

# Why Can't I Have Sugar? All About Diabetes

Eric DeJulio

University of Washington

In collaboration with Pamela Allweiss, National Center for Chronic Disease Prevention and Health Promotion, Centers for Disease Control and Prevention

*Disclaimer: The findings and conclusions in this report are those of the author(s) and do not necessarily represent the views of the Centers for Disease Control and Prevention.*

## **Why Can't I Have Sugar? All About Diabetes**

Eric DeJulio  
University of Washington  
Seattle, WA

### **Summary**

This lesson is designed to be part of a unit about how the body works. Students will learn about diabetes and glucose metabolism through skits and class discussion. They will assume the role of doctor and develop scripts to explain diabetes to patients and construct models of glucose metabolism to demonstrate how diabetes works. This lesson also serves as an opportunity to educate students about an important public health concern—the worldwide epidemic of diabetes.

### **Learning Outcomes**

- Students will build models representing the differences in sugar metabolism of people with type 1 diabetes, with type 2 diabetes, and without diabetes.
- Students will develop and interpret a model about the development of diabetes.
- Students will learn about risk factors for diabetes.

### **Social Outcomes**

- Students will learn the importance of group interdependence and skills to achieve a result when working towards a collaborative goal.

### **Materials**

1. Supplies for the modeling component of the “Diabetes Doctors” activity in Step 4 (styrofoam balls, toothpicks, marshmallows, tape, construction paper, butcher paper, markers, and any other materials easily available.)
2. Nametags (or other fun identifying markers) for students involved in skit
3. Optional: props for the skit in Step 1, as listed in the introduction of the skit
4. Butcher paper or a large pad of paper
5. Markers
6. Computers with Internet access for student research (one computer per group)

### **Total Duration**

3 hours, 20 minutes (2 x 100-minute blocks)

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## **Procedures**

### **Teacher Preparation**

Review the websites listed in the Web Resources section for background information on diabetes and glucose metabolism. Familiarize yourself with the “Desperately Seeking Insulin” skit used in the Introduction. The script provides a list of props; you may choose to use the props or not. Before starting the class, gather and make ready all necessary props for the skit. Additionally, assemble supplies for the modeling component of the “Diabetes Doctors” activity. Make sure computers with Internet access will be available for student research. Prepare class copies of the “Desperately Seeking Insulin” skit, found in the Introduction, and the “Diabetes Doctors” and “Diabetes Doctors Rubric” used in Step 4.

## Web Resources

Title: Insulin Resistance and Pre-Diabetes

URL: <http://diabetes.niddk.nih.gov/dm/pubs/insulinresistance/index.htm>

Description: This NIH website contains information about insulin resistance and how it is related to type 2 diabetes. It also gives some information about preventing type 2 diabetes.

Title: Insulin Resistance

URL: <http://syndromex.stanford.edu/InsulinResistance.htm>

Description: This website from Stanford University contains background information about insulin resistance, which is the one defining factor of type 2 diabetes.

Title: National Diabetes Fact Sheet

URL: [www.cdc.gov/diabetes/pubs/factsheet.htm](http://www.cdc.gov/diabetes/pubs/factsheet.htm)

Description: This Centers for Disease Control and Prevention website contains background information and statistics about diabetes. It also includes links to many other sites that have useful diabetes background information.

Title: Medline Plus Interactive Tutorials: Diabetes Introduction

URL: [www.nlm.nih.gov/medlineplus/tutorials/diabetesintroduction/htm/index.htm](http://www.nlm.nih.gov/medlineplus/tutorials/diabetesintroduction/htm/index.htm)

Description: Medline Plus has a variety of tutorials on health topics and conditions. This link contains a diabetes overview that can be viewed as an interactive tutorial or as a PDF document.

### Step 1: Introduction

**Duration: 50 minutes**

Begin the class by asking students to do a quick-write answering the following questions: Do you know someone with diabetes? What is diabetes? What are its causes? Instruct students to write for 3 minutes. Because of the prevalence of diabetes, it is likely that students might have diabetes or have family members with diabetes. Therefore, it is important to treat this topic in a sensitive manner and encourage students to share their knowledge of diabetes.

Next, inform students that they will be performing a two-act skit to learn more about diabetes and how the human body metabolizes glucose. Pass out copies of the "Desperately Seeking Insulin" script (see the Web Resource section that follows). Assign or ask for volunteers for each part, then give students time to review the script. These skits can be adapted to include all students, although not every student will get a speaking role. Students will perform the skit to learn about the differences between type 1 and type 2 diabetes.

Note: If there are not enough roles for all students to participate in both acts, assign students to focus on a particular scene during the skit. Students who are observing could be given the following prompt: What is happening in this act that is similar or different from your act? This responsibility will focus student attention on the content of the skit, and you can use this information to lead into the class discussion of the skit.

### Web Resource

Title: Desperately Seeking Insulin

URL: <http://teachhealthk-12.uthscsa.edu/pa/pa10/1001A.htm>

Description: This short, two-act skit helps explain the process of glucose metabolism (or loss of glucose metabolism, or both), which occurs in both type 1 and type 2 diabetes.

### **Step 2**

**Duration: 40 minutes**

After the skit is completed, lead a comparative discussion about the two different types of diabetes using the “Discussion Guide for Teachers” provided in the Supplemental Documents section. Be sure to acknowledge students’ ideas because they might have experience dealing with people who have diabetes. Record the information discussed on butcher paper. As part of the discussion, students should create a visual model of type 1 and type 2 diabetes. The information should be recorded on butcher paper in a manner that allows for easy comparison of the two conditions.

Discussion points are included in the “Discussion Guide For Teachers.”

### **Supplemental Document**

Title: Discussion Guide for Teachers

File Name: Discussion Guide for Teachers.doc

Description: This Word document contains the teacher instructions for leading the class discussion about diabetes.

### **Step 3**

**Duration: 80 minutes**

In this step, students will solidify their understanding of diabetes by pretending that they are doctors counseling patients who have been diagnosed with type 1 or type 2 diabetes. Inform students that they are going to become doctors for the day. Pass out the “Diabetes Doctors” handout and review it with the students. Divide students into groups of three and assign each group a topic: either type 1 or type 2 diabetes. Explain that each group will present a script and model to another group that has researched the other type of diabetes. Instruct students to provide a brief description of their respective patient and the patient’s symptoms. Students should draw on their knowledge from the previous day’s class session and use the websites provided to write a script that explains to patients what is going on inside their bodies and how the patients can improve their health. Each group of students should also use the materials provided to design a visual model of the processes involved in disease development and prevention or treatment. Explain that each student in the group will be given a section to present: description of symptoms, description of process, and description of treatment options and how they work, as outlined in the “Diabetes Doctors” handout. Finally, go over the “Diabetes Doctors Rubric” to make sure that students understand what is expected of them. After students have prepared their presentations, pair up the groups to present their scripts and models to each other. Students will assess the other group’s presentation using the “Diabetes Doctors Rubric.” The teacher will assess students using the “Diabetes Doctors Worksheet” (85% of their grade) and the peer evaluations of their presentation (15% of their grade.)

### **Supplemental Document**

Title: Diabetes Doctors

File Name: Diabetes Doctors.doc

Description: This Word document contains the instructions for the Diabetes Doctors activity.

Title: Diabetes Doctors Rubric

File Name: Diabetes Doctors Rubric.doc

Description: This Word document contains the rubric for the diabetes lesson post-assessment.

Title: Diabetes Doctors Worksheet

File Name: DiabetesDoctorsWorksheet.doc

Description: This Word document contains the group worksheet for the Diabetes Doctors activity.

Title: Diabetes Doctors Worksheet Key

File Name: DiabetesDoctorsWorksheetKey.doc

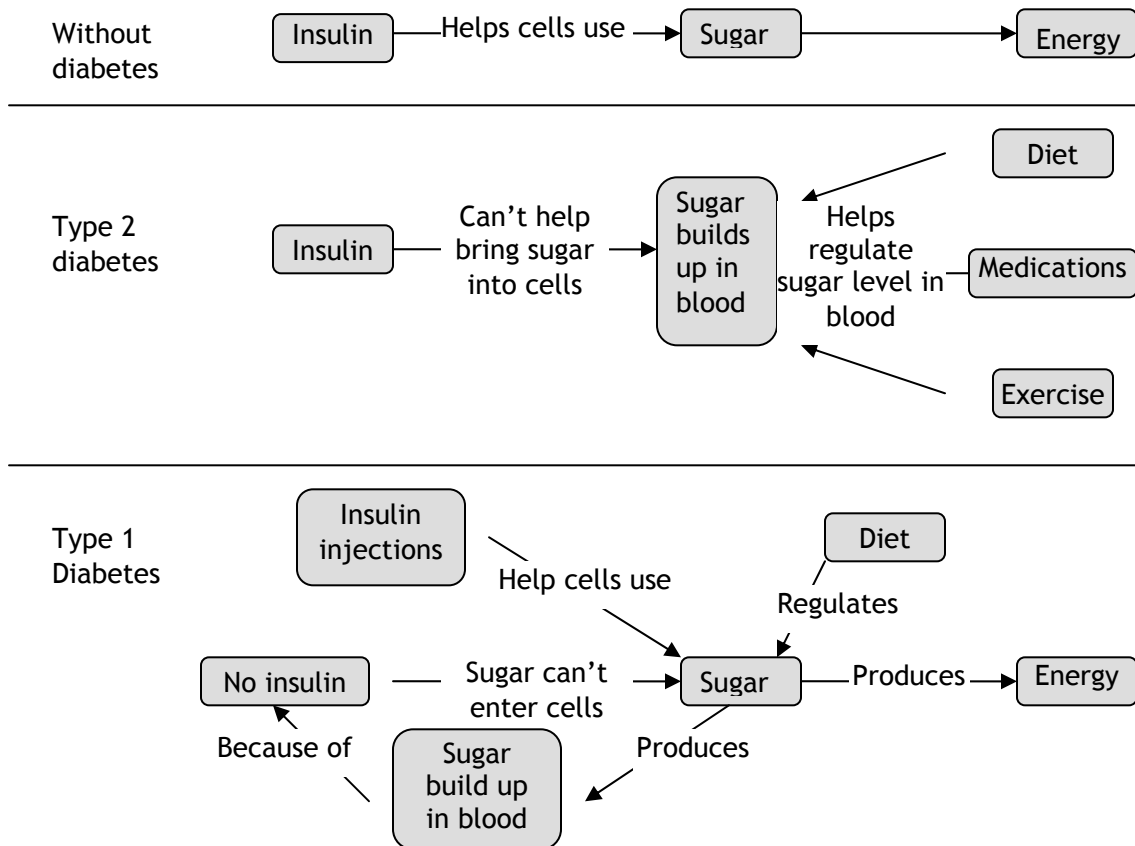
Description: This Word document contains the answer key for the group worksheet for the Diabetes Doctors activity.

#### Step 4

**Duration: 15 minutes**

Students will wrap up this learning activity by revisiting the models that were developed in Step 2.. Begin by debriefing the students and posing the following questions: What did we learn about the differences between type 1 and type 2 diabetes? What happens in the body to cause these diseases to occur? How do we prevent or treat these diseases?

Lead the students in revising the initial models to represent their new understanding. An example of a revised model follows:



**Conclusion****Duration: 5 minutes**

Because this is a social learning process as well as a content learning process, the students will be instructed to evaluate their group work skills. Have students answer the following questions about their participation and turn in their answers before they leave class:

- How did you contribute to your group's success?
- How can you improve your group work skills for next time?

**Assessment**

The students' knowledge of diabetes is assessed by having them complete a worksheet in Step 3. Additionally, students will be assessed by their peers on their group presentations in Step 3.

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**Modifications****Extension(s)**

Depending on the resources and contacts you have, there are many interesting, engaging activities that students can do with rat or mouse islets. Islets can be inspected under the microscope, dithizone stained to show zinc, and cultured in the presence of high and low glucose (see **Static culture of islets**) to cause insulin secretion (measured by ELISA test). Contact your local health department, hospital, diabetes research center, or university to try to locate these types of resources.

Possible questions the teacher might ask students:

1. Why did we put the islets in both high- and low-glucose-containing media? What will this tell us?
2. ELISA tests are used to detect all types of things in your body, especially infections. How do you think these tests can do this?

**Web Resources**

Title: Insulin ELISA

URL: [www.alpco.com/single.asp?CatNumber=08-10-1124-01](http://www.alpco.com/single.asp?CatNumber=08-10-1124-01)  
[www.lincoresearch.com/products/ezrmi-13k.html](http://www.lincoresearch.com/products/ezrmi-13k.html)

<http://www.crystalchem.com/products/RatMouseELISAKits.html>

Description: These are resources for ordering insulin ELISAs (to measure the amount of insulin in samples). These tests are expensive and resource intensive, but they would be a good biotechnology link as well.

**Supplemental Document**

Title: Static Culture of Islets

File Name: StaticInstructions.doc

Description: This document contains the instructions for performing a static insulin release assay with rat islets. This can be used as an extension biotechnology activity for this lesson.

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**Education Standards**

## **National Science Education Standards**

### LIFE SCIENCE, CONTENT STANDARD C:

As a result of activities in grades 5–8, all students should develop an understanding of

- **Structure and function in living systems**
- Reproduction and heredity
- **Regulation and behavior**
- Populations and ecosystems
- Diversity and adaptations of organisms

### SCIENCE IN PERSONAL AND SOCIAL PERSPECTIVES, CONTENT STANDARD F:

As a result of activities in grades 5–8, all students should develop an understanding of

- **Personal health**
- Populations, resources, and environments
- Natural hazards
- Risks and benefits
- Science and technology in society

## **Washington State Standards**

Essential Academic Learning Requirements (EALRs):

EALR 1.2.1—Analyze how the parts of a system interconnect and influence each other.

EALR 1.3.8—Understand how individual organisms, including cells, obtain matter and energy for life processes.

EALR 3.1.1—Analyze common problems or challenges in which scientific design can be or has been used to design solutions.

## Discussion Guide for Teachers

Why Can't I Have Sugar? All About Diabetes  
Eric DeJulio, CDC's 2005 Science Ambassador Program

Class discussion about diabetes in Step 2 should include the following:

- I. General overview of how our bodies use sugar: Our bodies turn the food that we eat into sugar (glucose). The blood circulating in the body carries glucose to the cells. The cells use the glucose, along with other materials, to make energy. Glucose leaves the bloodstream and enters the cells through "doors" called receptors. A substance called insulin works like a key to unlock the receptors. Insulin is a substance that your body makes in an organ called the pancreas. So, in general, diabetes is a disease that makes it difficult for the cells of the body to get the glucose they need to make energy. (Adapted from [www.nlm.nih.gov/medlineplus/tutorials/diabetesintroduction/id029102.pdf](http://www.nlm.nih.gov/medlineplus/tutorials/diabetesintroduction/id029102.pdf))
- II. General overview of different types of diabetes (should include a visual representation)
  - A. Type 1 diabetes: This form of diabetes occurs when the body produces little to no insulin and, therefore, is unable to process sugar. People with this form of diabetes are dependent on insulin injections to provide insulin for the body. Type 1 diabetes was once called juvenile diabetes because it is frequently diagnosed in children and young adults.
    1. Symptoms:
      - a. Constant hunger; excessive urination; sudden weight loss for no reason; rapid, hard breathing; sudden vision changes; weakness; drowsiness or exhaustion; fruity odor of breath.
  - B. Type 2 diabetes: This is the form of diabetes in which the body does not respond properly to insulin. Cells might not have enough receptors present to allow glucose to enter. In the beginning, the body is able to keep up with the demands of insulin production, but it loses this ability over time. This form of diabetes was previously termed adult-onset diabetes, although it can occur in children, too.
    1. Risk factors and symptoms
      - a. Risk factors include age, obesity, family history of diabetes, history of gestational diabetes, impaired glucose metabolism, physical inactivity, and race or ethnicity.
      - b. Symptoms include frequent urination, excessive thirst, unexplained weight loss, extreme hunger, sudden vision changes, tingling or numbness in the hands or feet, feeling very tired much of the time, very dry skin, sores that are slow to heal, or having more infections than usual.
- Note: The visual representation of diabetes can be of many different types. A few examples include:
  - a. A flow chart of the movement of sugar in the body.
  - b. Following the path of sugars inside a drawing of the human body.
- III. Discussion of how the two different types of diabetes are similar or different. Be sure to include a discussion of how each type of diabetes affects insulin production.
  - A. Type 1 diabetes: No beta cells in the islets results in no insulin production.



- B. Type 2 diabetes: Cells are not able to use up all of the insulin that was secreted. Pancreas responds by releasing more insulin. Individuals will have high levels of insulin and blood glucose at the same time.
- IV. Effects of the disease
  - A. Emotional
    - 1. Not being the same as peers
    - 2. Possible peer alienation
    - 3. Having to take insulin injections or medications
    - 4. Just wanting to be “normal”
  - B. Physical
    - 1. Having to take insulin injections or medications
    - 2. Controlled diet
    - 3. Tiredness from low blood sugar (hypoglycemia)
    - 4. Frequent urination
    - 5. Risk of complications
- V. Diabetes treatment discussion (Step 3) should contain the following information:
  - A. Insulin injections (types 1 and 2)—Because the body is not producing a sufficient amount of insulin, people with diabetes inject insulin to help break down sugar. People with type 1 diabetes use insulin to control their blood sugar. Sometimes people with type 2 diabetes need to use insulin, too.
  - B. Exercise (types 1 and 2)—Exercise can lower glucose levels in the body because cells use more energy when you exercise than when you rest.
  - C. Controlled diet—By controlling the types of food they eat, people with diabetes can help regulate the amount of insulin required to break down the food.
  - D. Drug therapy (type 2)—There are many medications available to help people with type 2 diabetes achieve better glucose control. These medications work in many different ways. Some increase insulin production. Some increase the cell’s sensitivity to insulin, helping the body to use its own insulin more efficiently. Some help the liver metabolize sugar. Some alter the absorption of sugar. Some newer medications can also alter satiety (how the body feels “full”) and can alter other hormones such as glucagon, which also affect how the body uses food.

Discussion information adapted from:

[www.nlm.nih.gov/medlineplus/tutorials/diabetesintroduction/id029102.pdf](http://www.nlm.nih.gov/medlineplus/tutorials/diabetesintroduction/id029102.pdf)

[www.kidslearnaboutdiabetes.org/what\\_is.html](http://www.kidslearnaboutdiabetes.org/what_is.html)

[www.health.ri.gov/disease/diabetes/children.php](http://www.health.ri.gov/disease/diabetes/children.php)

<http://diabetes.niddk.nih.gov/dm/pubs/insulinresistance/index.htm#7>

## **Diabetes Doctors General Instructions**

Why Can't I Have Sugar? All About Diabetes  
Eric DeJulio, CDC's 2005 Science Ambassador Program

### **Background:**

Food gives people energy to live. What happens when a person's ability to break down food doesn't work correctly? How can diabetes be prevented or treated?

### **Your Task/Your Audience:**

Imagine that you are a doctor who is seeing a patient. You diagnose that the patient has diabetes (your group will be assigned either type 1 or type 2 diabetes to work with). Your task is to develop a script to explain your diagnosis and a model to teach the patient about diabetes. This person has little or no knowledge about the disease, so you should develop your explanation to help this patient.

### **Why Am I Doing This?**

It's fun! You get to be the expert on the topic and help teach others about this disease. The focus for this presentation is on getting the content to the patient in an way he or she can understand—the patient really needs to know how to help control his or her condition. Teaching someone else is a great way for you to really learn the material, and it's definitely more fun than listening to me stand and talk. Learning to explain a complex system in a simplified manner is an important skill for you to learn.

### **Materials**

You can use computers (and the websites provided) to research your topic, but the information you've learned in class should be sufficient for this activity. There are also many different materials at the demonstration station that can be used for building your teaching models. These materials include:

- Styrofoam balls
- Toothpicks
- Tape
- Construction paper
- Marshmallows (come see me for these—you get a limited number per group)
- Butcher paper
- Markers

If there are any other supplies that you need, come ask me, and I will try to find something that will work for you.

### **What You Will Do**

You will be assigned either type 1 or type 2 diabetes for your activity.

You will create a script to help teach your patient about diabetes (you recently diagnosed him or her with the disease). This script should include the following components:

1. Description of the symptoms that led you to the diagnosis
2. Explanation of what is going on inside the patient's body
  - a. Visual model of this process (drawn or built with materials provided)
  - b. Verbal description of the process to accompany the model
3. Explanation of treatment or prevention options
  - a. What options are available? (Provide at least two options)

- b. What do these treatments actually do? Tie it in to the process described in component 2.

After you complete this activity, you will be teamed up with another group that was assigned the other topic. You will share your script with the other group, and members of that group will evaluate your presentation using a rubric. You will then switch roles and repeat the presentation and evaluation process.

Each person in the group will be responsible for presenting one of the following sections:

- Description of the symptoms (component 1)
- Processes inside the body (component 2)
- Treatment options and what they do (component 3)

You should use your model to help in the explanation of each section. This will help the patient better understand what you are talking about.

Remember that you are a doctor. It is important to come across as caring, thoughtful, and knowledgeable! You want your patient to trust you.

### **Web Resources:**

*Type 1 diabetes:*

General overview:

[www.health.ri.gov/disease/diabetes/children.php](http://www.health.ri.gov/disease/diabetes/children.php)

Helpful information for kids with type 1 diabetes:

<http://kids.jdrf.org/>

[www.kidslernaboutdiabetes.org/what\\_is.html](http://www.kidslernaboutdiabetes.org/what_is.html)

*Type 2 diabetes:*

General overview:

[www.health.ri.gov/disease/diabetes/children.php](http://www.health.ri.gov/disease/diabetes/children.php)

[www.kidslernaboutdiabetes.org/what\\_is.html](http://www.kidslernaboutdiabetes.org/what_is.html)

Tips for children to help prevent type 2 diabetes:

[www.ndep.nih.gov/diabetes/pubs/Youth\\_Tips\\_Active.pdf](http://www.ndep.nih.gov/diabetes/pubs/Youth_Tips_Active.pdf)

[http://win.niddk.nih.gov/publications/take\\_charge.htm](http://win.niddk.nih.gov/publications/take_charge.htm)

Insulin resistance:

<http://syndromex.stanford.edu/InsulinResistance.htm>

<http://diabetes.niddk.nih.gov/dm/pubs/insulinresistance/index.htm>

### **How You Will Be Graded**

You will be graded on the worksheet answers that you fill in (85% of grade), as well as on your individual presentations (15% of grade). You will be answering the questions in your presentation anyway, so it's easy to get credit for this activity!

## Diabetes Doctors Rubric

Why Can't I Have Sugar? All About Diabetes  
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Presenter name \_\_\_\_\_

Evaluator name \_\_\_\_\_

(10 points total)

Individual presenter:

\_\_\_\_ 1. Logical and clear explanation of section.

Whole group:

\_\_\_\_ 2. Overall understanding based upon presentation.

	✓ (5 pts)	✓ - (4 pts)	- (3 pts)
1	Student gave a logical and clear explanation of his or her section. The model was used to support the explanation.	Student gave an explanation of his or her section, but logic or clarity might be lacking. The model was used to support the explanation, but still not everything was understood.	Student did not give a logical and clear explanation of his or her section. The model was not used in the explanation, or nothing the person said was understood.
2	Students gave a good background overall about diabetes. Each section contained excellent information.	Students gave a fairly good understanding about diabetes. One section lacked some information that I wanted.	Students did not portray an adequate understanding about diabetes. Two or more sections lacked important information.

What were two strengths of this person's presentation?

Give participants two areas for improvement.

## Diabetes Doctors Group Worksheet

### Why Can't I Have Sugar? All About Diabetes Eric DeJulio, CDC's 2005 Science Ambassador Program

Group Members\_\_\_\_\_

Date\_\_\_\_\_

Period\_\_\_\_\_

Which diabetes type are you working with? \_\_\_\_\_

1. What symptoms or risk factors did the patient have that led you to the diagnosis of this type of diabetes? (List at least 3) *1.5 pts*
2. What is going on inside of the patient's body? (For this answer, you should turn in your model, as well as write a verbal description of your model.)  
*5 pts – 2.5 each for model and explanation*
3. What treatment or prevention options are available for the disease? (Describe at least two of each and tie them in to the model described in question 2.) *2 pts*

## Diabetes Doctors Group Worksheet Key

### Why Can't I Have Sugar? All About Diabetes Eric DeJulio, CDC's 2005 Science Ambassador Program

Group Members \_\_\_\_\_

Date \_\_\_\_\_

Period \_\_\_\_\_

Which diabetes type are you working with? \_\_\_\_\_

1. What symptoms or risk factors did the patient have that led you to the diagnosis of this type of diabetes? (list at least 3) *1.5 pts*

Some possible answers include

Type 1:

- Constant hunger
- Excessive urination
- Sudden weight loss for no reason
- Rapid, hard breathing
- Sudden vision changes
- Weakness
- Drowsiness or exhaustion
- Fruity odor of breath

Type 2:

- Obesity
- Insulin resistance
- Strong family history for type 2 diabetes
- Acanthosis nigricans (a thick, dark brown-looking skin lesion that appears on the neck, underarm, or groin area)

Answers from:

[www.health.ri.gov/disease/diabetes/children.php#Type%201%20Diabetes.](http://www.health.ri.gov/disease/diabetes/children.php#Type%201%20Diabetes)

2. What is going on inside of the patient's body? (For this answer, you should turn in your model, as well as write a verbal description of your model.) 5 pts – 2.5 each for model and explanation

The models that students will develop will vary. An example of the verbal description for both type 1 and 2 diabetes follows.

Type 1: There are no beta cells in the islets, which results in no insulin production. Therefore, the body is not able to break down sugar when it is eaten (or produced).

Type 2: Cells are not able to use all of the insulin that is secreted. The pancreas responds by releasing more insulin. People with type 2 diabetes will have high levels of insulin and blood glucose at the same time.

3. What treatment or prevention options are available for the disease? (Describe at least two of each and tie them in to the model you described in question 2.) 2 pts

Some possible answers include:

- Insulin injections (type 1 diabetes)—Because insulin is not being produced by the body, people with diabetes inject insulin to help break down sugar.
- Controlled diet (type 1 and 2)—By controlling the types of food that a person eats, he or she can help regulate the amount of insulin required to break down the food.
- Exercise (types 1 and 2)—Exercise can lower glucose levels in the body because cells use more energy when you exercise than when you rest.
- Drug therapy (type 2)—Increase insulin production or slow increase of blood sugar by restricting absorption of carbohydrates

Answers adapted from:

[www.kidslearnaboutdiabetes.org/what\\_is.html](http://www.kidslearnaboutdiabetes.org/what_is.html)

[www.health.ri.gov/disease/diabetes/children.php](http://www.health.ri.gov/disease/diabetes/children.php)

<http://diabetes.niddk.nih.gov/dm/pubs/insulinresistance/index.htm#7>

## Static Culture of Rat Islets

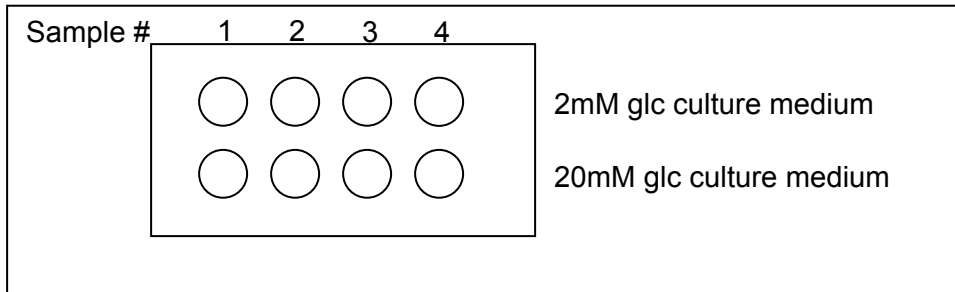
### Materials:

- Micropipette
- 2mM and 20 mM glucose-containing RPMI-1640 culture medium containing 10% newborn calf serum + 1% Penicillin or Streptomycin
- 96-well plate
- Carbon dioxide (CO<sub>2</sub>) incubator
- Refrigerator to store samples (samples can be stored for several days at 4 degrees Celsius, maybe even a week)

### Protocol:

1. Wash 1: Pick 100 rat islets and place into 10 mL of 2mM glucose-containing culture medium (RPMI-1640 + 10% NBCS + pen/strep)
2. Incubate for 10 min at 37 C + 5% CO<sub>2</sub>.
3. Wash 2: Re-pick 100 islets and place into 10 mL 2mM glucose-containing culture medium once again.
4. Incubate for 10 min at 37°C + 5% CO<sub>2</sub>.
5. Static incubation: Pick 10 islets in 10 µL of wash 2 and place into a well on a 96-well plate that contains 190 µL of media.

Your test plate will look similar to the following:



6. Incubate at 37°C/5% CO<sub>2</sub> for 1 hour.
7. Using a micropipette, remove 125 µL of supernatant from each well and transfer it to a new well.
8. Using a micropipette, remove 80 µL of supernatant from each *new* well and transfer it to a new well.
9. These samples can then be stored at 4°C (in refrigerator) until the insulin assay is performed.