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 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 in the liver and skeletal muscle, and 3) the conversion of glucose into triglycerides (white fat) in adipocytes and the liver by lipogenesis, 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”). Insulin is a hormone with a relatively short half-life, and it is 50% degraded within 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 free 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 consuming carbohydrates (especially simple carbohydrates), blood glucose increases within 15 – 10 mintues. Pancreatic insulin secretion increases in response to rising blood glucose, which is absorbed from the GI tract usually within 30 – 60 minutes of eating. 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.
[*Warning!* - if you have a glucose metabolism disorder you should only be part of the sugar-free group.]

**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 (not on top of the strip) – capillary action will draw the blood into the strip. An infrared light scans the strip and in a matter of 5-6 seconds, blood glucose is indicated on the screen in mg/dL. After this first (Time 0) reading, drink a 12-ounce (roughly 350 mL) serving of one of the beverages provided, then record blood glucose at 30, 60 and 90 minutes afterwards. Yes, you have to prick your finger 4 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 hazards container and get a new one. All materials that touch blood are collected in the red biohazards container.

Record data for yourself and your labmate in the table below.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Blood Glucose (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Full Sugar Drink</td>
</tr>
<tr>
<td>0</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td></td>
</tr>
<tr>
<td>90</td>
<td></td>
</tr>
</tbody>
</table>

**Data analysis:**

Enter your data in the excel template at the instructor’s station into either the full sugar drink or the sugar-free drink column for each time interval. The instructor will compare the time intervals for each experimental group using 4 sets of paired T-tests for each experimental group. The following time intervals will be contrasted for both the full sugar and the sugar-free groups: time 0 versus time 30, time 30 versus time 60, time 60 versus time 90, and then time 0 versus time 90.

**Questions:**

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

2. What is “Syndrome X”?
3. In general, how soon after eating (especially simple carbohydrates) can blood glucose increases be seen?

4. a) In your experiment did blood glucose increase in the group that drank full sugar drinks? 

   b) If so, at what time interval was the increase observed?

5. a) In your experiment did blood glucose decrease in the group that drank full sugar drinks? 

   b) If so, at what time interval was the decrease observed?

6. Based on when blood glucose levels dropped in the full-sugar group, when would you hypothesize that insulin secretion started during the experiment?

7. In your experiment did blood glucose increase at any time interval for the group that drank sugar-free drinks?

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

9. What is meant by endocrine and exocrine pancreas?