Supplemental Material

CBE—Life Sciences Education

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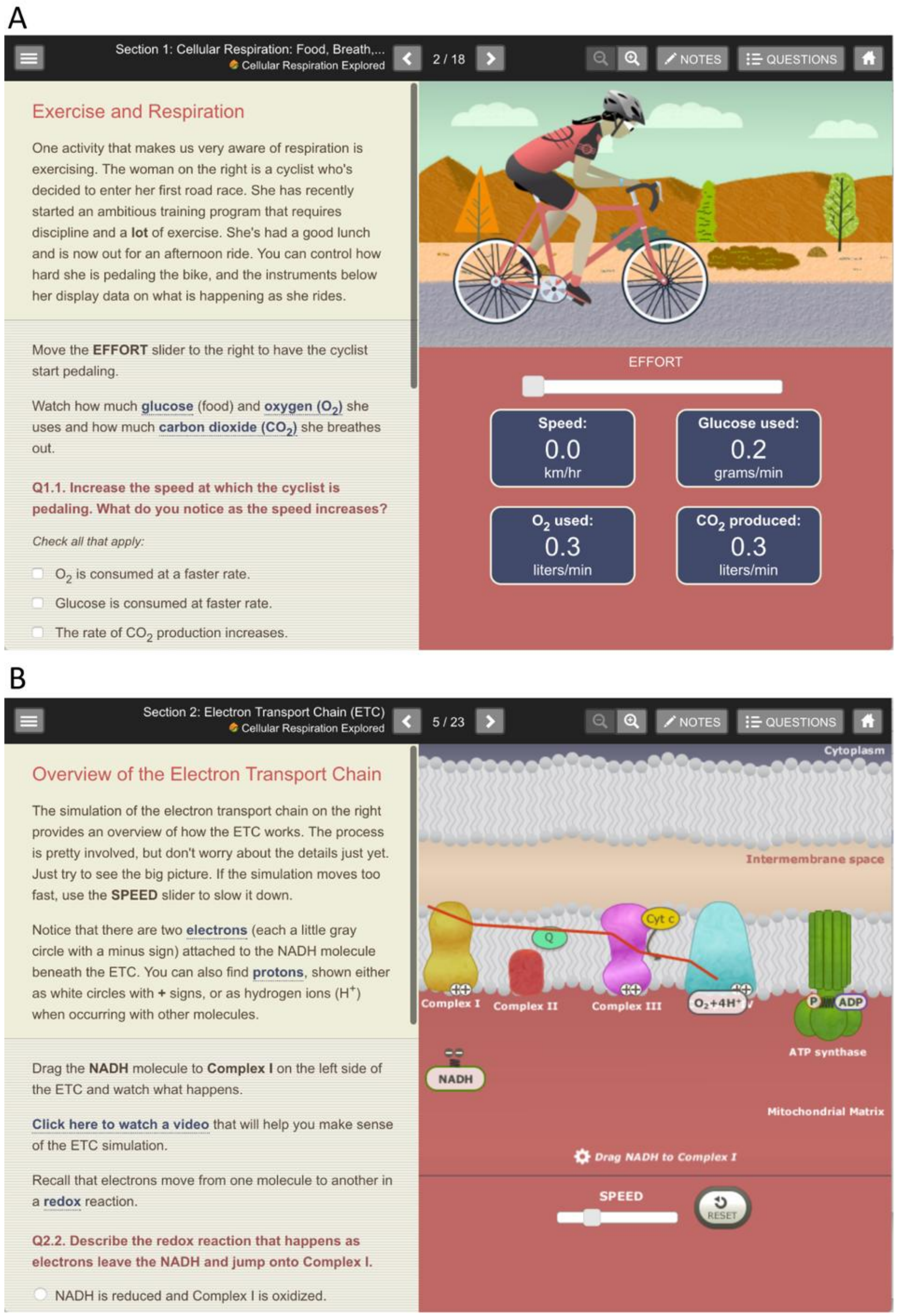
Supplemental Methods

Cellular Respiration Explored Tutorial by SimBio

The tutorial targets eleven learning objectives (LOs), three of which are most relevant to the Weight Loss and Energy from Glucose questions. The LOs were available for instructor review but not visible to students. One LO requires knowledge of the molecular processes involved in cellular respiration: "Identify processes in respiration where potential energy is transferred from one place to another, and draw a diagram showing the flow of energy between molecules or other stores of potential energy in each process (e.g., NADH → ETC → proton gradient → ATP)." One LO aims to help dispel a common misconception: "Avoid the misconception that energy is converted into matter." Another LO involves understanding the mass and energy inputs and outputs of cellular respiration: "Write down and explain the overall glucose → CO_2 equation (glucose + 6 O_2 → 6 CO_2 + 6 H_2O) and identify that this reaction releases energy."

The tutorial has two sections which we estimate in total take students one and half to two hours to complete. The first section provides an overview of cellular respiration, including the key processes (glycolysis, pyruvate processing, etc.), potential energy and how it can be stored in molecules, oxidation-reduction (redox) reactions, and the major carriers of energy in respiration (ATP, NADH, $FADH_2$). The second section focuses on the electron transport chain (ETC) and asks students to experiment with a simulation to explore the role of proton gradients, the different components of the ETC, and oxygen in transferring energy to ATP. Students interact with several simulations throughout the tutorial, including a whole organism scale simulation of a bicyclist training for a race (Supplemental Figure 1A) and a molecular-level simulation of the electron transport chain (Supplemental Figure 1B).



Supplemental Figure 1. Screenshots from the Cellular Respiration Explored tutorial. As students complete the interactive tutorial, they complete simulations and interact with and manipulate molecules and processes of cellular respiration at the (A) whole organism level and the (B) molecular level.

Throughout the tutorial, students answer a total of 51 questions that provide them with feedback, help them stay on track, and provide evidence of their progress to their instructors. Of those, 49 are multiple-choice (MC) questions, and two are more complex intermediate constraint questions (“LabLibs” per Meir et al, 2019). One of the intermediate constraint questions providing feedback is parallel to the Weight Loss question, with a stem “When your body uses glucose for energy during exercise, you weigh less afterwards. Why?” The student is asked to select from pre-populated phrases for each blank in the sentence, “You weigh less because glucose is transformed into _____ that is _____”. The tutorial ends with a 10-question MC quiz. One of these quiz questions targets whether students have the misconception that matter is converted to energy, and this is the MC question analyzed in this paper. Students complete the tutorial either at home or in the lab, depending on how it is assigned by their instructor, and most complete the tutorial individually.

In the research version of the tutorial used in this study, the three CR questions were given to students at the very beginning of the tutorial, prior to any instruction from the tutorial, and at the very end, after the tutorial and MC quiz. Faculty were asked to assign the pre-assessment within 0 – 3 days prior to students doing the tutorial, and the post-assessment within 0-3 days after completing the tutorial. Students did not have access to the tutorial while completing the pre-assessment but did have access while completing the post-assessment. However, students were not able to see whether their answers to the quiz questions were correct prior to completing the post-assessment. While we believe most participating faculty followed these instructions, we do not have independent data to verify that. The Cellular Respiration Explored tutorial, without the CR questions, is available for examination through the SimBio Preview application available at SimBio’s website simbio.com.

Automated Constructed Response Categorization Rubrics

Rubric descriptions of the Energy from Glucose and Enzyme Binding questions, two of the three constructed response (CR) questions used to assess student learning after completion of the Cellular Respiration Explored Tutorial. (Supplemental Tables 1 & 2).

Supplemental Table 1. Rubric for the automated categorization model used to identify ideas in responses to the Energy from Glucose question. From beyondmultiplechoice.org

Rubric category	Description	Cohen's Kappa
ATP with Correct Process	Uses ATP and gives a correct metabolic process; a process name or description is acceptable	0.861
ATP without Process or with Incorrect Process	No additional information	0.712
Correct Processes without ATP	Named metabolic process, correct use of "catabolism" or process description is acceptable; response must not use ATP	0.364
Sugar Converted to Energy	Response provides no other correct process (e.g. respiration); Response may refer to heat or fuel as energy; Response does not include an intermediate energy source (e.g. ATP)	0.562
Physiological Processes	Response uses physiological or whole organism process in explanation (e.g., circulation, chewing, digestion, muscle contraction description, enzymes breaking down sugar) without a molecular description	0.630

Supplemental Table 2. Rubric for the automated categorization model used to identify ideas in responses to the Enzyme Binding question. From beyondmultiplechoice.org

Rubric category	Description	Cohen's Kappa
Lock-and-Key	Student uses the phrase "lock-and-key," "lock and key" or uses the words "lock" and/or "key"	0.955
Induced Fit, Conformational Change	Student uses the words "induced fit" or describes induced fit, or uses/described some change in conformation	0.821
Transition state	Student uses the words "transition state" or attempts to describe a transition state in their answer.	0.809
Other Analogy	Student describes an enzyme and its substrate as a puzzle or puzzle pieces This bin include any other comparison (ex: hand/glove, Legos, hug, ball/glove)	0.684
Energy	Student describes an enzyme (lowering activation energy), discusses other activation energy, energy efficiency, or energy savings for the context of the reaction (or "function" of the enzyme).	0.955
Physical Fit	Student describes anything related to the shape of the interacting enzyme and substrate. Key terms include active site, orientation, similar, pocket, polarity, complimentary, location, orient, fit, shape, recognize, enter, surface	0.813
Amino acid interactions/sequence	Student discusses that or how the amino acid sequence influences the interaction between the enzyme and substrate or related mention that the amino acids are important in the interaction.	0.890
Bonding	Any specific, named mention of a type of bonding. Key types include hydrogen, covalent, intermolecular, intramolecular, weak interactions	0.754
Physical Change	Student discusses some physical or environmental change that has an impact on the enzyme. Key terms include denature, pH, temperature	0.890
Other regulation	Student discusses regulation and impact on enzyme. Key terms include cofactor, signaling, allosteric sites, competitive/noncompetitive regulation	0.786
Increase reaction rate	Student discusses that an enzyme increases the reaction rate	0.810

Supplemental Results

Descriptive thinking models included in responses to the Weight Loss question

We examined the percent of students from each institution type who used each of the three Descriptive thinking models in their responses (Supplemental Table 3). We examined which ideas (Supplemental Table 4) and descriptive models were present in CRs to the Weight Loss question associated with students' selection from a multiple-choice (MC) question that also targets the idea of where mass goes during weight loss (Supplemental Table 5). Many (41%) students who made the correct MC selection were classified as having a Mixed description in their written responses to the Weight Loss question. We also found similar percentages of students classified as having a Scientific description in their written responses regardless of MC selection. Of students who made no selection in the MC question, most (59%) wrote responses to the Weight Loss CR question that were classified as Developing descriptions.

Supplemental Table 3. Percent of student responses to the Weight Loss question categorized within descriptive thinking models by institutional type. Two-year colleges (TYC), n = 69; primarily undergraduate institutions (PUI), n = 212; research-intensive colleges and universities (RICU), n = 560.

	TYC, percent (number)		PUI, percent (number)		RICU, percent (number)	
Descriptive Model	pre-tutorial	post-tutorial	pre-tutorial	post-tutorial	pre-tutorial	post-tutorial
Scientific	4% (3)	20% (14)	25% (52)	33% (70)	12% (67)	34% (190)
Mixed	23% (16)	45% (31)	11% (23)	37% (78)	14% (81)	39% (218)
Developing	55% (38)	29% (20)	49% (104)	23% (48)	56% (316)	21% (116)
None	17% (12)	6% (4)	16% (33)	8% (16)	17% (96)	6% (36)

Supplemental Table 4. Ideas included in student written responses associated with multiple-choice (MC) selection. Correct Molecular Products, n = 678; General Metabolism, n = 70; Matter to Energy, n = 66; No selection, n = 23. Scientific ideas as defined by Sripathi et al. (2019) [b]Correct MC option

CR Rubric Category	MC Options, Percent (Number)		
	Correct Molecular Products[b]	General Metabolism	Matter Converted to Energy
Correct Molecular Products[a]	71% (479)	36% (27)	35% (23)
Exhalation[a]	56% (377)	37% (26)	29% (19)
Molecular Mechanism[a]	14% (93)	7% (5)	8% (5)
General Metabolism	9% (60)	14% (10)	12% (8)
Matter Converted to Energy	19% (126)	36% (25)	52% (34)
Excretion	36% (242)	24% (17)	20% (13)
How to Lose Weight	15% (103)	11% (8)	17% (11)

Supplemental Table 5. Thinking models in student CRs associated with multiple-choice (MC) selection. Correct Molecular Products, n = 678; General Metabolism, n = 70; Matter Converted to Energy, n = 66 [a]Correct MC option

Descriptive model	Responses, percent (number)		
	[a]Correct Molecular Products	General Metabolism	Matter Converted to Energy
Scientific	33% (239)	36% (5)	26% (22)
Mixed	41% (296)	21% (3)	32% (27)
Developing	20% (143)	29% (4)	28% (24)
None	6% (42)	14% (2)	14% (12)