Got Lactase?

The Co-evolution of Genes and Culture

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LESSON

STUDENT HANDOUT

DIET AND THE EVOLUTION OF SALIVARY AMYLASE

INTRODUCTION

Over the 200,000 years or so that modern humans have existed, human populations have adapted to a wide range of environments and foods. The availability of new energy-rich foods has resulted in different selection pressures affecting human evolution. For example, when some human populations started consuming milk as adults, the ability to digest lactose, the sugar in milk, provided a survival advantage to individuals with the trait. Over time, the lactose-tolerance trait increased in frequency.

Another example of an evolutionarily important change in diet was the increased availability of starch-rich foods, beginning with the agricultural revolution about 10,000 years ago. Once starch-rich foods became common staples in the human diet, people who were able to effectively digest starch may have had a survival advantage.

Starch is a plant polysaccharide (also called amylose) composed of many building blocks of glucose (a monosaccharide). Starch digestion begins in the mouth with the enzyme alpha-amylase (α-amylase), which is produced by both the salivary glands and the pancreas. Salivary α-amylase breaks the covalent bonds between glucose units in starch by adding a water molecule; this chemical reaction is called hydrolysis. The reaction produces maltose, a glucose-glucose disaccharide. Maltose is further broken down into glucose in the small intestine by the enzyme maltase.

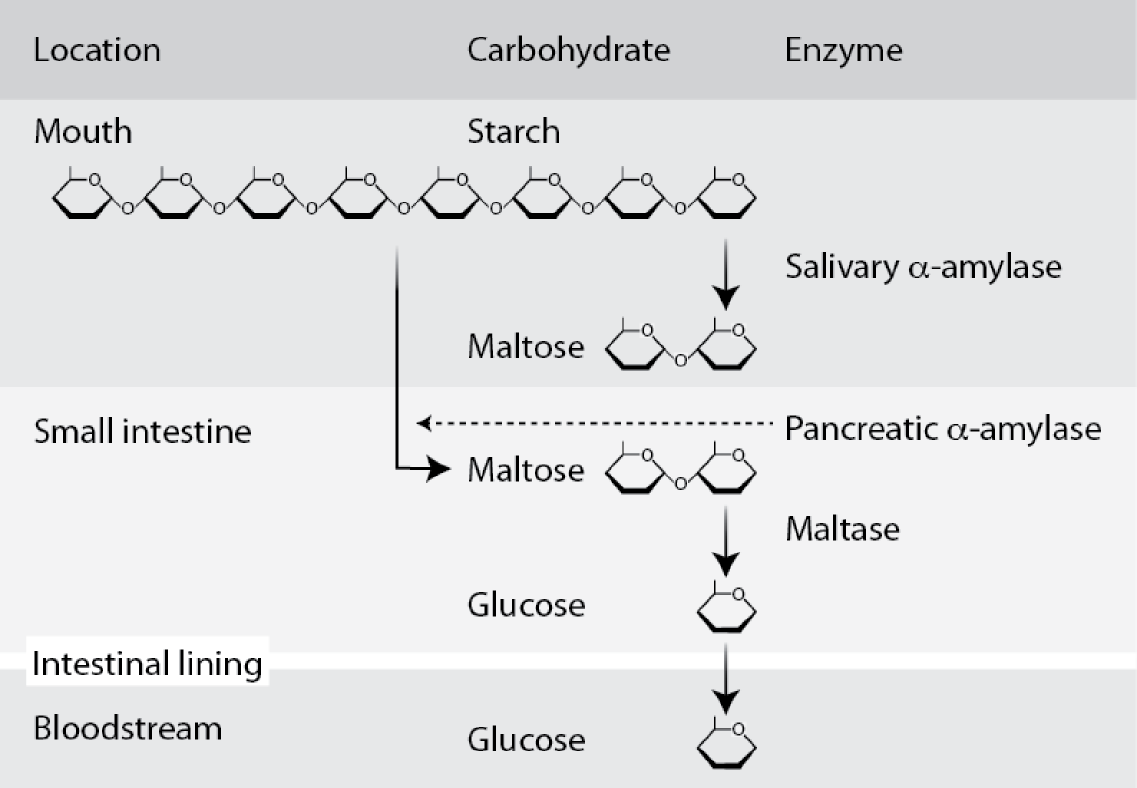

Figure 1. Steps in carbohydrate digestion. Carbohydrate digestion begins in the mouth, where salivary α-amylase attacks the α-glycosidic linkages in starch (or amylose), the main carbohydrate ingested by humans. The digestion of starch by α-amylase produces maltose. The secretion of α-amylase in the small intestine converts any remaining starch molecules to maltose. Maltose is then cleaved into two glucose molecules by maltase. (Disaccharides such as lactose are not digested until they reach the small intestine, where they are cleaved to produce glucose and galactose.) Monosaccharides, such as glucose, are absorbed through the wall of the small intestine into the bloodstream.

In humans, salivary α-amylase is produced by the *AMY1* gene on chromosome 1. Humans are diploid organisms, meaning that they generally have two copies of genes that are not present on the X and Y chromosomes—one copy inherited from each parent. However, genetic studies show that people can have anywhere from two to 15 copies of the *AMY1* gene on each chromosome 1, suggesting that the gene has been duplicated during human evolution.

Why would this be? Researcher George Perry and his colleagues hypothesized that increased consumption of starch-rich foods during human evolutionary history gave individuals with multiple copies of the *AMY1* gene a slight advantage over individuals with only a few copies. If this hypothesis is correct, then you might expect that people living in populations with starch-rich diets would have more copies of the *AMY1* gene than people living in populations lacking starch-rich foods.

To test their hypothesis, Perry and colleagues analyzed DNA collected from two groups of populations. The first group consisted of populations that have historically consumed a diet rich in protein and low in starch, such as hunter-gatherers living in the rainforests or near the Arctic Circle. The other group consisted of populations from agricultural societies and hunter-gatherers living in arid environments, which traditionally eat high-starch foods. The researchers measured the number of copies of the *AMY1* gene in individuals from these different populations.

In this activity, you will analyze some of the data collected by Perry and colleagues, which is shown in Tables 1 and 2.

MATERIALS

- scientific calculator and/or a computer with a spreadsheet program like Excel
- accompanying "Salivary Amylase Data" Excel file, if using a computer
- graphing paper if not using a computer program
- colored pencils for graphing if not using a computer program
- ruler for graphing if not using a computer program

PROCEDURE

Part A. Relationship Between *AMY1* Copy Number and Amylase Production

Before looking at *AMY1* copy number in different populations, Perry and colleagues investigated whether the number of copies of the *AMY1* gene is associated with the amount of amylase in saliva.

The data in Table 1 show the number of *AMY1* gene copies and milligrams (mg) of AMY1 protein per milliliter (ml) of saliva for 25 adult Americans of European descent. The individuals listed in Table 1 were randomly selected from a larger group analyzed in the study by Perry and colleagues (Perry, G.H., *et al*. *Nature Genet*. 2007).

Table 1. *AMY1* Copy Number and Amylase Production Among European-American Populations

Individual	# of *AMY1* Gene Copies	AMY1 Protein in Saliva (mg/ml)
1	7	3.85
2	5	1.09
3	12	5.17
4	6	3.24
5	8	2.80
6	6	3.30
7	7	2.89
8	11	3.76
9	6	2.65
10	3	0.93
11	8	2.46
12	5	1.37
13	5	2.33
14	7	3.37
15	9	3.72
16	7	5.67
17	6	4.61
18	6	4.33
19	3	3.13
20	4	4.24
21	7	4.33
22	8	1.89
23	8	3.48
24	4	1.83
25	7	3.41

1. The data in Table 1 show the number of *AMY1* gene copies and milligrams of amylase protein per milliliter of saliva in 25 adults. On a separate sheet of paper, construct and label a graph that illustrates the relationship between these two measured variables. Include a title for your graph and labels for the *x*- and *y*-axes.

2. From the graph, do the two variables appear to be associated? ______ Explain your answer.

3. Based on the graph, make a claim regarding the number of *AMY1* gene copies and how they relate to the concentration of AMY1 protein in saliva. Explain the claim using scientific reasoning.

4. Based on your claim, suggest a hypothesis to explain why more or fewer copies of a gene would affect the amount of protein produced.

5. Suppose that you analyzed the number of copies of the *AMY1* gene in two individuals. Individual A has four diploid copies of the *AMY1* gene, and individual B has eight diploid copies of the *AMY1* gene. Can you predict with certainty who will have more amylase enzyme in their saliva? Use evidence from your graph and/or Table 1 to support your answer.

Math Extension

Just looking and exploring the graph allowed you to make claims about the relationship between *AMY1* gene copy number and salivary amylase production. Statistical tests are used to provide supporting evidence for these claims by estimating uncertainty.

1. Use Pearson's correlation coefficient from a linear regression test to test the null hypothesis that these two variables listed in Table 1 are not correlated ($r = 0$). What do you conclude based on your calculations?

2. Use a statistical test to determine the strength of the correlation between *AMY1* gene copy number and salivary amylase production. What do you conclude based on your calculations?

Part B: Relationship Between *AMY1* Copy Number and Dietary Starch

Table 2 shows some of the data Perry and colleagues collected on *AMY1* gene copy numbers from different populations. The first group of individuals studied included 11 adult Americans of European descent, six Hadza (Tanzania), and eight Japanese, all of whom eat a high-starch diet. The second group of individuals studied included nine Biaka (Central African Republic), six Mbuti (Democratic Republic of Congo), eight Yakut (Siberia), and two Datog (Tanzania), all of whom eat a low-starch diet. All individuals listed in Table 2 were randomly selected from the larger study published by Perry and colleagues (2007).

Table 2. *AMY1* Copy Number and Dietary Starch Levels

High-Starch Diet Profile		**Low-Starch Diet Profile**	
Population	# of *AMY1* Gene Copies	Population	# of *AMY1* Gene Copies
European-American	4	Biaka	8
European-American	8	Biaka	4
European-American	11	Biaka	2
European-American	6	Biaka	5
European-American	5	Biaka	4
European-American	6	Biaka	4
European-American	6	Biaka	6
European-American	15	Biaka	7
European-American	8	Biaka	4
European-American	8	Mbuti	4
European-American	7	Mbuti	7
Hadza	15	Mbuti	4
Hadza	5	Mbuti	4
Hadza	7	Mbuti	5
Hadza	6	Mbuti	4
Hadza	3	Yakut	9
Hadza	7	Yakut	4
Japanese	10	Yakut	5
Japanese	6	Yakut	5
Japanese	6	Yakut	9
Japanese	5	Yakut	10
Japanese	6	Yakut	8
Japanese	5	Yakut	5
Japanese	6	Datog	2
Japanese	7	Datog	8

1. The data in Table 2 represent the number of *AMY1* gene copies in two groups of populations with different diets. For each diet-profile population, determine the sample sizes and then calculate the means, standard deviations, and 95% confidence intervals of the data. Enter your answers in the box below.

	High-Starch Diet Profile	**Low-Starch Diet Profile**
Sample Size (n)		
Mean ($\bar{x}$)		
Standard Deviation (s)		
95% Confidence Interval $\left(\frac{2s}{\sqrt{n}}\right)$		

2. On a separate sheet of paper, construct and label a graph that summarizes the data in Table 2, illustrating the differences between the two groups. Include 95% confidence interval error bars as well as a title for your graph and labels for the *x*- and *y*-axes.

3. From the graph, how do the two diet profiles compare? Explain your answer.

4. What do the 95% confidence interval error bars tell you about each mean?

5. Suggest a scientific hypothesis (a testable explanation) to explain your answer to question 3 above.

Math Extension

The graphs suggest that populations that consume a high-starch diet and populations on low-starch diets have a different number of copies of the *AMY1* gene.

1. Perform a *t*-test to find out whether this difference is real and not simply due to chance. Explain your results.

2. Based on the data and statistics, make a claim regarding what might explain the difference in *AMY1* copy number between the two groups of populations.

Part C. Design an Experiment

Design a study you could conduct to investigate further the evolutionary relationship between diet and amylase production. With your plan, include

- a new research question;
- the hypothesis you are testing;
- the variables you will measure, including units;
- a measurable prediction.

AUTHOR

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