**What Causes Obesity?**

**Introduction and Background Information**

Obesity occurs when there is an imbalance between the amount of energy taken in (food eaten) and the amount of energy consumed (activity). There are many factors that play a role in the development of obesity. These include lifestyle, environmental factors, and sometimes genetics.

There is a signal molecule called leptin that is produced by fat cells. Leptin is received by brain cells and is an important signal for maintaining the correct energy balance. In order for leptin to be received by brain cells, the correct cell membrane receptor must be present and functioning normally. High levels of leptin are present when the receptors

do not function properly. We call this condition leptin resistance.

In this simulation experiment, you will be a researcher testing samples from two adolescents participating in a research study investigating leptin resistance in obese adolescents. Leptin levels will be tested using a simulated ELISA (Enzyme-Linked Immunosorbent Assay) procedure.

Your role as a researcher is to:

determine if either subject is leptin resistant

decide how to share this information with the research study participants

recommend some behaviors that would help these subjects prevent or combat obesity

Your group will prepare a short report to share with the “subjects” that includes conclusions, with supporting evidence, and recommendations.

In this case, rabbit antibodies specific to human leptin are first added to the reaction wells. The antibodies are allowed to bind to the plastic wells. Excess antibodies are washed off. Samples containing leptin are added to the wells. Leptin will bind to the antibodies. Washing is performed to remove unbound material. Enzyme-linked anti- leptin antibodies are added, which will bind to the bound leptin. Again, washing is performed to remove unbound material. Substrate is added and color is detected which is proportional to the amount of leptin in the sample. A color change from colorless to

red will indicate the presence of leptin.

**Safety Note:**

 Use gloves and eye protection.

 Follow all chemical safety rules!

**Procedure**

1. Label the bottom of wells of the microtiter or spot plate according to the chart

below.

Well 1 = negative control Well 2 = positive controls Well 3 = subject 1

Well 4 = subject 2

2. Rinse a micropipette in a beaker of distilled water (dH O). Practice squeezing the pipet slowly to get one drop at a time. When you are comfortable with using the pipet, remove any remaining water before starting the experiment.

2

3. Carefully place two (2) drops of rabbit anti-human leptin antigen (A) into each of the eight wells of the microtiter strip. Replace unused sample back into the tube from the pipet. Flush and rinse the pipet several times in dH O. Discard and

2

replace the water used for washing the pipet.

4. Incubate the plate for 2 minutes at room temperature.

5. Place two (2) drops of the negative control (B) sample into each of the two negative control wells (1). Replace unused sample back into the tube from the pipet. Flush and rinse the pipet several times in dH O. Discard and replace the

2

water used for washing the pipet.

6. Place two drops (2) of the positive control (C) sample into each of the two positive control wells (2). Replace unused sample back into the tube from the pipet. Flush and rinse the pipet several times in dH O. Discard and replace the

2

water used for washing the pipet.

7. Place two drops (2) of the subject 1 sample (D) into each of the two subject 1 wells (3). Replace unused sample back into the tube from the pipet. Flush and rinse the pipet several times in dH O. Discard and replace the water used for

2

washing the pipet.

8. Place two (2) drops of the subject 2 sample (E) into the subject 2 wells (4).

Replace unused sample back into the tube from the pipet. Discard this pipet.

9. Incubate the plate for 2 minutes at room temperature. This is a simplified version and we will not wash off any unbound antibodies.

10. Using a new pipet, place two (2) drops of the secondary antibody (F) into all wells. Replace unused sample back into the tube. Flush and rinse the pipet several times in dH O. Discard and replace the water used for washing the pipet.

2

11. Incubate the plate for 2 minutes at room temperature.

12. Place two (2) drops of substrate (G) into all wells.

13. Observe and record results.

color for negative control color for positive control color for subject 1 color for subject 2

14. Interpretation of results:

Key: blue = normal leptin pink = high leptin

Subject 1 has leptin. Subject 2 has leptin.

**Analysis Questions:**

What would you tell the parents?

What are some behaviors that would help these subjects prevent or combat obesity?

How do you feel about conducting such testing on subjects?

Do you think that this would help the subject? Why, or why not?

What is the difference between subject care and clinical research? Which best describes this activity?

How might this test be useful in a clinical research study?