Bio 236 Lab – Blood Glucose Tolerance Test

Blood glucose is regulated in large measure by two pancreatic hormones, **insulin** and **glucagon**. Both are peptides secreted by the pancreas (as an “endocrine function”) in response to changes in blood glucose outside of its normal physiological range, and both cause biological changes in distant target cells by binding to protein receptor molecules embedded in the plasma membrane. The pancreas also serves an “exocrine function” in that it can secrete digestive enzymes into the nearby duodenum of the small intestine. Virtually all body tissues have receptors for – and therefore respond to – insulin. Insulin causes a wide range of responses including gene regulation, changes in intracellular ion concentration, and changes in fuel metabolism. In terms of glucose utilization, it is convenient to think of three major insulin targets: skeletal muscle, adipose, and liver. In these tissues, insulin stimulates glucose uptake from the blood and encourages glucose metabolism in three major pathways: 1) breakdown by **glycolysis** and cell respiration for ATP production; 2) uptake of “extra” blood glucose in order to store it as glycogen, and 3) the transduction of glucose into triglycerides in adipocytes for long-term energy storage. When blood glucose increases (after eating) the pancreas responds by secreting insulin into the blood. **Insulin** stimulates blood glucose uptake by body tissues, which functionally will reduce blood glucose levels. When the muscles and liver take up blood glucose, and extra blood glucose not needed for cell metabolism can be converted to a storage form of glucose called **glycogen** (a process called “**glycogenesis**”). If blood glucose drops (after skipping a couple of meals) the pancreas respond by secreting **glucagon**, which stimulates the liver to break down glycogen stores into glucose (a process termed “**glycogenolysis**”) and release glucose into the blood, functionally increasing blood glucose levels. Thus, regulation of blood glucose to within a normal range involves both the pancreas and the liver. **Normal “fasting levels”** of blood glucose ranges from 70 – 130 mg/dl but in a diabetic patient these levels range much higher (> 126 ml/dl). Shortly after eating, **post-prandial blood glucose** levels are slightly elevated to 170 – 180 mg/dl, and in a diabetic patient might be well over 200 mg/dl.

**Diabetes mellitus** is a disorder of fuel metabolism. The two major syndromes are classified as **Type I diabetes** (formerly **insulin-dependent diabetes mellitus**) and **Type II diabetes** (formerly **non-insulin-dependent diabetes mellitus** and more recently referred to as “**insulin resistance**”). Both are characterized by **hyperglycemia** (high blood glucose) and inability to properly metabolize glucose. In uncontrolled diabetes, excess blood glucose is excreted in urine (**glucosuria**), resulting in the well-known clinical sign that gives name to the disease: diabetes mellitus is Greek for “sweet urine”. Type 1 diabetes results from autoimmune destruction of the **Beta cells** of the pancreas, thus type 1 diabetics make little or no insulin. Type II diabetes is far more common - 95% of diabetics in the US are type II - and in some ways far more complex. The precise problem in Type II appears to be that body tissues fail to respond to insulin correctly, even though insulin is produced and circulated. It is useful to think about diabetes as a cruel paradox, much in the manner of a shipwreck survivor with no potable water though he floats in an oceanic expanse. In someone suffering from diabetes - the blood is overloaded with glucose, but tissues starve as they are unable to use it. “**Metabolic syndrome**” (a.k.a. “**Syndrome X**”) is a generalized term to describe the combination of medical disorders (e.g. age, weight, activity level, heredity, etc...) the increase a person’s risk for developing diabetes.

To assess insulin performance, clinicians use the **oral glucose tolerance test** (OGTT). Before arriving at the clinic, the patient drinks nothing and eats nothing for 12 hours (an overnight ‘fast’). Blood samples are taken before and 2 hours after drinking a 75 gram glucose solution, which allows measurements of both fasting and post-prandial blood glucose.

In interpreting the test, imagine the negative feedback regulation of insulin: After the meal, insulin secretion increases in response to rising blood glucose, which is absorbed from the GI tract. In a normal individual, insulin clears glucose from the blood by promoting uptake into skeletal muscle, liver and adipose. As glucose declines, the signal for insulin secretion diminishes. In the case of diabetes, glucose remains in the blood because insulin secretion is insufficient, and/or insulin has inadequate effect on target tissues.

**Procedure:** In groups of 3-4, students will simulate the OGTT. Each group requires a pen-prick with lancets, glucose strips, and a digital glucose-meter. Obtain a droplet of blood from a finger by lancing. [It helps if you squeeze your
fingertip to trap blood first before using the lancet.] A partner can prepare the glucose-meter by placing a strip in the meter. A few seconds are required before the meter indicates it is ready to read a sample. When the droplet appears on the LCD, apply the blood drop to the end of the glucose strip – capillary action will draw the blood into the strip. An infrared light scans the strip and in a matter of seconds, blood glucose is indicated on the screen in mg/dL. After this first reading, drink a 12-ounce (roughly 355 mL) serving of one of the beverages provided, then record blood glucose at 30, and 60 minutes afterwards. Yes, you have to prick your finger 3 times! Please be careful with the strips - they cost about 70 cents each, and are not reusable once they've been inserted into the reader. Always use a fresh lancet for each time interval – if you have any doubt, dispose it in the sharps hazards container and get a new one. All soft materials that touch blood are collected in the red biohazards plastic bag container. Record data in Table 1 below.

Table 1

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Blood Glucose (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Full Sugar Lemonade</td>
</tr>
<tr>
<td></td>
<td>Sugar-Free Lemonade</td>
</tr>
<tr>
<td>0</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td></td>
</tr>
<tr>
<td>60</td>
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</tbody>
</table>

Data Analysis

Data on blood glucose over time, for sugar, and sugar-free beverages will be plotted in an Excel spreadsheet. Data will be summarized (to produce average blood glucose values over time), and analyzed using paired T-tests. If the P-value for a T-test is $\geq 0.05$ the two data sets are not significantly different, but if the P-value is < 0.05 then the two data sets are significantly different.

Questions:
1. What are normal glucose levels for fasting and post-prandial periods? For a diabetic?

2. What is the half-life of endogenous insulin, and how is it removed from the body?

3. What is meant by endocrine and exocrine pancreas? Which cells are involved in each role?

4. What is “Syndrome X”?

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5. In this experiment, some individuals consumed sugar-free beverages. How did their glucose over time compare to those who consumed regular beverages? How can you explain this?

6. What are some other factors that might have affected the results of this exercise?