Evaluating the Quick Fix: Weight loss drugs and cellular respiration

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Abstract

One key to student success in introductory and cell biology courses is a foundational knowledge of cellular respiration. This is a content area in which students often harbor misconceptions that make cellular respiration particularly challenging to teach. Conventional approaches presenting cellular respiration as a complex series of isolated steps creates a situation where students tend to memorize the steps but fail to appreciate the bigger picture of how cells transform and utilize energy. Instructors frequently struggle to find ways to motivate students and encourage deeper learning. The learning goals of this cellular respiration lesson are to understand energy transfer in a biological system, develop data analysis skills, practice hypothesis generation, and appreciate the importance of cellular respiration in everyday life. These goals are achieved by using a case study as the focal point. The case-based lesson is supported with student-centered instructional strategies, such as individual and group activity sheets, in-class group discussions and debate, and in-class clicker questions. This lesson has been implemented at two institutions in large enrollment introductory biology courses and in a smaller upper-division biochemistry course.

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Supporting Materials: S1. Weight Loss Drugs-Pre-class homework, S2. Weight Loss Drugs-Pre-class homework instructor's key, S3. Weight Loss Drugs-In-class worksheet/Case study, S4. Weight Loss Drugs-In-class worksheet/Case study instructor's key, S5. Weight Loss Drugs-PowerPoint slides, S6. Weight Loss Drugs-Additional teacher notes-The Bhopal Disaster

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Learning Goal(s)

- Students will understand how cells transform energy via cellular respiration.
- Students will develop data analysis skills.
- Students will practice hypothesis development.
- Students will discover that knowledge of cellular respiration can inform lifestyle decisions.

Learning Objective(s)

- Students will be able to explain how the energy from sugars is transformed into ATP via cellular respiration.
- Students will be able to predict an outcome if there is a perturbation in the cellular respiration pathway.
- Students will be able to state and evaluate a hypothesis.
- Students will be able to interpret data from a graph, and use that data to make inferences about the action of a drug.

INTRODUCTION

Origin of Lesson

We developed this lesson at the 2014 National Academies Northstar Summer Institute. Our team had a common interest in teaching biochemistry and cell biology content and developed a unit on cellular respiration based on scientific teaching principles (1). The foundational concepts of energy transfer and mass conservation can be difficult to conceptualize, particularly for learners in introductory biology courses. Students struggle to understand the connections among the various components of cellular respiration (glycolysis, the citric acid cycle, electron transport chain and oxidative phosphorylation) as well as the interdependence of cellular respiration and photosynthesis (2). To confront these challenges biology educators have developed and implemented role-plays, case-based learning exercises, and laboratory modules (3-9). We decided that a case-based lesson would be the best fit for the structures of the courses that we teach and the learners at our respective institutions.

We identified a case (10,11) from the National Center for Case Study Teaching in Science (12) about the weight loss drug 2,4-dinitrophenol (DNP) and adapted it for our courses. The DNP example is frequently used to reinforce the concepts of cellular respiration and highlights the severity of perturbations to cellular respiration reactions. The first death associated with DNP was reported in 1918 (13), and the DNP example appears in some introductory biology textbooks (e.g. 14, 15) as a way to encourage students to think more deeply about cellular energy transformations. We developed a modified set of learning goals and objectives that targeted our student populations, large classes at a public land grant institution and small classes at a private college. An effort was made to align some of our goals with the core concepts outlined in Vision and Change (16,17). When we adapted this case for our lesson, we increased student engagement, we added a component that asks students to apply their knowledge of biochemical pathways under normal conditions, and we asked them to make a prediction using a visual representation about the biochemical outcome in the presence of a chemical perturbation to the pathway. Providing students with the opportunity to predict an outcome before the answer is revealed is an evidenced-based practice that supports conceptual learning in biology (18).

Context and Rationale

The weight loss drug in the case study contains the compound 2,4-dinitrophenol (DNP). DNP acts as a weight loss agent by uncoupling the mitochondrial proton gradient from ATP synthesis. This uncoupling disrupts the cellular respiration pathway and results in a rapid consumption of energy by the cell without the commensurate production of ATP (19). Despite numerous adverse side effects and health risks, including death, attributed to DNP (20), some populations, like bodybuilders, continue to utilize DNP to meet their performance goals (21,22).

In this lesson, the students are provided with a partial dataset from the primary literature (23) and are asked to sketch a prediction for what happens when DNP is introduced to the pathway. This prediction and hypothesis generation is a powerful hook to engage the students in discussions of their reasoning before the rest of the data is revealed.

Intended Audience

The lesson as described is targeted for introductory biology students. It can be modified for use in upper-division cell biology, biochemistry, and physiology courses. It has been utilized in large (400 students) and small (20-50 student) classes at a large public university and a small, primarily undergraduate institution. In the large sections, trained undergraduate learning assistants circulated among the groups to address questions, keep the students on task, and encourage deeper thinking as appropriate.

Required Learning Time

The required in-class learning time can be as little 30 minutes. Students may complete a homework assignment (Supporting Materials S1) that sets the stage and introduces the central concepts of cellular respiration. Students complete an additional worksheet (Supporting Materials S3) in class as they work through the lesson. Optional activities intended to generate more in depth exploration and discussion can easily be added, expanding the lesson to an entire class session or multiple class sessions.

Pre-requisite Student Knowledge

This lesson is most effective if introductory students already have a foundational understanding of the basics of cellular respiration and have completed a pre-activity homework assignment. Advanced students who have already studied glycolysis, the citric acid cycle, electron transport and oxidative phosphorylation in detail, may use this activity as a culminating experience or a test of knowledge before going into deeper discussions.

Pre-requisite Teacher Knowledge

Teachers should have an understanding of the components of cellular respiration so they can facilitate discussions about perturbations to the system. Introductory biology textbooks usually have chapters with titles such as, "Cellular Respiration and Fermentation", "Pathways that Harvest Chemical Energy", or "How Cells Make ATP." They do not need to be experts in cell biology or biochemistry.

SCIENTIFIC TEACHING THEMES

Active learning

In this lesson, student learning occurs through individual, self-paced homework assignments and in-class team learning activities. We designed the individual homework assignments to reinforce content knowledge and prepare students for discussions that take place during the lesson. The students work together in teams to interpret graphical representations of data and make predictions. We use team problem-solving activities in class to encourage peer discussions that lead to a whole class debate about the possible outcomes of perturbations to biological systems.

Assessment

We assess student learning and readiness for class through a pre-class homework assignment (Supporting Materials S1) and an online pre-class quiz designed to encourage review of the fundamental aspects of cellular respiration. During class, clicker questions provide formative assessments and feedback about the types and frequency of student predictions. An interrupted case study allows ongoing assessment of conceptual understanding and clarification of misconceptions as the students advance through the case. Optional post-lesson activities include individual homework application questions and an assignment to generate possible exam questions. The question writing assignment is intended to get the students to work with the important points of cellular respiration, not necessarily to generate actual questions for an exam.

Inclusive teaching

The framing story used in the case study about weight loss drugs provides relevance to a variety of learners, as many students are familiar with body image issues. The supplemental application question on cyanide gas exposure offers an opportunity to incorporate a discussion of environmental social justice. The lesson design allows students to engage with the content in a variety of ways: individual self-paced homework, clicker questions, peer discussion, data interpretation, and graphing. Clicker questions are a low-stakes learning opportunity that helps teachers identify misconceptions and generate class discussion to address misconceptions. Students may be placed into heterogeneous learning teams at the beginning of the semester (or class) to encourage cooperative learning and peer mentoring.

LESSON PLAN

An overview and timeline for the lesson plan are provided in Table 1.

Teacher Preparation

Teachers should assign the pre-class homework (Supporting Materials S1) and hand it out to students the class period before you use the case study. A hand drawn version, or the image provided in Supporting Material S1 (https://commons.wikimedia.org/wiki/File:Mitochondrial_electron_transport...), might be preferable to one taken from their textbook as it requires additional interpretation and deeper understanding, as opposed to just copying information to a replica of a book figure. We often couple this with an online quiz to encourage reading of the background material and to assess the student's preparation. Our online quiz questions are available to valid instructors upon request.

Student Preparation

Students complete a homework assignment (Supporting Materials S1) and an online quiz prior to class. Check to see that students have completed the assignment to ensure that they are prepared for the activity. You may choose to assign points in order to hold students accountable. We use a simple complete or incomplete check off as students arrive at class. Before class begins, you can ask the students if they have questions about the homework assignment. It is helpful to have the students keep the homework as a reference while they work through the activity.

Class set up

We designed the in-class case study worksheet to be brief (Supporting Materials S3). The introductory paragraphs are short enough that students can read through them during class and they can take the sheet with them after class as a study reference. Do not hand out this worksheet or presentation slides ahead of time (i.e. the class period before), as some students may look up information about 2,4-dinitrophenol (DNP) before coming to class. While it is encouraging to have students investigate DNP, students who come with knowledge of the mechanism miss out on the surprise of the unexpected results shown in class and do not generate as many hypotheses about the DNP mechanism. We strongly discourage the use of cell phones and laptops while working on this case study. We find that when students are asked questions, some immediately query the internet and volunteer a fine answer, but they miss the opportunity to think about and fully engage with the material.

Students should work in groups for this activity. Depending on enrollments and how your classroom is set up, you can assign groups, use pre-existing groups, or you can ask students to self select into groups of three to seven. In both our large and small classes, groups are established at the beginning of the semester, and the students have practice with group dynamics and group worksheets. Trained undergraduate learning assistants facilitate the group interactions in the large (400 student) class. The exercise works well in both the large and small class with pre-existing groups as well as in small classes without pre-existing groups.

Presentation slides (Supporting Materials S5) with embedded questions for individual student responses (clickers or a simple estimate of raised hands) are necessary to get the students to think about the questions and to commit to an answer. Student commitment to an answer and a display of the result is key to the success of the exercise.

Introducing the case study

At the start of class, we inform the students that they will engage in an activity that will require them to use what they have learned about cellular respiration. They will need to apply their knowledge to a new situation, make decisions, and give advice. We also let students know that they will work with data that arises from primary literature and that data interpretation is an important skill they will use throughout the course and their lives. These introductory statements prepare the students for the challenge and the discomfort they may feel. This acknowledgement seems to ease the fear some students have of case studies.

Hand out the case study worksheet and instruct the students to read the first three introductory paragraphs and answer Question 1 on their own. They can stop after Question 1 (end of page 1). Allow several minutes for this. If you ask the students to do the first question on their own, it encourages them to think for themselves and it also allows enough time for students who might be slower readers to digest the material. Alternatively, you can ask the groups to read the narrative out loud together then individually answer the question. After students have considered Question 1 individually, instruct them to discuss their answer with their group.

Experimental methodology for figures used in the lesson

Human myotubes can be generated from skeletal muscle biopsies and represent a model system for intact human skeletal muscle. In the Gaster 2007 study (23), muscle biopsies were obtained from ten control subjects. The cells were cultured and allowed to differentiate into myotubes prior to treatment with DNP. The N values for the experiments represent replicate experiments (N=8 for Figure 1 and N=3 for Figure 2).

Discussion of Figures 1 and 2 and Questions 1 and 2

After students consider Figure 1, have a quick group discussion. This discussion serves as a formative assessment and gives students an opportunity to ask questions about the introductory content before they move on to other ideas. Some engagement prompts that you may want to include during these discussions are: summarize Figure 1 in a single sentence; state other pieces of information that can be gleaned from the figure and caption; explain the meaning of rate (rate of glucose oxidation as that compares to overall amount of glucose), P-values (what does $p < 0.01$ mean, is this significant, is this important, etc.), and standard error.

After the Figure 1 discussion, direct the students to individually predict the concentration of ATP in the presence of DNP and to draw their prediction on Figure 2 (Question 2). In our experience, if students commit to a prediction on paper it is more powerful than if they wait for the answer and draw the real data in later. Students can discuss their prediction with their group after they draw their prediction. The majority of students will draw a bar for ATP concentration in the presence of DNP as much larger than that of the control cells. This prediction demonstrates their understanding of how glucose oxidation and ATP production are normally linked. We poll the students with a clicker question about their predicted values. You can poll the students' individual predictions or group predictions.

Present the poll data to the students. We ask student representatives from the groups to explain why they chose their answer. Students will likely predict that the ATP concentration will be greater in the DNP treated cells than the control cells, citing that increased oxidation of glucose in DNP treated cells would result in increased ATP synthesis (i.e. more cellular respiration, so more ATP). Once the students explain their answers, we reveal the actual data. When the majority of the class gets the wrong answer (approximately 80% in our classes), students are surprised and motivated to learn more about what is happening in the presence of DNP. This contradiction of expectations is very powerful and draws the students into the subsequent discussions. Give students time to ponder why there might be a difference in their prediction and the actual data.

If the case study is used in an upper-division class, a more nuanced discussion of ATP levels and homeostasis should also be included. In the presence of DNP, the adenylate kinase catalyzed reaction 2ADP <==> ATP + AMP, will stabilize the ATP levels and concurrently lead to an increase in cellular AMP concentration. The concept of energy charge should also be addressed (equation below). Energy charge is maintained within an experimentally determined range of 0.7-0.95 in all cellular organisms (24).

Finally, advanced students with a broad understanding of metabolism would also benefit from the study of the AMP-activated protein kinase and its role in energy homeostasis throughout the body (25,26).

The y-axis scale in Figure 2 allows room for a prediction of all possible ATP concentrations. In the presentation slides, we include a second version of Figure 2 (animated) with an adjusted axis to help illustrate the data. Note that the p value is intentionally left blank on the student worksheet but appears on the animated PowerPoint slide. This strategy prevents bias in the students' drawings but allows the instructor to emphasize the importance of statistical analysis when discussing data.

Once students are better able to visualize the data in Figure 2, we ask them to interpret the figure and then move on to Question 3. We ask them to come up with as many

$$energy\ charge = \frac{[ATP] + \frac{1}{2}[ADP]}{[ATP] + [ADP] + [AMP]}$$

different hypotheses as they can. If time allows, they can share their group list with another group. We solicit hypotheses from the groups, discuss and record them on the board or document imager. Possible and probable hypotheses include, perturbations in glycolysis, pyruvate processing, the citric acid cycle, the electron transport chain, ATP synthase, feedback loops/inhibitor regulation, movement of molecules into the mitochondria, and destruction of the proton gradient. There are numerous and varied responses at this stage and students are encouraged to come up with a number of plausible explanations for what DNP may do in the cell, e.g. DNP blocks the O2 in the cell, DNP blocks the electron carriers or pyruvate, DNP requires a good deal of ATP so ATP gets used up in the cell before detection, heat denatures the proteins of the electron transport chain, DNP acts as a non-competitive inhibitor.

Options for hypotheses discussion

After you consolidate the groups' hypotheses, ask groups to evaluate each hypothesis on the list. This analysis will help students compare their reasoning with their peers'. Alternatively, if you are short on time, instead of having students generate their own hypotheses, give students a list of potential hypotheses and ask them to evaluate each one. This task allows practice deciding whether a given hypothesis is reasonable and testable.

Explanation of DNP mechanism

At this point in the class, we find that the students' interest is piqued and they want to know the exact mechanism of DNP. Success! Students are engaged and curious and are asking for more details so they can understand why there was a difference in their expectations and the experimental data. Before we explain the mechanism, we ask a clicker question about the relative pH in various compartments of the mitochondrion. This question prompts students to review what pH represents, and we follow with a reminder about how proton pumping leads to this pH difference. Many students struggle with the concept of pH and what it means with respect to the concentrations of H(+) and OH(-), so we find it helpful to reinforce this concept. During respiration, the cell has worked to pump protons from the matrix to the intermembrane space. The lower H(+) concentration in the matrix means it has a higher pH relative to the cytoplasm.

Display the DNP molecule and discuss how it is a small lipophilic compound capable of crossing the inner mitochondrial membrane and entering the matrix via simple diffusion (Presentation slides are animated to demonstrate this process). DNP is also a weakly acidic compound. If you are teaching advanced undergraduates, you may ask them to discuss in small groups how this pH difference and DNP's ability to cross the membrane into the matrix might give enough information to generate a more specific hypothesis for DNP's action. If your students don't have sufficient background, you can explain the mechanism at this point.

Line structure of 2,4-Dinitrophenol

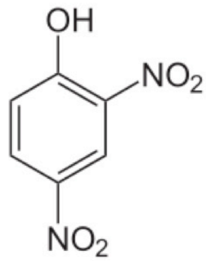

Figure 1. Line structure of 2,4-Dinitrophenol

The 2,4-dinitrophenol figure is from https://en.wikipedia.org/wiki/2,4-Dinitrophenol#/media/File:2,4-Dinitrop. The original uploader was NEUROtiker. This simple structural formula is ineligible for copyright and therefore in the public domain.

The lower H(+) concentration (higher pH or more basic) encountered in the matrix allows DNP to act as an acid and donate its proton (H(+); animated effect on presentation slides). This proton release diminishes the gradient between the matrix and intermembrane space, leading to a proportional decrease in the amount of ATP that can be generated by ATP synthase. We ask the students to look back at Figures 1 and 2 on their worksheets and consider if it makes sense that in the presence of DNP, glucose oxidation increases without an increase in ATP synthesis. This phenomenon is the uncoupling of the pathways. Some students may pick up on the uncoupling terminology from the second paragraph of the worksheet, but most do not. Additionally, you can ask the students what is happening if, despite the increase in glucose oxidation, no additional ATP is produced? Where does the energy go? Some students may be able to articulate that normally the energy associated with H(+) moving down the H(+) gradient is utilized by ATP synthase to phosphorylate ADP, but with DNP there is a smaller H(+) gradient and energy is released as heat.

Conclusion of the case study

To bring the discussion back to Miguel, we ask the students what kind of advice they would give him. The next clicker question (Question 4) gives introverted students and the often minority of students who would tell Miguel to take the drug (approximately 15%) a chance to answer anonymously.

To further explore energy transformation concepts, you can pose this question for students to answer before the next class meeting, "Where does the mass go when someone loses weight?" (Question 5). When fuel (glucose and other biomolecules) is oxidized, carbon dioxide, one of the final products, is exhaled. Mass exhaled away as CO2 leaves the body. Students struggle with this concept as carbon dioxide is a gas and they do not often think about the fact that it has mass. In the case study, more fuel must be oxidized to generate a comparable level of ATP. The extra fuel is exhaled as carbon dioxide. An individual taking DNP will lose weight quite effortlessly, though they might also suffer major side effects or die. We have found that it is important to complete the unveiling of the mechanism during a single class period as the students lose interest if they have to wait a day before they learn the answer.

Optional homework assignments after the case study

If time allows, you may choose to assign homework after completion of the case study. This assignment encourages the students to continue to think about cellular respiration and reinforces the objectives of the lesson. Ask the students to go home and write three original exam questions that incorporate some aspect of cellular respiration. Questions may address lower level remembering and understanding of material (e.g. inputs and outputs of cellular respiration), apply knowledge to a new situation (e.g. a mitochondria defect or DNP use as a pesticide), and/or determine the problem when given specific data (e.g. normal vs. affected acetyl CoA levels or NAD(+)/NADH ratios). These questions can be multiple choice, data interpretation, graphing, or short answer questions based on the structure of your exams and the experience level of your students. Additionally, you may ask students to bring these questions to class so they can discuss and polish them with their group members. You may want to provide an incentive for questions that end up being used on the exams. Additional application questions pertaining to the Union Carbide Bhopal pesticide plant disaster can be found in the Supporting Materials (S6). This assignment can be used as a homework assignment or as an in-class extension to reinforce the concepts or provide an opportunity to discuss social justice issues.

TEACHING DISCUSSION

This lesson has been implemented successfully in introductory biology courses at two institutions and in a smaller upper-division biochemistry course. With modifications, it would also be appropriate for cell biology and physiology courses that include exploration of cellular respiration. We find that the case study format helps to make cellular respiration, an area often underappreciated by students, more interesting. If the instructor wishes to extend the lesson, some of the optional exercises discussed above could be included, or additional exercises could be created.

One of the fundamental learning objectives for this lesson is the ability to interpret graphical representations of data and make inferences. This exercise has the students interpret a graph, think about how that information applies to cellular respiration, and make inferences about how DNP impacts ATP production. When confronted with data that runs counter to their predictions (approximately 80% of students indicated that ATP production should increase in the presence of DNP), the students, while momentarily frustrated and confused, soon start to seek out additional information that clarifies the situation. They become energized and engaged. The exercise generates animated discussions, and it creates multiple teachable moments allowing you to stress the importance of cellular respiration, data interpretation, and hypothesis generation. When you take the time to analyze the mechanism of DNP action, it provides opportunities to review how pH affects the ionization state of molecules, the properties of membranes, the molecules that can or cannot cross membranes, and the role of the proton gradient in ATP synthesis. It also allows students to transfer their understanding of cellular respiration concepts to new contexts.

Student performance on exam questions that require an understanding of enzyme kinetics and interpretation of graphs has improved since the introduction of this cellular respiration teaching module. We used embedded exam questions to assess student performance across the two institutions and to determine whether the activity impacted student learning. In fall 2013 (prior to the development of the case study teaching module) approximately 35% of students at one of our institutions correctly answered questions about energy transfer and cellular respiration. From 2014 through 2016, after the introduction of this module, students at both institutions responded correctly 50-85% of the time to a series of exam questions about energy transfer and cellular respiration.

Our students often ask what kind of details they need to know about the case study and DNP. The immediate answer to this question depends on the desired outcomes. In this context, we use the case study as an example to discuss the difference between a learner's perception of knowing or understanding a concept compared to being *familiar* with a concept. A student who is familiar with cellular respiration will recognize the terminology of the mechanism and the final products, but a student who understands is able to work through perturbations of the system or can apply the ideas to a new situation. This example seems to resonate with most students and they seem to have a better idea or feeling about what they should gain from the case study experience.

There are, of course, some students who are not well prepared for case study activities. The stronger members of the working group often carry these students along. To ensure that less prepared students or less strong groups are engaged in the activity, it is good to check with each group and ensure each group is on track. This can be handled by the instructor and/or the learning assistants.

SUPPORTING MATERIALS

- S1. Weight Loss Drugs-Pre-class homework
- S2. Weight Loss Drugs-Pre-class homework instructor's key
- S3. Weight Loss Drugs-In-class worksheet/Case study
- S4. Weight Loss Drugs-In-class worksheet/Case study instructor's key
- S5. Weight Loss Drugs-PowerPoint slides
- S6. Weight Loss Drugs-Additional teacher notes-The Bhopal Disaster

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Table 1. Weight Loss- Teaching Timeline

Activity	Description	Time (min)
Preparation for class		
Teacher prep	Review the provided PowerPoints and adjust to fit your course goals. Make copies of pre-class homework assignment (Supplemental Materials) and in-class worksheet (Supplemental Materials).	30-60
Student prep	Complete homework assignment before arrival at class.	15
Class meeting (based on 50 minute class period)		
Lesson introduction	Introduce the case study and answer any questions about homework. Hand out the in-class worksheet and instruct students to read the first page and complete Question 1.	2
Question 1	Determine and summarize what happens to the rate of glucose oxidation in the presence of DNP. Students work individually and then share with their group.	5-8
Discussion	Class discussion for Question 1 serves as formative assessment.	5-8
Question 2	Predict what will happen to the concentration of ATP in the cells exposed to DNP. Explain your reasoning and plot prediction. Discuss answers with group. Poll students.	5-10
Discussion	Discuss clicker results. Reveal ATP concentration data and discuss the figure. Be prepared for a range of student reactions. Use the student responses to encourage further questions and hypothesis generation.	4
Question 3	What might be happening in the presence of DNP? Generate multiple hypotheses that might explain the results. Students share within groups and then discuss with the class.	8-12
Question and discussion	In what area of the mitochondria will the pH be higher? Reveal clicker results and discuss pH concepts.	4
Explanation of mechanism	Demonstrate the mechanism of the DNP model with animated PowerPoint slides.	3-5
Question 4	What advice would you give to Miguel about whether he should take the diet pills?	2
Question 5	Where does the weight go when someone loses weight?	Take home question