Biology 104: Human Biology
Spring 2019 Lab-Like
Activity One

A scientific study: How to collect data, analysis, graph, and interpret the significance of the study

This is to be handed in, in class, on the due date. NO OTHER TIME WILL BE ACCEPTABLE, and will result in zero points for this activity if not handed in as directed.

Print name CLEARLY:_____

Laboratory Objectives

After completing this lab, you should be able to:

1) Interrupt sets of data and develop an understanding of how to graphicly depict the data set.
2) Calculate the average distance from the mean (the average value of the data), known as the standard deviation and identify error and uncertainty.
3) Carry out the task of collecting further data from a graph to produce a second graph in order to determine the K_m of an enzymatic reaction.
4) Communicate your experimental findings, analyses, and interpretations in writing.

Introduction

The main goal of this lab-like activity is to fully understand the analysis and interruption of scientific information obtained from an experiment and to fully explain and

understand the overall trend of the data given to you. For this exercise, you will be looking at data obtained from a study on enzyme activity. The metabolism of a human requires enzymes. Why? Consider that a molecule of the disaccharide sucrose can break down to glucose and fructose on its own, but the process might take days. That same conversion takes just seconds inside your cells. Enzymes make the difference. An enzyme makes a reaction run much faster than it would on its own. The enzyme is unchanged by participating in the reaction, so it can work again and again.

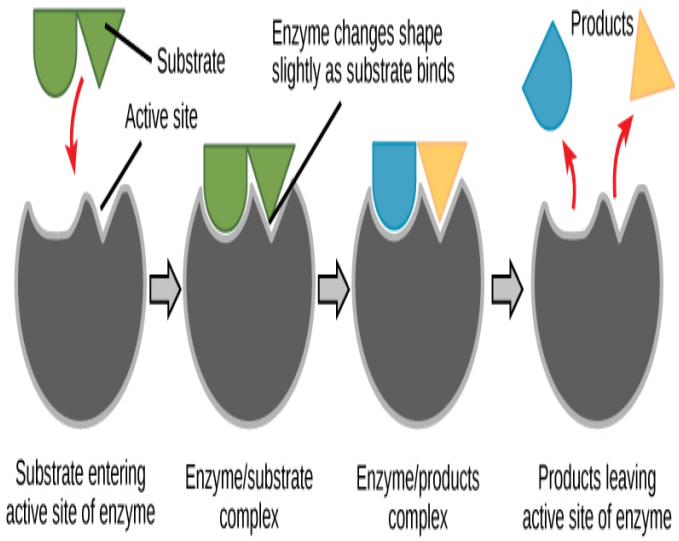

Figure 1: *According to the induced-fit model, both enzyme and substrate undergo dynamic conformational changes upon binding. The enzyme contorts the substrate into its transition state, thereby increasing the rate of the reaction.*

Each kind of enzyme interacts only with specific reactants, or **substrates**, and alters them in a specific way. Such specificity occurs because an enzyme's polypeptide (or nucleotide) chains fold to form a pocket called an **active site**, where substrates bind and a reaction proceeds. An active site is complementary in shape, size, polarity, and charge to the enzyme's substrate. That fit is the reason why each enzyme acts in a specific way on a specific substrate (Figure 1).

An enzyme speeds a reaction by reducing activation energy, so it lowers the barrier that prevents the reaction from proceeding. When we talk about activation energy, we are really talking about the energy required to bring reactant bonds to the breaking point. An active site can bring reactants to this state by (for example) holding them in a certain position, squeezing them, or redistributing their charge.

There are two ways to measure enzyme activity: (1) determine the rate of disappearance of the substrate, and (2) determine the rate of appearance of the product. The experiment that you have been given the data for was on the *in vitro* activity of the enzyme sucrase on the substrate of common sucrose (table sugar). The assay was set up to measure to release and appearance of fructose as the enzyme broke the glycosidic linkage between glucose and fructose (which is the disaccharide sucrose) as shown in Figure 2.

In Humans, sucrase is secreted by the tips of the villi of the epithelium in the small intestine. This enzymatic reaction is an example of hydrolysis, a chemical reaction in which water is used to break down a compound; this is achieved by breaking a covalent bond in the compound by inserting a water molecule across the bond.

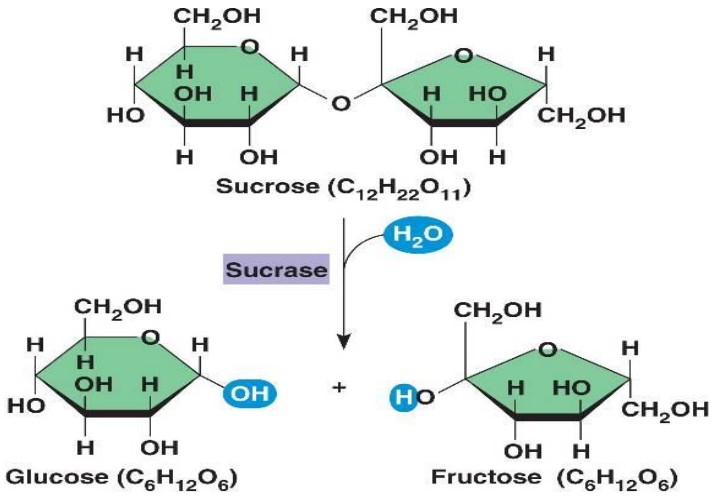

Figure 2: *Hydrolysis enzyme reaction of sucrase to produce glucose and fructose from the disaccharide sucrose.*

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The glucose is easy to metabolize in cell respiration or it can be stored in the liver as glycogen. However, excessive consumption of fructose has been shown to contribute to insulin resistance, obesity, an elevation of LDL cholesterol, and triglycerides - all leading to the onset of metabolic syndrome (Malik and Hu, 2015), type 2 diabetes, and cardiovascular disease (Rippe and Angelopoulos, 2015).

The experiment and the data

The experiment was set up to investigate the rate (speed) of the activity of the enzyme sucrase on the substrate (the disaccharide sucrose). Four different concentrations of sucrose were used with a constant concentration of enzyme (0.5 mg ml^{-1}) in a constant reaction volume. The experiment was set up in triplicate (n =3) and a control containing no enzyme was used in each data set. At each time course a 2ml sample was taken. To each sample was added 1ml of Resorcinol reagent (1g resorcinol and 0.25 g thiourea in 100 ml glacial acid acid) followed by 7 ml of 80% HCl. Each sample was mixed, incubated at 90°C for 15min, cooled and the absorbance read at 520 nm (Foreman *et al.*, 1973).

In each case, the control indicated an absorbance at 520 nm of zero.

So, to analysis the data

- Calculate the average absorbance value **Mean ($\bar{X}$)** for the three repeats at each time course for each concentration of sucrose and add them into tables 1A to 1D respectively.

- Calculate the Difference from the mean **$(X-\bar{X})$** for the three repeats at each time course for each concentration of sucrose and add them into tables 1A to 1D respectively.

- Calculate the (Difference from mean)^2 **$(X-\bar{X})^2$** for the three repeats at each time course for each concentration of sucrose and add them into tables 1A to 1D respectively.

- Calculate the sun (∑) of the (Difference from the mean)^2 **$\sum(X-\bar{X})^2$** for each concentration of sucrose and add them into tables 1A to 1D respectively.

- Calculate the Variance. The term *variance* was first introduced by Sir Ronald Aylmer Fisher, FRS (1918), and is a measurement of how far a data set are spread out from their average value. This is the Sum of (Difference from the Mean)^2 divided by the degrees of freedom (n – 1).
  So: $\frac{\sum(X-\bar{X})^2}{(n-1)}$
  Do this for each concentration of sucrose and add them into tables 1A to 1D respectively.

- Now onto the final step: Determine the standard deviation. This is a measure that is used to quantify the amount of variation or dispersion of a set of data values. A low standard deviation indicates that the data points tend to be close to the mean (also called the expected value) of the set, while a high standard deviation indicates that the data points are spread out over a wider range of values. (Bland and Altman, 1996). The standard deviation is calculated as the square root of the variance (the value you just calculated). So: $\sqrt{\frac{\sum(x-\bar{x})^2}{(n-1)}} =$
  Do this for each concentration of sucrose and add them into tables 1A to 1D respectively.

Graphing the Data

On the first page of graph paper plot the time (in mins) on the horizontal X – axis against the mean **$(\bar{X})$** values for the absorbance (at 520 nm) for each time period on the vertical Y - axis. Do this for each of the different concentrations of substrate, so a line plot for**1 mg ml^{-1}** sucrose, **2 mg ml^{-1}** sucrose, **3 mg ml^{-1}** sucrose, and lastly 4 **mg ml^{-1}** sucrose. You will have four line plots on this one sheet of graph paper. Label each one clearly with its concentration and units.

Table 1A. Absorbance 520nm Values over Time – 1 mg ml^{-1} Sucrose substrate

Sample/time	0 min	5 min	10 min	15 min	20 min
1st run	0.000	0.234	0.264	0.344	0.408
2nd run	0.021	0.246	0.276	0.354	0.411
3rd run	0.009	0.239	0.304	0.328	0.403
Mean ($\overline{X}$)					
($X-\overline{X}$)	1) 2) 3)	1) 2) 3)	1) 2) 3)	1) 2) 3)	1) 2) 3)
$(X-\overline{X})^2$	1) 2) 3)	1) 2) 3)	1) 2) 3)	1) 2) 3)	1) 2) 3)
$\Sigma(X-\overline{X})^2$					
$\frac{\Sigma(X-\overline{X})^2}{(n-1)}$					
$\sqrt{\frac{\sum(x-\overline{x})^2}{(n-1)}}$					

Table 1B. Absorbance 520nm Values over Time – 2 mg ml^{-1} Sucrose substrate

Sample/time	0 min	5 min	10 min	15 min	20 min
1st run	0.003	0.356	0.498	0.588	0.644
2nd run	0.009	0.347	0.501	0.576	0.650
3rd run	0.017	0.343	0.509	0.598	0.639
Mean ($\overline{X}$)					
($X-\overline{X}$)	1) 2) 3)	1) 2) 3)	1) 2) 3)	1) 2) 3)	1) 2) 3)
$(X-\overline{X})^2$	1) 2) 3)	1) 2) 3)	1) 2) 3)	1) 2) 3)	1) 2) 3)
$\Sigma(X-\overline{X})^2$					
$\frac{\Sigma(X-\overline{X})^2}{(n-1)}$					
$\sqrt{\frac{\sum(x-\overline{x})^2}{(n-1)}}$					

Table 1C. Absorbance 520nm Values over Time – 3 mg ml^{-1} Sucrose substrate

Sample/time	0 min	5 min	10 min	15 min	20 min
1st run	0.011	0.456	0.655	0.733	0.783
2nd run	0.005	0.501	0.621	0.698	0.795
3rd run	0.004	0.464	0.638	0.721	0.788
Mean ($\overline{X}$)					
$(X-\overline{X})$	1) 2) 3)	1) 2) 3)	1) 2) 3)	1) 2) 3)	1) 2) 3)
$(X-\overline{X})^2$	1) 2) 3)	1) 2) 3)	1) 2) 3)	1) 2) 3)	1) 2) 3)
$\Sigma(X-\overline{X})^2$					
$\frac{\Sigma(X-\overline{X})^2}{(n-1)}$					
$\sqrt{\frac{\sum(x-\overline{x})^2}{(n-1)}}$					

Table 1D. Absorbance 520nm Values over Time – 4 mg ml^{-1} Sucrose substrate

Sample/time	0 min	5 min	10 min	15 min	20 min
1st run	0.000	0.655	0.802	0.842	0.840
2nd run	0.002	0.661	0.818	0.839	0.838
3rd run	0.002	0.670	0.811	0.836	0.838
Mean ($\overline{X}$)					
$(X-\overline{X})$	1) 2) 3)	1) 2) 3)	1) 2) 3)	1) 2) 3)	1) 2) 3)
$(X-\overline{X})^2$	1) 2) 3)	1) 2) 3)	1) 2) 3)	1) 2) 3)	1) 2) 3)
$\Sigma(X-\overline{X})^2$					
$\frac{\Sigma(X-\overline{X})^2}{(n-1)}$					
$\sqrt{\frac{\sum(x-\overline{x})^2}{(n-1)}}$					

Using the standard deviation values you have calculated, accurately draw in these values from the Y axis scale above and below the data points for each time period and for each sucrose concentration line plot. This bar graphically depicts the variation around the mean for the data set.

To determine the speed or velocity of the enzyme reaction you need to first determine the initial rate of the reaction in each of the concentrations of substrate used. The initial rate is calculated over the region of the reaction where product formation is occurring in a linear fashion, as shown in figure 3.

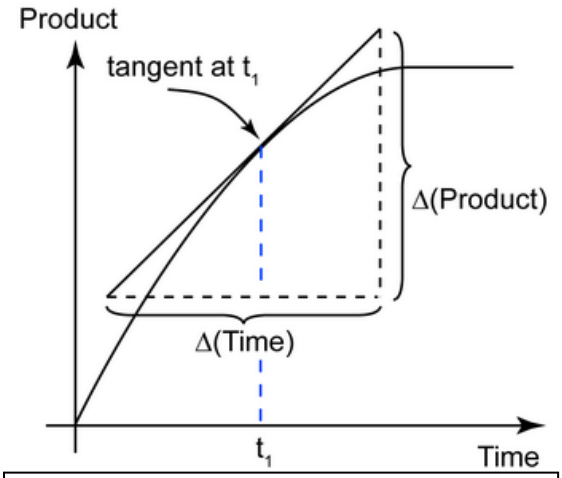

Figure 3: *Example plot of how to determine the fastest initial rate of the production of the product of an enzymatic reaction.*

This is where there is the most significant change in the production of the product being measured in the experiment. In other words, where the largest change in absorbance at 520 nm can be seen.

To make this a little simpler, from the data provided in tables 1A through 1D and from your first plotted graph, the most significant change in absorbance, and thus the quickest formation of product produced from the enzyme reaction, occurs within the first 5 mins for each concentration of sucrose used.

So for each substrate concentration used determine the change in absorbance which occurs over that time. **In this case, the absorbance value at 5 mins minus the absorbance value at 0 mins divided by 5**.

$$\text{Initial Rate} = \frac{\text{Change in Absorbance}}{\text{Time}} = \frac{\Delta \text{ Abs}}{\text{min}}$$

Now you should have four rate values – one for each of the four concentrations of sucrose used in the reaction.

On the second page of graph paper plot the four concentrations of the substrate sucrose (**mg ml^{-1} sucrose**) on the horizontal X – axis against the rate values on the vertical Y - axis. Draw a line to connect the data points, starting at zero. This is a Michaelis-Menten plot, as shown in figure 4. The Michaelis–Menten kinetic model is one of the best-known explanations of enzyme kinetics, describing the rate of enzymatic reactions, by relating reaction rate (rate of formation of product) to the concentration of a substrate (Michaelis and Menten, 1913).

The substrate concentration that produces a V_i that is one-half of V_{max} is designated the Michaelis-Menten constant, K_m This K_m value is (roughly) an inverse measure of the affinity or strength of binding between the enzyme and its substrate. The lower the K_m, the greater the affinity (so the lower the concentration of substrate needed to achieve a given rate).

From your second graph plot, draw a line to the V_{max} value shown on your graph (use figure 4 as a guide) and clearly label it. From the value of your V_{max} on the Y axis determine half of its value. Draw a line from the Y axis from the $\frac{1}{2}$ V_{max} value to the plot curve and then draw a line direction down to the X axis, as shown in figure 4, to determine the K_m of the enzyme sucrose and its affinity to release fructose from the breakdown of sucrose.

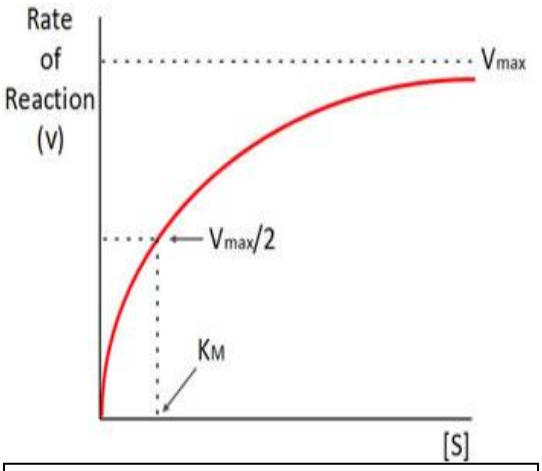

Figure 4: *Example of a Michaelis-Menton plot, including how to determine the V_{max} and the K_m*

Questions

All questions must be fully answered for maximum possible points awarded.

1) What is the k_m of the sucrase used in this experiment?

2) What is the significance of this K_m value to the enzymatic breakdown of sucrose in this experiment?

3) The release of fructose appeared to remain constant from 15 mins to 20 mins when a substrate concentration of 4 mg ml^{-1} sucrose was used. Explain why.

4) In each case, the control indicated an absorbance at 520 nm of zero. What is the significance of this to the experimental set up?

5) The excessive consumption of fructose has been shown to contribute to many health problems in humans. Pick one, describe it fully in your own words, describing what organs are affected and all detrimental aspects to human health which occur. Cite at least two separate primary references. Type this up and staple it to this lab activity.

References

Bland, J.M. and Altman, D.G. (1996). Statistics notes: measurement error. *British Medical Journal*.**312** (7047): 1654.

Fisher, R.A. (1918) The Correlation Between Relatives on the Supposition of Mendelian Inheritance. *Translations of the Royal Society of Edinburgh*, **52**: 399 – 433.

Foreman. D. Gaylor. L. Evans. E. & Trella. C. (1973) A modification of the Roe procedure for determination of fructose in tissues with increased specificity. *Analytical Biochemistry,* **56:** 584-589.

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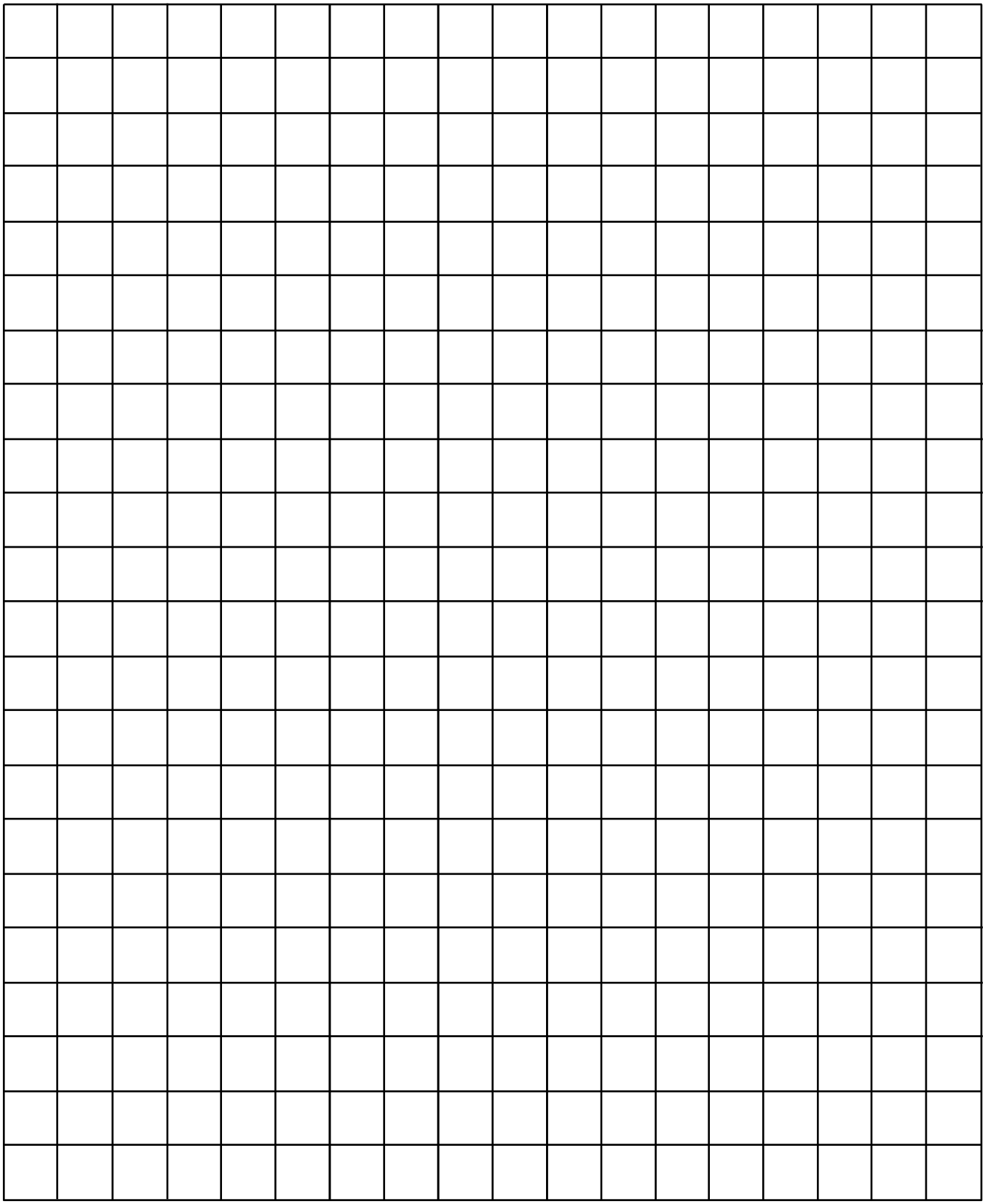
Malik, V.S. and Hu, F.B. (2015). Fructose and Cardiometabolic Health: What the Evidence from Sugar-Sweetened Beverages Tells Us. *Journal of the American College of Cardiology,* **66 (14):** 1615–1624.

Rippe, J.M. and Angelopoulos, T.J. (2015). Fructose-containing sugars and cardiovascular disease. *Advances in Nutrition,* **6 (4):** 430–439.

1-CENTIMETER GRID PAPER



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