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, including different 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 in some human populations.

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 composed of many building blocks of glucose, which is a monosaccharide. Starch cannot be dissolved in water or in stomach acid so an enzyme is needed to break it up. Starch digestion begins in the mouth with the enzyme amylase. Salivary 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.

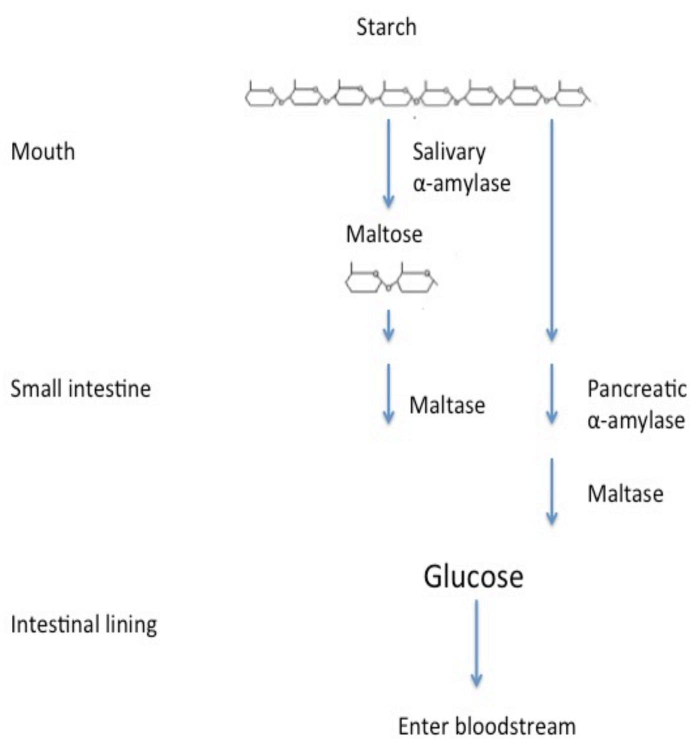

Figure 1. Steps in carbohydrate digestion. Carbohydrate digestion begins in the mouth, where salivary α-amylase attacks the α-glycosidic linkages in starch, the main carbohydrate ingested by humans, to produce maltose. The enzyme α-amylase is also produced in the pancreas (pancreatic α-amylase) and dispensed to the small intestine, where it converts any remaining starch molecules to maltose. Maltose is then cleaved into two glucose molecules by maltase. Monosaccharides, such as glucose, are absorbed through the wall of the small intestine into the bloodstream.

In humans, the *AMY1* gene on chromosome 1 produces salivary amylase. Humans are diploid organisms, meaning that, except for the genes on the X and Y chromosomes, they have two copies of most genes—one copy inherited from each parent. However, genetic studies show that people can have anywhere from two to 15 diploid 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 as some groups of people started consuming more starch, individuals with more copies of the *AMY1* gene would have had a survival advantage. It follows that people living in populations with starch-rich diets would have more copies of the *AMY1* gene than people in populations that don't eat a lot of starch-rich foods (G. H. Perry et al., *Nature Genet.* 2007).

QUESTIONS

1. List the enzymes involved in starch digestion, where they work, and what they do.

2. Name the gene that produces salivary amylase. How many copies of the gene do humans have?

3. The researchers hypothesized that increased consumption of starch-rich foods during human evolutionary history gave individuals with multiple copies of the salivary amylase gene an "advantage." In this context, what does advantage mean?

To test their hypothesis, Perry and colleagues analyzed DNA collected from two groups. 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 tropical 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.

MATERIALS

- Scientific calculator and/or a computer with a spreadsheet program like Excel
- Graph paper, pencils, and a ruler if not using a spreadsheet 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 in different individuals and the milligrams (mg) of AMY1 protein per milliliter (ml) of saliva. These measurements were taken in 25 adult Americans of European descent.

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

Individual	Number 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

Source: G. H. Perry et al., *Nature Genet.* 2007.

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 say with certainty who will have more amylase enzyme in their saliva? Use evidence from your graph and/or Table 1 to support your answer.

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.

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

Source: G. H. Perry et al., *Nature Genet.* 2007.

1. The data in Table 2 represent the number of *AMY1* gene copies in two groups with different diets. For each diet-profile group (i.e. high-starch or low-starch), determine the sample size and then calculate the mean, standard deviation, and 95% confidence interval. Enter your answers in Table 3 below.

Note: The mean you will calculate below is the average number of *AMY1* gene copies for that group and provides an estimate of the average gene copy number of the entire high-starch or low-starch diet population. To know how close this sample mean is to the actual mean of the whole population you can calculate the 95% confidence interval. Basically the 95% confidence interval tells you that the actual population mean is likely to be a value within that interval. The formula provided below for calculating the 95% confidence interval is an approximation of the actual formula. You may use a calculator or spreadsheet program to calculate 95% confidence intervals, which will give you a slightly different result.

Table 3. Calculations for Sample Groups from Table 2

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

Math Extension for Part A (Optional)

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 the claim 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?

Math Extension for Part B (Optional)

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.