermittent exercise.” In the case of rejection through empirical results, students were directed to elaborate another explanation for the fatigue and describe their explanation in the classroom, integrating the content explored in their discipline.

**Materials**

Students were instructed to propose an experiment to answer the following questions:

1. Which metabolic pathways of energy production are responsible for meeting the high ATP demand during high-intensity intermittent exercise?

2. Which major metabolic pathways are active during the pauses (at rest), and how do they influence phosphocreatine (PCr) synthesis?

3. Is lactate production related to muscular fatigue?

These questions were also used as a theoretical guide for teachers and students to assemble the experimental protocol together. Along with the questions, students had access to an array of materials available for the experiment: six pairs of photocells hardwired to a notebook computer for recording running time and velocity, a portable lactate meter (Accusport) and reactive lactate strips (Roche), 100-m measuring tape with minimum divisions of 1 mm, a regulation-size track with an extension of 400 m, a Reflotron analyzer and reactive strips for blood uric acid (Roche), and materials for blood analysis [capillaries (30 μl) with heparin, cotton, alcohol, rubber gloves, blood lancets (Roche), and waste containers for blood and sharp materials].

**Orientation in the Assembly of the Experiment**

After stating the questions and relating the materials available, the teacher has to guide the students in designing the experiment. However, for full understanding, students must feel responsible for the elaboration of the experiment. Thus, the teacher must not give the experimental protocol but instead lead a debate using biochemical principles based on the questions and materials. There is no need for students to undertake additional reading of empirical papers to answer the questions. In this way, the students use knowledge acquired in the classroom, fostering the perception of autonomy.

**Answering the Questions**

**Question 1:** which metabolic pathways of energy production are responsible for meeting the high demand for ATP during high intensity exercise? The number of chemical reactions needed for ATP production can be used to guide the students to answer this question.

ATP production through the transfer of phosphate from PCr to ADP is a one-step reaction. Moreover, PCr is a high energy compound [standard free energy change ($\Delta G^{\circ}$) = −43 kJ/mol] available for immediate use for ATP synthesis ($\Delta G^{\circ}$ = +31 kJ/mol) inside muscle cells. The high levels of ATP hydrolysis by myosin heavy chains during high-intensity exercise increase the availability of ADP in the cytosol, favoring the formation of ATP through the reaction catalyzed by creatine kinase (CK). However, as the supply of PCr is limited, the efficiency of muscle contraction is only maintained over a short period of intense activity. After this first debate, the students realize that the available materials do not allow them the possibility of measuring the consumption of PCr directly, an issue that will be addressed in questions 2 and 3.

For increasing exercise intensities, there is also an increase in the second reaction of the phosphagen system: the adenylate kinase (AK) reaction. In the presence of AK, two molecules of ADP are converted to one molecule of ATP and one molecule of AMP. The reaction can take place in both directions, but the formation of ATP is favored if there is an excess of free ADP and the AMP is removed, a situation that occurs during intense activity. AMP is removed through the purine degradation pathway, increasing the formation of inosine. Inosine is metabolized by xanthine oxidase forming uric acid, a unique end product.
product of purine degradation in humans. From the list of materials, it is possible to suggest the indirect measurement of the AK reaction through the quantification of uric acid.

Students need to make a comparative analysis between concentrations before and after the exercise, since a basal concentration of uric acid is present in the blood. It would be suitable to perform a kinetic analysis to estimate the peak of uric acid in blood after its production inside the muscle and transport into the bloodstream. To solve this problem without further experiments, we suggest blood analysis 15 and 60 min after the completion of the exercise (1).

Anaerobic glycolysis is composed of 11 reactions, producing 2 ATP molecules (glycogenolysis produces 3 ATP molecules) and 2 lactate molecules. Students are prompted to remember the allosteric compounds involved in glycolysis. Due to the high demand for ATP required for the muscular contraction of type II muscular fibers in intense exercises, there is an increased production of ADP and P_i (by myosin ATP hydrolysis) and AMP (by AK). AMP, ADP, and P_i are positive allosteric regulators of glycogen phosphorylase and PFK. In this situation, the electron transport chain (ETC) is fully reduced, due to the high availability of NADH and FADH_2, largely produced by the tricarboxylic acid cycle. The reduction of ETC complexes causes a rise in the NADH concentration in the cytosol, favoring the reduction of pyruvate to lactate through the oxidation of NADH to NAD^+.

The students also recall that lactate produced in this situation will be removed from muscle cells and enter the blood by symport with H^+ through monocarboxylate transporters (MCTs). Checking the list of materials once more, students note that they can measure blood lactate concentrations before and after intense exercise.

**Question 2:** which major metabolic pathways are active during pauses (at rest), and how do they influence PCR synthesis? During pauses, the intracellular ATP availability increases due to oxidative synthesis in the mitochondria. The reduced coenzymes NADH and FADH_2 produced during exercise are reoxidized through the mitochondrial ETC, which reduces O_2 to H_2O. The increase in ATP levels during the pause inverts the reaction catalyzed by CK into the direction of PCR synthesis, storing PCR in muscle cells for the next muscular contraction. Teachers can use the simple proportion of time if pauses between efforts are in the linear range of PCR synthesis [until ~120 s (7)]. If PCR synthesized is proportional to the rest time, then subsequent muscular action would be equally efficient if the PCR stores are full; the inverse is also possible. The students concluded that performance may be an indirect way to analyze if more or less PCR is restored in accordance with the length of pauses.

**Question 3:** is lactate production related to muscular fatigue? The amount of PCR restored can influence the concentrations of ADP and, consequently, AMP in the subsequent exercises. Thus, PCR stores can indirectly modulate the AK reaction and glycolytic pathway and lactate and uric acid production during exercise. The students discussed that, by modulating the pause duration, it is possible to restore more or less PCR and, in theory, to increase or diminish lactate production, which could be related to fatigue. To investigate this hypothesis, they proposed to analyze the correlation of the lactate production to its appearance in the blood with performance, as measured by photocells.

**Experimental Protocol**

After the discussion guided by the three questions listed above, teachers and students can propose two tests with the same intensity (maximum voluntary effort) and an equal number of sprints, differing only in the length of the pauses between the sprints. The tests must be long enough to promote high lactate production.

We carried out this procedure in 10 different groups of students. The dimensioning of the experiment can be variable with similar results. We suggest 6–10 sprints (at maximum voluntary intensity) of 30–40 m, with volunteers standing still before each sprint. The pause between each sprint can vary between 20 and 30 s for shorter pauses and between 120 and 150 s for longer pauses. Pauses above 120 s promote an almost complete recovery of PCR (7). The students must be instructed to run the distance as fast as they can. The pauses between sprints are passive (volunteers must remain motionless and on their feet until the next sprint). The start direction changes in each sprint, with the final position of one sprint becoming the beginning of the next. Sprint times were measured by six photocells hardwired to a computer. The software (Labex, Velocity) registers the time and velocity with a precision of 0.001 s. It is also possible to carry out the experiment with chronometers. A warmup of ~15 min consisting of low-intensity running and stretching must precede the tests. After the experimental design proposed by the students, the volunteers provided written informed consent.

**Blood Lactate and Uric Acid Measurements**

Students used the Accusport portable analyzer (Roche) for the measurement of blood lactate concentration and the Reflotron analyzer (Roche) for the analysis of blood uric acid levels. Blood samples were collected for lactate analysis from the tip of the finger with heparinized capillaries before and immediately after the end of the experiment. Uric acid was analyzed using 25-μl blood samples before and 15 and 60 min after the conclusion of each test.

It is important that the students have been previously trained in safe procedures for the collection of blood and blood analysis, such as the use of gloves, proper waste disposal, and local asepsis before and after sampling.

**General Procedures**

We suggest at least 16 working hours for the completion of the task, including a period of 4 h before the experiments for theoretical discussion, forming groups, training in blood sampling, and familiarization with the equipments. Students can be divided into groups of 10 subjects: 1 operator of the software hardwired to the photocells or 2 timers (one for the pause and another for sprints), 1 operator for the lactate meter, 1 student responsible for uric acid analysis, 1 student for blood sampling, 1 student recording data, and 4–6 volunteers for intermittent exercise tests. The two tests must be carried on different days, with the same volunteers, in two 4-h periods, with the latter at least 72 h after the first test. Other duties can be switched. Finally, a period of 4 h for data analysis, discussion of the results, and oral presentations is required.
Statistics

Usually, the number of volunteers is too low to consider variability and statistical significance. Also, we don't discriminate the volunteers by gender, age, or physical status, which could interfere in the variability. Thus, we use only the means of each variable. Considering first-year graduate students, we found that this approach is sufficient for the discussion of data and understanding of the metabolic principles involved in the experiment. Graphical presentation can be performed using Microsoft Excel, Origin, or similar software.

RESULTS

An example of the possible experimental results is shown in Fig. 1. The protocol of the tests in this case was eight 30-m sprints with pauses of 20 s (R20) and 120 s (R120). Data represent the mean of five student volunteers for each variable. Figure 1A shows the velocity in each sprint, Fig. 1B shows blood lactate levels before and after 8 sprints, and Fig. 1C shows blood uric acid levels before and 15 and 60 min after the completion of each test.

Blood lactate level after the two tests were similar (11.4 mM for R20 and 10.3 mM for R120). However, the velocity in the R20 test decreased over the course of the eight sprints compared with the fairly consistent velocities recorded in the R120 test. It is important to note that in the case of a reduced number of sprints, the velocity curves are similar to those shown in Fig. 1A, with lower blood lactate concentrations. An example of this occurred with another group of students who performed a protocol of six 30-m sprints with pauses of 20 and 120 s. In their experiments, the mean blood lactate concentration was 7.5 mM in the R20 test and 7.8 mM in the R120 test (n = 4).

We observed that the blood uric acid level after 60 min was higher in the R20 test (7.3 mg/dl) than in the R120 test (5.8 mg/dl). In the two experiments using six 30-m sprints with pauses of 20 and 120 s, the difference in blood uric acid concentrations was not evident after 60 min (5.6 mg/dl for R20 and 5.0 mg/dl for R120), showing only a trend of increasing in the R20 test in relation to the R120 test after 15 min (4.9 and 3.9 mg/dl, respectively).

DISCUSSION

With the results shown in Fig. 1, the students rejected the cause and effect relationship between lactate production and muscular fatigue, showing the lack of universality of the historical theory that relates lactate production with an increase in muscular acidosis and fatigue. This fact compelled them to propose a new biochemical theory to explain the fatigue observed in Fig. 1A. The high blood lactate in both tests (R20 and R120) cannot explain the observed fatigue.

The proposal of another theory is led by the teacher using, once more, the allosteric regulators of glycolysis. This is an appropriate time to explore P_i as a positive allosteric regulator of the glycolytic pathway. P_i is not consumed in the CK or AK reaction. In addition, intracellular Ca^{2+} (high levels of which signal the coupling of myosin and actin for muscular contraction) is also an important positive allosteric regulator of glycogen phosphorylases A and B during muscular activity (3), regardless of the length of the pause. The high intracellular concentrations of P_i (due to ATP hydrolysis) and Ca^{2+} (during
the exercise) allow for the suggestion that the glycolytic pathway could be highly stimulated even when PCr stores are full, leading to similar lactate production under R20 and R120 conditions. The initial hypothesis, “lactate production is responsible for muscular fatigue and performance decrease during high-intensity intermittent exercise,” does not fit this theory.

After data analysis, students concluded that the replacement of less PCr in the R20 test than in the R120 test is the main cause of the difference in performance observed in Fig. 1A, and not the supposed acidosis caused by the production of lactate. The modestly increased concentration of uric acid in the R20 test can be used to support the theory that the incomplete restoration of PCr between sprints may have led to a greater increase of ADP due to CK equilibrium and, thus, greater activation of AK. If there is acidosis, it may be caused by the increased hydrolysis of ATP in this type of exercise and not by lactate production.

As Robergs’ proposition (13) had been discussed during the course, students realize that prior experiments involved a coincidental relationship between muscle acidosis (caused by the high rate of ATP hydrolysis) and the production of lactate and muscle fatigue in high-intensity exercise, not a relationship of cause and effect (14). Based on these empirical data, we think that is not necessary to make a review of experts’ arguments about the traditional acid lactic theory.

Another pedagogical proposal to explore similar data would be one in which the teacher does not interfere in the formation of the theory (as proposed in answering the questions). Students are encouraged to use their own biochemical knowledge and information of the experimental activity and to compare the results with the literature, giving support to the theory proposed by the group. Considering that this pedagogical proposal has been applied to first-year graduate students, we suggest that the teacher compiles some information from the literature in a simple and direct manner. For example,

- Gaitanos et al. (6) observed that, in high-intensity intermittent exercise, ATP-PCr and anaerobic glycogenolysis have almost equal contributions in a single effort of 6 s (~30–40 m).
- Balsom et al. (1) observed similar increases in plasma uric acid concentrations after 15 × 40-m sprints with pauses of 30 and 120 s.
- Aerobic metabolism is the main pathway of ATP resynthesis during the pause. The synthesis of PCr depends on ATP concentration. The maintenance of muscle power during high-intensity intermittent exercise of a short duration depends on the recovery of PCr stores during periods of rest (7).
- Wadley and Le Rossignol. (16) found no correlation between aerobic power and the magnitude of fatigue using a protocol of 12 series of 20-m sprints with pauses of 20 s.
- Wenger and Tomlin (17) suggested that greater aerobic power is related to a greater ability to recover between efforts of high-intensity intermittent exercises, due to increased PCr synthesis.
- McMahon and Wenger (12) found that subjects with higher aerobic power showed little fatigue during a test with six 5-s sprints with pauses of 90 s.
- It was suggested that the efficiency of oxidative metabolism is not evident in pauses of <20 s (17).
- Balsom et al. (1) observed similar blood lactate concentrations after 15 × 40-m sprints with pauses of 30 and 120 s.
- Ca\textsuperscript{2+} and P\textsubscript{i} are positive allosteric regulators of glycolysis and glycogenolysis (3). P\textsubscript{i} is not consumed by the one-step reactions catalyzed by CK and AK. Ca\textsuperscript{2+} is released from the sarcoplasmic reticulum into the cytosol, promoting the formation of cross bridges (the binding of myosin with actin) and, thus, muscular contraction.

This is an activity that introduces the methodology of inductive empirical science. The inductive method starts with observations of nature, with the goal of finding a few concise statements about how nature works. Because one cannot access the interior of the muscle during the tests and observe what is occurring within metabolic pathways, there is only one path to choose: imagine what is occurring and propose a theory capable of explaining the observations after each test (R20 and R120) supported by additional empirical data.

Theoretical knowledge is useful when it has the power to predict the future. For example, students could use the theory proposed by the observed facts and plan: “If my goal is to use a training method aimed at the maintenance of muscle power, velocity, or acceleration in successive intensive efforts with high lactate production, I must consider pauses of 120 s to restore PCr.” The physical education student, in his/her professional life, can raise a hypothesis based on a theory that he or she proposed in the past with classmates. Nevertheless, more experiments are needed to support other hypotheses that can sustain the theory, giving confidence to the predictive power of the theory. We propose that the empirical inductive method can be used as a pedagogical tool. The experiment proposed in this work gives students the opportunity to return to the origin of knowledge production and consider its limitations.

Students also can produce a report comparing the experimental data with the literature. In this case, the teacher plays the role of a scientist working in the same area, discussing the theory and proposing corrections for the report and the oral presentation. This activity can simulate future participation in conferences and promote knowledge of the current methods of producing and reviewing scientific work. It is an enlightening experience for all, and some may even be called to a scientific vocation.

Conclusions

Students engaged in scientific practice and are encouraged to review and discuss the results obtained from the biochemical knowledge acquired in classroom. The theoretical knowledge and observations of the experiments allow the student to apply this content in their professional lives. At the end of this practice, the teacher can demystify a controversial concept in physical education, rejecting the cause and effect relationship paradigm between lactate-based acidosis and muscle fatigue.

Besides the integration of the formal content of biochemistry applied to exercise, the student begins to acquire critical conscience about the origin, limitations, and interpretation of the theories that are presented along their professional development. This implies that the teacher guides students according
to the principles of empirical inductive scientific methodology, assisting in the accuracy for data collection, tabulation, graphical presentation, and interpretation of results.

REFERENCES