MILK—HOW SWEET IS IT?

OVERVIEW

This hands-on activity is a supplement to the short film *The Making of the Fittest: Got Lactase? The Co-evolution of Genes and Culture*. Students will simulate a lactose tolerance test, similar to the one shown in the film. Instead of measuring glucose in patients' blood samples, students will combine milk with "patients' intestinal fluid samples" (which consist of either water or a lactase solution) and measure the amount of glucose produced over time. Students will work in pairs to collect, analyze, and graph their data to determine which "patients" are lactase persistent (lactose tolerant) and lactase nonpersistent (lactose intolerant). This activity complements the related lesson entitled "Got Lactase? Blood Glucose Data Analysis," in which students graph and analyze actual blood glucose data.

KEY CONCEPTS AND LEARNING OBJECTIVES

- Compounds in food are sources of energy for cells in the body. They first have to be broken down into simple molecules that can be absorbed and used by cells.
- Digestive enzymes, such as lactase, facilitate the breakdown of food molecules, including carbohydrates, proteins, and lipids.
- Enzymes have specificity. Lactase only breaks down lactose, but not other carbohydrates, such as sucrose or maltose.
- To measure whether an enzyme is active, scientists can measure the end product(s) of an enzymatic reaction.

At the end of this activity, students should be able to:

- plan the steps in an experimental procedure.
- test for the presence of glucose in a sample using glucose test strips.
- visually represent data using graphs.
- collect, analyze, and interpret data to draw a conclusion.
- understand the importance of using controls in an experiment.

CURRICULUM CONNECTIONS

Curriculum	Curriculum Topics
NGSS	HS-LS1-3, HS-LS3-1, HS-LS4-1, HS-LS4-2, HS-LS4-3, (LS4.B, LS4-C), HS-LS4-5
Common Core*	CCSS.ELA-Literacy.RST.9-10.3, CCSS.ELA-Literacy.RST.9-10.4
AP (2012-13 Standards)	2.D.3, 2.D.4, 3.A.1, 3.C.1, 4.A.1, 4.A.4, .4.B.1, 4.B.2
IB (2016 Standards)	2.3, 2.4, 2.5, 2.7, 6.1, D1, D2

KEY TERMS

Lactose, lactase, persistence, intolerance, enzyme, monosaccharide, disaccharide, glucose, galactose, allergy

TIME REQUIREMENT

This hands-on activity can be conducted in two 50-minute periods. This estimate does not include teacher preparation time or the time required for watching the short film.

SUGGESTED AUDIENCE

This activity is appropriate for middle-school life science and high-school biology (all levels including AP and IB).

PRIOR KNOWLEDGE

Students should know that an enzyme is a molecule that speeds up a chemical reaction and that enzymes are specific for particular substrates. Knowing that traits are inherited and that some traits provide a selective advantage to individuals who possess them will help prepare students for viewing the short film *Got Lactase? The Co-evolution of Genes and Culture*.

MATERIALS

Each group of students will need:

12 30-mL plastic medicine cups—a sleeve of 250 can be purchased from BioRx Laboratories and other suppliers. The medicine cups should be calibrated in milliliters.
1 permanent marker
18 glucose reagent test strips—some of the less expensive brands include 1 Parameter Glucose Test (URS) Urinalysis Reagent Strip and Diastix Urinalysis Reagent Strips, which are available in quantities of 100 strips per jar.
1 glucose color chart—this will need to be copied from the glucose reagent strips container. Once copied, color charts can be laminated or mounted between two pieces of clear packing tape to protect them so that they can be used many times.
6 plastic stirring rods or coffee stir sticks
1 graduated cylinder that can measure at least 5-mL samples—this is optional if the 30-mL medicine cups have milliliter markings.
1 timer—students will need to wait 30 seconds before reading their test strips; instead of a timer, they can use a clock or watch that measures seconds.
1 clock or watch—students will need to record the times for taking their measurements.
paper towels
colored pencils (optional)
graphing paper (optional)

The entire class will need solutions of patients' samples and controls, as well as milk. The following materials are needed to make these solutions:

4 or more	large beakers or containers with a pouring spout that can hold a 100-mL volume or more
1 per group	smaller beakers or containers with a pouring spout that can hold a 35-mL volume or more
1	graduated cylinder (50 mL or larger)
1	kitchen sieve or funnel
1	box of lactase capsules or Lactaid caplets (Note: If you use an off-brand version, please run a few trials before having students do the lab, as some field testers ran into false-positive issues with generic brand capsules.)
1	coffee filter
1	mortar and pestle (optional)
	tap water

PROCEDURE

Before class, you will need to set up containers with the following solutions: milk, lactase-persistent patient samples, lactase-nonpersistent patient samples, negative control, and positive control. The lactase-persistent patient samples and positive control consist of a solution of water and lactase. The lactase-nonpersistent patient samples and negative control consist of water only.

Lactase-Persistent Patients and Positive Control

- If using lactase capsules, dissolve the contents of three lactase capsules in 50 mL of water. If using Lactaid, grind four Lactaid capsules with a mortar and pestle and dissolve in 50 mL of water.

- Filter the samples through a coffee filter held in a kitchen sieve or funnel. This is necessary because cellulose is often used as a binder in the capsules. The final mixture should look like clear water.
- Every student group will need 5 mL of lactase solution for the positive control and 5 mL for each lactase-persistent patient. For example, **if you have 12 groups of students in your classroom and you designate three lactase-persistent patients, you should make (5 mL × 12 for the positive control) + (5 mL × 12 × 3 for the patients' samples) = 240 mL lactase solution for the whole class.** You may want to make extra in case some solutions are spilled.
- The lactase solution remains viable for an entire day and overnight at room temperature.
- Aliquot enough solution for the positive control and each of the patients that you designate as lactase persistent. Label the containers accordingly (for example, "Patient #1, "Patient #3," "Patient 4," "Positive Control"). For your own reference, note which patients are lactase persistent in the chart below.

Lactase-Nonpersistent Patients and Negative Control

- The lactose-intolerant patient samples and the negative control are just water.
- Every student group will need 5 mL of water for the negative control and 5 mL for each lactase-nonpersistent patient. For example, **if you have 12 groups in your classroom and you designate one lactase-nonpersistent patient, you should make (5 mL × 12 for the negative control) + (5 mL × 12 for the patient sample) = 120 mL water for the whole class.**
- Aliquot enough water for the negative control and each of the patients that you designate as lactose intolerant. Label the containers accordingly (for example, "Patient #2," "Negative Control").

Milk

Each group of students will need 30 mL of milk. You can use skim, low fat, or whole milk. Do not use lactose-free milk or soy milk. Pour 35 mL of milk in as many containers as there are groups of students in the class. **For example, if you have 12 groups in your class, you will need about 420 mL of milk.**

	Example for 12 groups in a class	**Patient #1**	**Patient #2**	**Patient #3**	**Patient #4**	**Positive Control**	**Negative Control**
Amount of solution per container	60 mL						
Type of solution	lactase solution					Lactase solution	Water

TEACHING TIPS

- Have students watch the film *Got Lactase? The Co-evolution of Genes and Culture* before doing this activity. They can watch the film in class, or you could assign it as homework the day before doing the experiment.
- Review the In-Depth Film Guide that accompanies the short film. The guide will provide additional background information that you may want to discuss with your students.
- Include a vocabulary review for students who might get confused by the terms. Some important terms to review might include:
    - enzyme (including clarifying that lactose is the sugar and lactase is the enzyme)
    - lactose intolerant and lactase nonpersistent,
    - lactose tolerant and lactase persistent.
    - control, positive control, and negative control.
- If resources are limited, you may choose to have each group of students test two samples from patients (one lactose intolerant and one lactase persistent) rather than four.
- To speed the lab up, consider modifying how the data is collected.
    - Have each group of students do one patient and one control and then pool the class data.
    - Cut the number of patients to two.
- Questions 8-10 in the analysis section might be challenging for students with limited understanding of digestion, enzymes and/or allergies. You may choose not to include these questions or provide students with additional background information.
- After doing this hands-on activity, you could have your students complete the complementary worksheet entitled "Got Lactase? Blood Glucose Data Analysis." In that activity, students graph actual blood glucose data from several individuals.

ANSWER KEY

Sample Data Table: Student data will vary depending on which patients were designated as persistent and nonpersistent. The glucose amounts may also vary depending on the types of glucose test strips used, the strength of the lactase solution, and variations in experimental procedures.

	0 minutes		**2 minutes**		**7 minutes**	
Samples	**Glucose levels in patient intestinal fluid at 0 minutes. (mg/dL)**	**Start time**	**Time to read 2-min. test strip**	**Glucose levels at 2 minutes (mg/dL)**	**Time to read 7-min. test strip**	**Glucose levels at 7 minutes (mg/dL)**
1 (example of lactase-persistent patient)	0	12:40	12:42	250	12:47	2000
2 (example of lactase-intolerant patient)	0	12:45	12:47	0	12:52	0
Negative Control	0	12:50	12:52	0	12:57	0
Positive Control	0	1:00	1:02	250	1:07	2000

1. *When you were measuring patients' samples, why did you have to use a clean graduated cylinder each time?*
You use a new or clean graduated cylinder each time you measure a solution to prevent contamination.

2. *The chemical reaction that takes place in the positive control sample is shown below.*

Lactose → Glucose + Galactose
↑
enzyme

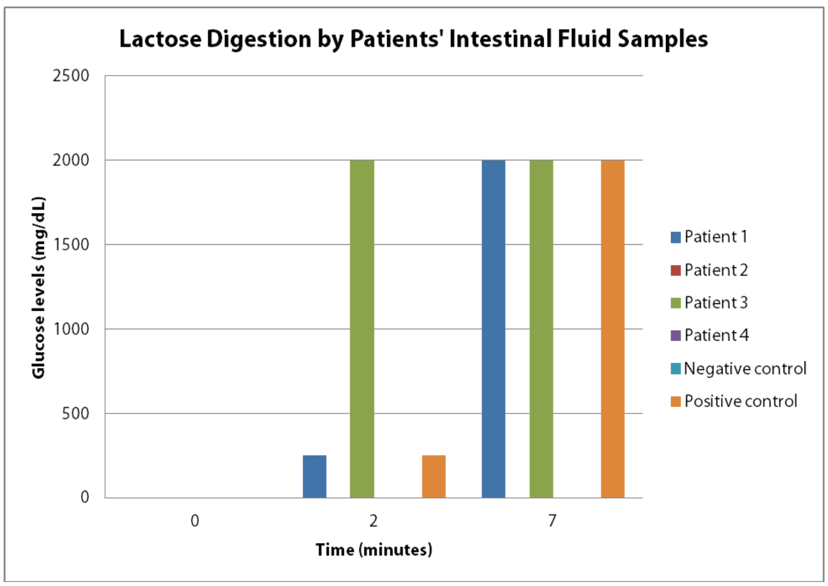
a. *What is the source of lactose?* **Milk**

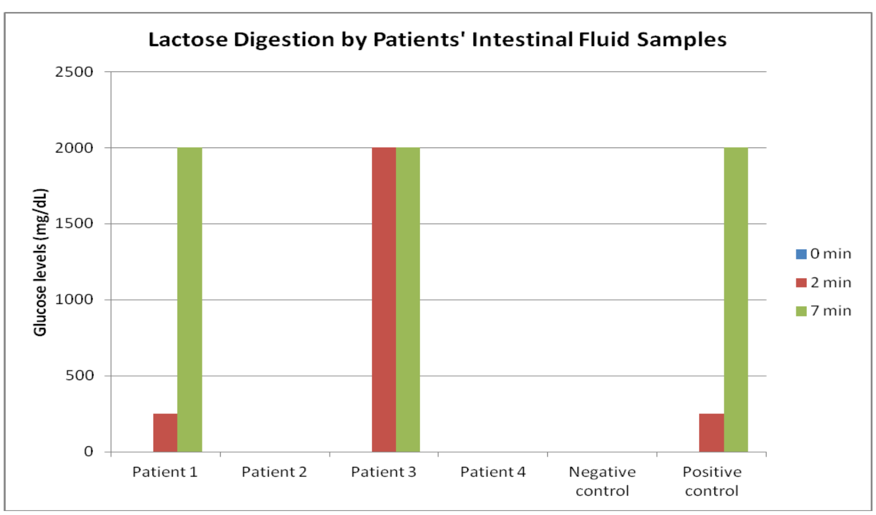
b. *What is the name of the enzyme that digests lactose into glucose and galactose?* **Lactase**

c. *Which component of the chemical reaction did you measure using the test strip?* **Glucose**

d. *What compound in the chemical reaction above is missing from the negative control sample?* **Enzyme (lactase)**

3. *Use colored pencils to construct a bar graph based on your data . The graph should show the results of the 0, 2-, and 7-minute glucose test for all your samples, including the controls. Be sure to provide an appropriate title, labels for the x- and y-axes, and legend.*
Examples of possible student graphs with four patients and the controls are shown below. Results will vary depending on experimental protocols and conditions.





4. a. *Which patient(s)appear to be lactase persistent (lactose tolerant)?*
In this example, Patient #1 and Patient #3. Answers will vary depending on how the lab was set up.

b. *Describe the evidence supporting this claim.*
Glucose levels increased when the intestinal fluids from Patient #1 and Patient #3 were combined with the milk. This indicates that the intestinal fluids contained enough lactase to break down the lactose into glucose and galactose.

5. a. *Which patient(s) appear to be lactase nonpersistent (lactose intolerant)?*
In this example, Patient #2 and Patient #4. Answers will vary depending on how the lab was set up.

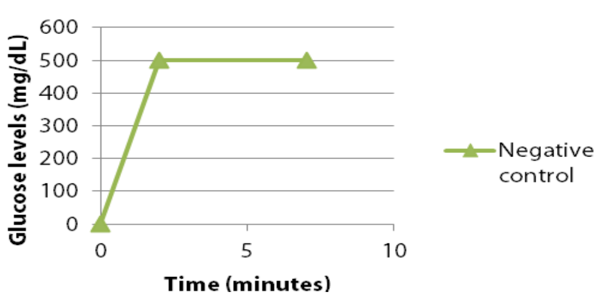
b. *Describe the evidence supporting this claim.*
Glucose levels did not increase when the intestinal fluids from Patient #2 and Patient#4 were combined with the milk. This indicates that the intestinal fluid did not contain lactase.

6. a. *Explain why it was necessary to measure glucose levels in a sample of milk to which 5 mL of water was added (the negative control).*
The water added to the milk serves as a negative control. Water does not contain any lactase, so it should not produce any glucose when combined with milk. If a student detected glucose during this step, it suggests that something went wrong. For example, the water was contaminated, the test strips were not working, or there was an error in the procedure.

b. *Explain why it was necessary to measure glucose levels in a sample of milk to which 5 mL of lactase solution was added (the positive control).*
The lactase solution added to the milk serves as a positive control. Adding lactase to milk should result in the breakdown of lactose into glucose and galactose. If no glucose is detected in the positive control, it's possible that the lactase solution was inactive or the glucose strips were not working.

c. *Why do you need both negative and positive controls?*
Student answers will vary, but they should indicate that the two controls detect different potential errors. For example, if the glucose strips are not detecting glucose, they will always result in a 0 mg/dL reading. The negative control is supposed to give a 0 mg/dL reading, so it would not indicate that the glucose strips are not working. But if the positive control provides a reading of 0 mg/dL, you would know something is wrong.

d. *If the negative control had resulted in the data shown in the graph below, what would you conclude? Provide two possible explanations to account for these results.*
Possible explanations are: There is lactase in the water; there is lactase or glucose in the milk; the test strip is not working properly; a mistake was made when recording the data.
You may want to ask more advanced students how they might differentiate between these possibilities.



7. Instead of measuring glucose levels in the milk, identify another compound you could have measured the levels of to determine whether lactase was active?
Similar results should have been obtained by measuring galactose levels. (In the body, galactose is eventually converted to glucose.)

8. *The glucose test you performed using simulated intestinal fluids required 5 mL of milk and 2 to 7 minutes to obtain results. Explain why in the film Dr. Wells had to consume a liter of milk and blood glucose levels were measured periodically for 40 minutes, a much longer period of time.*
In the test with the simulated intestinal fluids, lactase is in immediate contact with the lactose and quickly breaks it down into glucose and galactose. After a person drinks a liter of milk, the milk must first travel to the small intestine, be broken down by lactase, and then the glucose has to diffuse into the bloodstream where it can be measured. This process takes time. The increase in blood glucose levels is gradual since the milk is not digested all at once.

9. a. *Read the nutrition label on a container of milk. In addition to carbohydrates, which include lactose, list the other nutrients present.*
Depending on the source, students may list: fat, protein, sodium, cholesterol, vitamin A, C, D, and calcium.

b. *If lactose intolerance did not cause any symptoms, lactose-intolerant individuals would be able to use milk as a source of protein. Explain why it is possible for them to digest the proteins in milk even though they lack the lactase enzyme.*
Enzymes are specific. Lactase only digests lactose. Other enzymes are responsible for the digestion of proteins.

c. *Sucrose is a disaccharide present in many cow milk substitutes, such as soy, rice, and almond milk. It is composed of glucose and fructose. Explain why lactose-intolerant individuals may be able to digest sucrose without any problems.*
Students should make the point that sucrose digestion probably requires a different enzyme than ____ lactase. Lactose-intolerant individuals only lack the lactase enzyme but may still produce the enzyme for digesting sucrose.

10. *A milk allergy is the result of an immune reaction to one or more of the components of milk, such as the protein casein. Explain how an allergy to milk is different from lactose intolerance.*
A milk allergy is an immune response which involves the production of antibodies specific to substances in milk, such as casein. Lactose intolerance does not involve the immune system but is the result of an inability to produce the enzyme lactase. You may explain to students that people with milk allergies suffer from different symptoms than people who are lactose intolerant. The symptoms of a milk allergy include hives, wheezing, and vomiting, whereas the symptoms of lactose intolerance are gastrointestinal distress, including gas and diarrhea.

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