

SIMULATED DIABETES TESTING EXPERIMENT USING
ARTIFICIALLY MANIPULATED LIVESTOCK BLOOD

by
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ABSTRACT

Hollie Elizabeth Ables: Simulated Diabetes Testing Experiment Using Artificially Manipulated Livestock Blood

(Under the direction of Carol Britson)

Due to the prevalence of diabetes and lack of simple endocrine experiments, a laboratory protocol was developed in order to increase student knowledge of the disease and the diabetic testing process. In this paper, we present the protocol for manipulating the glucose concentration of commercially available bovine blood. The bovine blood was manipulated using Ringer's solution to which glucose was added, and six samples were prepared (pre-prandial and postprandial, normal; pre-prandial and postprandial, pre-diabetic; pre-prandial and postprandial, diabetic). This laboratory exercise provides a physical, hands-on student activity that highlights pancreatic endocrinology and the results of pancreatic malfunction. The laboratory exercise is relatively inexpensive and can be performed in under an hour. Students in Human Anatomy and Physiology II performed the experiment using glucose meters. When tested, the blood samples' glucose readings were consistently within the desired ranges. The post-laboratory questions were designed to assess student knowledge of diabetes upon completion of the experiment. The students also completed a pre-laboratory and post-laboratory survey, and these results were also recorded and tabulated. Upon completion, the majority of students (164 of 209) reported an increase in knowledge concerning diabetes. An overwhelming majority (190 of 209) of the students enjoyed the laboratory exercise. Bovine blood was used in the experiment to ensure realism, and 194 of the students believed that this

experiment was realistic given the constraints (i.e., using simulated blood). Due to student results and survey answers, it was deduced that this laboratory exercise was successful.

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Introduction

Objectives

According to the American Diabetes Association, over eight percent of the American population has diabetes, and every 17 seconds someone is diagnosed with diabetes in the United States (14). It is important for everyone, including college students entering the health profession, to become familiar with the disease and the diabetic testing process. However, experimental protocols designed for undergraduate students (students working toward a Bachelor's degree) that explore endocrine physiology yet avoid student exposure to biological fluids from the human body are lacking. In this paper, we present a protocol for manipulating the glucose concentration of commercially available bovine blood. By choosing to use bovine blood, we were able to ensure that the experiment was as realistic as possible. For example, the modified blood was similar to human blood in both appearance and flow. It is our aim that students become more knowledgeable about diabetes and diabetes testing using this modified blood.

Many of the currently available laboratory exercises that explore pancreatic physiology can be costly, take a great deal of time to complete, or are not physical, hands-on experiments. The following are examples of currently published endocrinology laboratory experiments: ovulation testing (39), virtual simulation using PowerPhys[®] experiments for blood glucose regulation and thyroid function and testing blood glucose concentrations and animal dissection (1), virtual simulation using Physiology Interactive

Lab Simulations ® to explore endocrine function: thyroid gland and metabolic rate (25), exercises requiring torso models, compound microscopes, and prepared slides and a cat or a pig for dissection (40), observing endocrine organs using a microscope, Thin-Layer Chromatography of steroid hormones, and insulin shock using fish (19), and self-testing one's glucose tolerance (31). Because animals are used in some of these experiments, one must follow animal care procedures and have such experiments approved if the subjects to be used are animals (e.g., Institutional Animal Care and Use Committee approval). Also, one must consider that self-testing body fluids could potentially be dangerous to the student.

The present experiment is relatively inexpensive. For a Human Anatomy and Physiology II class of 250 students spread across eight laboratory periods with eight groups per lab (four students per group), this experiment can be performed for \$22.65 per student group. After the initial experiment and the deduction of the cost of the glucose meters (meters can be reused year after year), the exercise can be performed for \$7.67 per student group.

Because student safety is of the utmost importance, instructors must ensure that students avoid exposure to human biological fluids. Human blood can contain blood borne pathogens (Hepatitis B Virus (HBV), Hepatitis C Virus, and Human Immunodeficiency Virus), and using needles or lancets can potentially expose students to these pathogens (30). When students use their own bodily fluids, many precautions must be taken in order to reduce the likelihood of student exposure. As a result, personal protective clothing and equipment must be used, training must be provided, vaccinations (HBV) must be taken, and signs and labels must be placed in appropriate locations. Many

students either do not understand safety protocols or incompletely/incorrectly follow said protocols. In order to ensure student safety, we developed this protocol to manipulate the glucose concentration of commercially available bovine blood for use in a simulated diabetes experiment. The bovine blood used in the experiment is sterile and safer than human blood.

Overview

Approximately 25.8 million people in the United States have diabetes (28). Of these individuals, about 7 million have not been formally diagnosed (28). It is estimated that 25.6 million Americans older than the age of 19 have the disease while 10.9 million of them are ages 65 and older, which accounts for 11.3% and 26.9% of these age groups, respectively (28). Only 215,000 Americans younger than 20 years of age have diabetes, and most have Type 1 (28). It has been estimated that 79 million people in the United States are pre-diabetic, “which is a condition in which an individual's blood glucose concentrations are higher than normal, but the concentrations are not high enough for the individuals to be diagnosed with diabetes” (28).

In 2010, there were 270,000 Mississippians with Type 2 diabetes, which accounts for 12% of the adult population of the state (27). It is estimated that over 100,000 of these individuals have diabetes but are unaware that they have the disease (27). In 2013, 12.5% of Mississippians had diabetes, and Mississippi ranked second in the nation for diabetes prevalence (37). In fact, Mississippi has been ranked in the top 5 since 1996 (37). The prevalence of diabetes in Lafayette County, MS is one of the lowest in the state, yet

individuals with diabetes still account for 10.4% of the population (12).

While Type 2 diabetes is the most prevalent form of the disease and accounts for 90-95% of all diagnosed cases (28), it is preventable and can be managed with lifestyle changes (36). Through increased exercise, weight loss, and a healthy diet that emphasizes fruits, vegetables, and whole grains, individuals can manage the condition (10). This diet also includes lean meats and beans and consists of foods low in fats, cholesterol, sodium, and added sugars (10).

There are many factors that affect the food choices of Americans including both food deserts and food insecurity. Food deserts are becoming more prevalent in America, and individuals that live in food deserts are more likely to develop diabetes (6). A township or county is designated a food desert if 50% or more of the population does not have convenient access to a supermarket, super center, or wholesale club (6). One should be mindful that this designation does not account for farmers who grow their own food and only accounts for large supermarkets that have 50 or more employees. Food insecurity exists “whenever the availability of nutritionally adequate and safe foods or the ability to acquire acceptable foods in socially acceptable ways (e.g., without resorting to emergency food supplies, scavenging, stealing, or other coping strategies) is limited or uncertain” (24). Residents are considered to have convenient access to food when they are no further than 10 miles from such a supermarket, super center, or wholesale club (6). Because people that live in food deserts have limited access to high quality food, they are more likely to develop the unhealthy eating habits that can lead to diabetes (6). Over 29% of southern counties are considered food deserts, accounting for 256 of the 873 non-

metro counties in the south. These counties are located across the states of Texas, Oklahoma, Louisiana, Arkansas, Mississippi, Alabama, Tennessee, Kentucky, Georgia, Florida, North Carolina, South Carolina, Virginia, West Virginia, and Maryland.

It is estimated that 23.5 million people reside in food deserts, and half of these individuals are low-income and thus at risk of food insecurity (13). Food insecurity is one of the most influential factors determining particular food choices among Americans and results from an inadequate supply of funds (24). Data suggests that almost 14% of the population in the United States is food insecure or at risk of running out of money for food (24). Furthermore, data suggest that food insecure adults are at risk of diabetes, and adults with diabetes are more likely to have their blood glucose reach either hyperglycemic and hypoglycemic levels due to unhealthy eating habits (24). Low-income adults who are food insecure are twice as likely to have diabetes, and the cost of diabetes further hinders their ability to afford high quality food (24). Both food insecurity and food deserts are risk factors for diabetes (24, 6).

There are multiple reasons why low-income individuals that live in food deserts develop unhealthy eating habits. For instance, in food desert counties, small retailers are more common; in fact only ten of the 256 counties considered to be food deserts in the south have a large supermarket (6). Small, local grocery stores generally have higher priced food, less variety for selection, and lower quality food that may not meet healthy dietary requirements (6). Other options include convenience stores where customers must pay even more for even lower quality goods (6). Therefore, individuals that live in a food desert and conjointly suffer from food insecurity must alter or reduce their dietary intake

or participate in meal skipping in order to counterbalance reduced food expenditures and increased food prices (24). Because many energy dense foods (i.e., high fat and sugar) are less expensive, individuals that are food insecure tend to purchase such foods since the price of fruits and vegetables are much higher than the cost of sweets and soft drinks (24). Increasing dietary intake of saturated fats and added sugars by 1% reduces food cost by as much as \$1.80 per person per day (24). Those that reside in food desert counties are 23.4% less likely to consume the recommended five servings of fruits and vegetables as compared to non-food desert residents (6). Because access to grocery stores and money can be sparse, patterns of food intake are cyclic or episodic for low-income families living in food deserts (24). These individuals commonly experience lengthy periods of food adequacy followed by food inadequacy and may participate in binge eating in order to compensate for future food shortages (24).

It is estimated that 20.9% of Mississippians are food insecure, and rural counties in the Lower Mississippi Delta generally only have one supermarket per 190.5 square miles (13, 6). In addition, almost 70% of the low-income populations in the Delta are required to travel 30 or more miles in order to gain access to the lower food prices that are offered by a large supermarket (6). While Oxford, MS is classified as a food desert by the U.S. Department of Agriculture, the city is attempting to counteract the label through the creation of Oxford City Market and Mid-Town Farmer's Market (26). The Oxford City Market has 29 vendors, and many of them accept food stamp (SNAP, Supplemental Nutrition Assistance Program) benefits as a form of payment, further assisting those suffering from food insecurity (26).

The University of Mississippi Food Bank was created in 2012 in order to “foster a healthy college community by providing nourishing food to students in need” (34). During a recent visit in February 2014, it was noted that the UM Food Bank offers a variety of vegetables including beans, peas, corn, and carrots as well as oatmeal, peanut butter, and a variety of soups. There was a small selection of meats available including tuna and chicken. However, the food bank provides very few choices of fruit. According to the CDC's definition of a healthy diet, the food offered at the UM Food Bank would not provide an appropriate diet for one with diabetes.

Diabetes can result in multiple, chronic complications such as micro vascular complications, retinopathy, neuropathy, nephropathy, and amputation. The predominant cause of blindness in those aged 20-74 is diabetes because individuals with the disease are more likely to develop retinopathy (11). In 2005-2008, 4.2 million diabetics over the age of 40 had diabetic retinopathy, and “655,000 had advanced diabetic retinopathy that could lead to severe vision loss” (11). Retinopathy develops when capillaries in the back of the eye become blocked due to high glucose concentrations, and as more and more vessels become blocked, the patient can develop non-proliferative retinopathy (2). If blood vessels become too damaged, new and weak blood vessels form allowing blood leakage into the eye, causing a block in vision (i.e., proliferative retinopathy) (2). Almost everyone with diabetes will get some form of non-proliferative retinopathy, yet individuals with Type 1 diabetes are more likely to develop non-proliferative retinopathy than individuals with Type 2 diabetes (29). Among those diagnosed with diabetes, 40 to 45 percent have developed some form of diabetic retinopathy (29). Patients are more likely to develop retinopathy the longer they have diabetes especially if the condition is

not adequately controlled (5).

Currently, “more than 60% of non-traumatic, lower-limb amputations are performed in people with diabetes” (11). Individuals with diabetes are more likely to have Peripheral Arterial Disease, which results when blood vessels of the legs are narrowed or blocked by fatty deposits (2). As a result, blood flow to patients' feet and legs decreases, and individuals are more likely to develop nerve disease (e.g., neuropathy), which reduces sensation (2). These problems make it easier to get ulcers and infections, which are harder to treat when glucose concentrations are high, that may lead to amputation (2). In the year 2006, approximately 65,700 individuals with diabetes underwent non-traumatic, lower-limb amputations as a result of the disease (11).

Diabetes can lead to multiple deaths each year and is the seventh leading cause of death listed on death certificates in America (28). However, one should be mindful that patients with diabetes do not typically die directly because of diabetes. Approximately 70% of the time patients die from complications resulting from the disease. From the years 2000 to 2004, 72,256 people (more females than males) died each year due to diabetes (15). In the same time period, 57,677 white Americans were affected and 12,548 African Americans were affected each year (15). In 2012, 246,000 deaths were attributable to diabetes of which 73,000 were directly caused by the disease (3, 20). In Mississippi alone, an average of 671 people died each year between 2000 and 2004 (15). In 2010, diabetes contributed to the deaths of 926 people in Mississippi (27). Data demonstrate that one in three children in the United States will develop diabetes (20). However in ethnic minorities, studies show that one in two children will develop diabetes

(20).

In 2025, it is predicted that more than 75% of individuals with diabetes will live in developed countries, and the United States of America will be home to the majority of those people (23). As the prevalence of diabetes increases, the cost of diabetes will continue to rise. In the United States, the baseline cost per year for a healthy, white male with no complications from the disease is \$1,684, yet the cost for women with the same characteristics is \$2,105 (year 2000 U.S. dollars) (8). In 2012, diabetes cost the nation \$245 billion: \$176 billion in direct medical costs and \$69 billion in lost productivity (2). The cost of diabetes in Mississippi alone totaled \$2.74 billion in 2012 (2). It is estimated that undiagnosed diabetes costs the nation \$18 billion, or \$2,864 per adult (41). The rate at which Americans are developing diabetes is extremely high, and as more people develop the disease, the collective cost of diabetes will continue to increase. For instance, in 2009 an estimated \$113 billion was spent on diabetes care, and America had 20.2 million individuals with diagnosed diabetes (9, 8). In a 2001 study, projections for the number of people with diabetes were calculated using data from the National Health Interview Survey to determine diabetes prevalence rates (7). It was projected that by 2025 20 million Americans would have diabetes; however, America surpassed this number in 2009 (7). It is projected that the number of adults in the world with diabetes will rise to 300 million by 2025 (23). Due to the rise in diabetes by 2034 the cost of diabetes is projected to be \$336 billion per year (year 2007 U.S. dollars) (22).

Because of the individual and collective cost and the risks of diabetes, college students with and without the disease should be educated about diabetes (32). The

majority of college students live sedentary lives and have a poor diet during undergraduate years; in fact, up to 23.3% of college students do not exercise, and 14.3% only exercise once a week while 16.8% exercise twice a week (32). A study performed in 2011 showed that student knowledge of the disease is low (32). Students incorrectly defined diabetes and could not name the risk factors of the disease. Studies demonstrate that college students have poor eating habits, in part, because for many students their first year of college is the first time they are allowed to make unregulated food choices (16). Students eat fewer fruits and vegetables and have a more high-fat, high-calorie diet, and according to a study performed by the American College Health Association in 2004 only 7.3% of students eat five or more serving of fruits and vegetables on a daily basis (16). Taste, time sufficiency, convenience, and budget influence students' eating habits while high stress and low self-esteem can drive college students to have unhealthy diets (16). Due to the large number of convenience stores, vending machines, and fast food outlets that are readily available, students are provided with unhealthy choices and encourage them to participate in skipping meals, eating away from home, and snacking (16).

Background

Diabetes mellitus refers to a metabolic disorder “resulting from defects in insulin secretion, defects in insulin action, or both” (17). Insulin is a hormone that facilitates glucose uptake in the cells of the body and is released by beta cells in the pancreas when an individual consumes a meal (4). Without insulin, the concentration of glucose in the blood increases greatly, and the body experiences abnormalities in carbohydrate, protein, and fat metabolism (4). There are three major forms of diabetes: Type 1, Type 2, and

gestational diabetes (17). However, pre-diabetes is becoming more prevalent in America. Table 1 lists the range of glucose values for normal, pre-diabetic, and diabetic patients. Type 2 is the most prevalent form of diabetes, and 90% of individuals diagnosed with the disease have this form (4). Only 5-10% of individuals are diagnosed with the more severe Type 1 diabetes (4). It is estimated that patients with gestational diabetes accounts for only 2-3% of patients with diabetes (4).

Type 1 diabetes, also known as juvenile-onset diabetes, describes a condition in which the pancreas does not produce a sufficient amount of insulin (17). Type 1 diabetes can result from an autoimmune disorder that attacks and destroys the insulin producing beta cells within the pancreas (4). Type 1 diabetes is most often seen in children and young adults (17). Individuals with Type 1 diabetes can experience acute symptoms such as excessive thirst (polydipsia) and frequent urination (polyuria) (17). Polyuria results from the body's attempt to purge excess glucose in the blood stream (4). Other symptoms include weight loss and abdominal pain (4). Individuals with severe cases of diabetes may develop ketoacidosis as a result of conversion of fats and proteins into ketones (17). This conversion can lead to a coma and even death if left untreated (4). As a result, insulin therapy is essential for individuals who suffer from Type 1 diabetes (17).

Type 2 diabetes is also known as maturity-onset diabetes (17). This form of diabetes results from reduced insulin production or a decrease in insulin utilization (17). As a result, blood glucose concentrations rise (4). Type 2 diabetes is most often seen in patients who are older, obese, have a family history of diabetes, or do not exercise (4). Individuals with Type 2 diabetes rarely experience weight loss or ketosis (17). Patients with Type 2 diabetes may have the disease for many years and experience no symptoms

Table 1: Blood Glucose Concentrations for Diagnosis of Diabetes

	Pre-prandial	Postprandial
Normal Glucose Concentration	<100 mg/dL	<140 mg/dL
Pre-diabetes	100 mg/dL – 126 mg/dL	140 mg/dL – 200 mg/dL
Diabetes	>126 mg/dL	>200 mg/dL*
Hypoglycemia	<70 mg/dL	<70 mg/dL

*Termed random blood glucose.

(4). However, those with more severe cases may experience polyuria, polydipsia, polyphagia, and recurring infections especially on the feet (4).

Treatment of diabetes targets symptom prevention, restoration of carbohydrate metabolism, and complication minimization. The concentration of glucose in the blood is used to give an indication of blood glucose control (4). Individuals with Type 1 diabetes are generally treated with insulin injections (17). On the other hand, Type 2 diabetes can be treated with diet, exercise, oral medications, or insulin injections depending on the severity of the disease (17). Individuals who treat the disease with insulin therapy often experience hypoglycemia (17). Hypoglycemia results from a decrease in blood glucose concentration. The condition can result in confusion, hunger, sweating, palpitations, and death if concentrations remain too low (17).

Individuals with diabetes must follow strict guidelines when treating their disease. For both Type 1 and Type 2 diabetes patients, blood testing is used to determine how well blood glucose concentrations are controlled with a healthy diet of vegetables, fruits, whole grains, and lean meats (4, 17). These foods help maintain a stable glucose concentration within the bloodstream because they have a low glycemic index (i.e., a measure of the increase in blood glucose two hours after a meal) (4). Meals should be spaced evenly throughout the day, and it is imperative that patients do not skip meals (4). Healthy weight loss can significantly help those with diabetes (17). Weight loss promotes better glucose uptake and improves blood glucose concentrations (4). If it is necessary for the patient to measure his or her blood glucose throughout the day, the patient will need a blood glucose meter. By using a small drop of blood, the meter can determine the glucose concentration in one's blood. Self-monitoring of blood glucose can help determine how

diet, exercise, and medicine affect one's diabetes and helps the individual make future choices. Blood glucose concentrations should be measured before eating or two hours after a meal, pre-prandial and postprandial glucose concentrations, respectively (4).

Individuals with Type 1 diabetes must be very vigilant when treating the disease (4). Because the pancreas no longer produces insulin, patients must check his or her blood glucose 2-3 hours after a meal and use 2-4 insulin injections each day to control the blood glucose concentrations. (4). The amount of insulin in each injection is adjusted depending on the patient's physical activities and diet (17). Recently, more individuals have been developing pre-diabetes which results in high blood glucose concentrations, but these concentrations are not high enough to be considered diabetes. Individuals who are overweight are more likely to develop pre-diabetes who are then more likely to develop Type 2 diabetes; in fact, those with Type 2 diabetes almost always have had pre-diabetes in the past. Those with pre-diabetes do not usually present with symptoms (4).

Learning Objectives

After completion of the experiment students should be able to perform the following tasks:

1. Define pre-diabetes and diabetes
2. List the risk factors of the disease
3. Differentiate between Type 1 and Type 2 diabetes
4. Predict pre-prandial and postprandial values for individuals with diabetic, pre-diabetic, and normal glucose concentrations
5. Operate a blood glucose meter, record, and interpret results

6. Display sufficient knowledge of diabetes

Activity Level

This laboratory exercise was designed for undergraduate Human Anatomy and Physiology students, specifically sophomore level students interested in health professions such as nursing, exercise science, occupational therapy, and nutrition and dietetics. The exercise can be modified for upper-level science students potentially entering the healthcare field and will be discussed late in the Wider Educational Applications section. Such modifications will increase the difficulty of the exercise and will allow the students to develop inquiry-based examination skills.

Prerequisite Student Knowledge

In order to complete the laboratory exercise, students should have a basic understanding of diabetes, which they can gain through pre-laboratory readings, a pre-lab introduction, and/or class lecture. At the beginning of the laboratory session, students should be instructed how to use a blood glucose meter and test strips to ensure accurate results. Students should also be trained in the safe handling and disposal of biological wastes.

Time Required

The present exercise can be easily conducted as part of one laboratory session and in less than an hour. On average, it took students 30 minutes to perform the experiment and complete the post-laboratory exercises.

Methods

Equipment and Supplies

The following chemicals and materials are needed to prepare for the experiment:

1. *Ringer's solution* (Table 2) will be used as a medium for the glucose added to the blood to simulate diabetic concentrations in each sample. Ringer's solution is used because it can be added to bovine blood without affecting the blood's flow, appearance, or consistency. The following supplies are needed to prepare the solution:

- Chemicals
 - sodium chloride, crystals; potassium chloride, crystals; calcium chloride, anhydrous granules; sodium bicarbonate, powder; and glucose, anhydrous flakes
- Distilled water¹
- 1 liter container
- A stir plate and a magnetic stir bar will be needed in order to mix the distilled water and dissolved chemicals thoroughly.
- An electronic balance

¹ Deionized water may also be used as some labs may have their own source of deionized water. When buying small amounts, commercially distilled water is inexpensive (e.g., \$0.23 per liter).

2. *Defibrinated bovine blood* can be purchased from a biological supply house such

Table 2: Ingredients Required for Mammalian Ringer's Solution

Distilled Water	1.00 liter
Sodium Chloride	9.00 grams
Potassium Chloride	0.42 grams
Calcium Chloride	0.24 grams
Sodium Bicarbonate	0.20 grams

as Carolina Biological. Avoid citrated blood, as it will coagulate due to the presence of calcium ions in the Ringer's Solution.

3. *1 mL transfer pipettes* will be used to correctly measure and transfer bovine blood and Ringer's solution to an appropriate container for mixing and before transferring samples to individual vials.
4. *Six 2 mL capped vials* will be needed per group of students. The individual blood samples will be placed in separate, labeled vials. Vials should be labeled with blood type (postprandial or pre-prandial) and patient number 1, 2, or 3.
5. *A vortex mixer* will be needed in order to thoroughly mix the Ringer's solution and blood samples. It will also be needed to remix the blood of each sample vial on the day of the experiment to ensure the mixture is homogenous. Because hematocrit values can adversely affect the glucose readings, it is imperative that the blood is mixed properly.

Materials needed per student group on the day of the laboratory experiment:

1. *One glucose meter* (We purchased ours from Ward's Science; however, one can possibly get meters and strips donated from a company since the exercise is educational.) is needed per group of students. The meter will be used to measure the blood glucose concentrations of the samples given to each group.
2. *Six glucose meter test strips* are needed per student group. One test strip will be needed for each sample that is to be tested. Extra strips should be made available in case students incorrectly test a sample of blood and need to retest.

3. *1 mL transfer pipettes* will be used to transfer one to two drops of blood to the appropriate application zone of each glucose meter test strip when the students are testing the blood.
4. *Sample vials of pre-prandial and postprandial blood for patients 1, 2, and 3* will be used to measure blood glucose concentrations. There will be a total of 6 samples for each student group.

Human Subjects

This laboratory experiment will be incorporated into the Human Anatomy and Physiology II laboratory curriculum and will be performed with other endocrinology laboratory exercises. Subjects that will perform this experiment will be Human Anatomy and Physiology II students most of whom are interested in health professions (e.g., nursing and exercise science). Students completed two assessment surveys (pre and post lab) to determine the success of the experiment. The laboratory protocol was approved as exempt by the University of Mississippi Institutional Review Board (Protocol #14x-068) under 45 CFR 46.101(b)(#1), and all students gave their consent.

Instructions

Laboratory Instructor

One week before the laboratory. One week before the laboratory exercise, the instructor or laboratory manager should prepare 1 liter of Ringer's solution. (1) Using Table 2, the instructor should add the appropriate amount of each chemical to approximately half of a liter of distilled water. (2) The mixture should be stirred thoroughly using the stir plate and magnetic stir bar until all chemicals are dissolved. (3) 2.0 grams of anhydrous glucose are then added to the Ringer's Solution. (4) The mixture

should be stirred thoroughly using the stir bar and stir plate. (5) Next, the instructor should add the remaining amount of water. Upon completion, there should be a total of 1 liter of water in the solution. (6) For best results, the mixture should be stirred for at least 15 minutes. (7) The glucose concentration of the Ringer's Solution should be tested before it is added to the blood. The target glucose reading is approximately 200 mg/dL. If the glucose reading is not at or near 200 mg/dL, the mixture must be stirred until the reading is at or near the desired concentration.

Using defibrinated bovine blood, the instructor should prepare blood solutions by mixing the appropriate amount of Ringer's solution to the bovine blood according to the ratios found in Tables 3 and 4, pre-prandial and postprandial values, respectively. Samples should be mixed thoroughly using a vortex mixer to ensure the samples' homogeneity. If the samples are not mixed thoroughly, settling of the hematocrit can significantly decrease the values. After preparing solutions, each sample should be tested and values should be recorded for the instructor's use. The readings may vary slightly from those listed in the table but will still be in range of each patient's physiological state. Such variability will add to the realism of the experiment since no two patients with diabetes demonstrate the same blood-glucose values when testing their blood. The values recorded by the students should be $\pm 15 \text{ mg/dL}$ of those listed in Tables 3 and 4 in order to ensure precision and accuracy. Lastly, the blood solutions should be placed in labeled vials. Each vial should be labeled with patient number and pre-prandial or postprandial depending on which ratio was used (i.e., Patient 1 – Pre-prandial).

Table 3: Pre-prandial Ringer's Solution to Blood Ratio

	Pre-prandial Ringer's Solution with Glucose to Blood Ratio	Expected Glucose Readings
Patient 1 - Normal Glucose Level	1.0: 2.5	71 mg/dL
Patient 2 - Pre-Diabetic	0.6: 1.0	123 mg/dL
Patient 3 - Diabetic	1.6: 1.0	191 mg/dL

Table 4: Postprandial Ringer's Solution to Blood Ratio

	Postprandial Ringer's Solution with Glucose to Blood Ratio	Expected Glucose Readings
Patient 1 - Normal Glucose Concentration	0.7: 1.0	125 mg/dL
Patient 2 - Pre-Diabetic	1.0: 1.0	145 mg/dL
Patient 3 - Diabetic	3.0: 1.0	216 mg/dL

Day of the laboratory exercise. On the day of the laboratory exercise, the instructor should gather the necessary supplies and ensure that the sample vials are thoroughly vortexed before testing.

Student Instructions. The student (or group) should obtain the pre-prandial and postprandial vials (6) of blood for each patient from the laboratory instructor as well as one glucose meter and six glucose meter test strips. The student should insert one meter test strip into the glucose meter. Using a pipette, a drop of blood should be placed on the tip of the meter strip and results recorded in a table provided by the instructor. Used meter strips should be disposed of appropriately. These steps are repeated for each blood sample. The complete lab protocol given to the students is presented in the appendix.

Troubleshooting

In order to provide the students with the best laboratory experience possible, there are a few additional considerations that should be taken into account.

For instance, glucose meters should be tested regularly to ensure that the meters are working properly. Batteries should be checked and changed as necessary. Because a measure of variation can be seen between different glucose meters, it would be beneficial if the instructor provided the students with the same brand of meters to ensure the most accurate results (21). Allowing students to use different brand meters may result in slight differences between students' reported values and the given values the instructor has recorded. Variation may be as much as 50-70 mg/dL (21).

Due to the sensitivity and complexity of the glucose meter and test strips, degradation of the meter and strips can result in different forms of variation. The

expiration date of the meter strips should be checked since old strips can lead to variation in readings. Strip and meter exposure to humidity, vibration, heat, or dirt can either increase or decrease the values measured by the glucose meters (33, 35). As a result, the meter and strips should be stored in a dry environment at room temperature. If the meter is exposed to extreme heat or extreme cold, an increase or decrease in glucose reading of approximately 5 to 7 percent will be seen (35). There is an inverse relationship between exposure to extreme temperatures and the value of readings in the test strips (33). As a result, if power failure occurs during harsh winter or summer weather one should consider disposing of the strips and the meter if values are distorted a great deal.

Because meters are programmed to respond to changes in glucose concentration at fixed hematocrit levels, measured glucose values may differ slightly from student and instructor values. An increased number of erythrocytes in the blood sample may impede the diffusion of plasma, which contains the glucose, into the reagent layer of each meter strip; increase in hematocrit may decrease readings up to 20%; and decrease in hematocrit may increase readings up to 40% (33). If the same blood is used to make the samples, the values of each sample tested should not vary between students.

The instructor should demonstrate how to correctly operate the glucose meter at the beginning of all lab sessions. However, variation can still result due to several forms of operator error. For instance, if mechanical stress is applied to the meter strips or the meters become dirty, inaccurate glucose readings will result (35). When students were performing the experiment during an initial test of the protocol (Summer 2013), they placed the portion of test strip, which had blood on it, into the meter itself. Also, if there

are bubbles in blood that is applied to the meter, or if there is an inadequate amount of blood placed onto the strip, there will be variation in the glucose readings (35).

Furthermore, students should be advised to use a different pipette to place the blood on the test strips when testing the different blood samples. Using the same pipette can result in greater variation among the readings; however the values are generally still within the required ranges.

Variation due to erythrocyte metabolism may also result after an extended amount of time (35). The blood used in this laboratory exercise is free of preservatives. As a result, glycolysis inhibitors such as fluoride and iodoacetate are not present, and some glycolysis can occur. However, data suggest that the glycolysis level is not sufficient enough to dramatically affect the glucose concentrations. Blood solutions were prepared and tested, and 28 days later the samples were tested again. At most, blood solutions differed by 12 mg/dL after one month. Generally, values were within $\pm 2 - 3 \text{ mg/dL}$.

Safety Considerations

The blood purchased from Carolina Biological Supply is sterile and is safe for student use. However, chemicals used to prepare the Ringer's Solution may cause eye or skin irritation and may be hazardous if ingested or inhaled. As a result, the instructor should use caution when preparing the solution.

Results

Expected Results

Glucose concentrations recorded will be similar to those in Table 5, which depicts data collected during February 2014. For Pre-prandial and Postprandial Patient 1, readings should be less than 100 mg/dL and less than 140 mg/dL, respectively. Similarly, pre-prandial and postprandial values for Patient 2 should be within 100 – 125 mg/dL and 140 – 200 mg/dL, respectively. Values for Patient 3 both pre-prandial and postprandial should be greater than 126 mg/dL and greater than 200 mg/dL.

Misconceptions

Values recorded by students will vary due to inherent variability of the glucose meters, and students will not record the same values as each other or the instructor. Instructors should be aware that the blood-glucose values for normal, pre-diabetic, and diabetic patients will differ but should fall in the appropriate ranges given in Table 1. The reliability of the readings is robust enough that student error (e.g., incorrectly recording data or inaccurately placing blood on the test strip) can easily be identified and corrected.

Evaluation of Student Work

Students will complete pre-laboratory questions in order to demonstrate they have met the following learning objectives:

1. Define pre-diabetes and diabetes
2. List the risk factors of the disease

Table 5: Mean and Standard Deviation of Pre-prandial and Postprandial Blood Glucose Values Recorded by Students

Sample	Pre-prandial Glucose Readings	Postprandial Glucose Readings
Patient 1 - Normal	86 mg/dL \pm 6.27	125 mg/dL \pm 6.52
Patient 2 - Pre-Diabetic	116 mg/dL \pm 4.85	146 mg/dL \pm 5.05
Patient 3 - Diabetic	190 mg/dL \pm 14.25	223 mg/dL \pm 14.48

3. Differentiate between Type 1 and Type 2 diabetes

Students will complete a data table and interpret results of the patients in order to demonstrate they have met the following learning objectives:

1. Predict pre-prandial and postprandial values for individuals with diabetic, pre-diabetic, and normal glucose concentrations
2. Operate a glucose meter and record and interpret results

Students will complete post-laboratory questions in order to demonstrate they have met the following learning objective:

1. Display sufficient knowledge of diabetes (i.e., students should be able to demonstrate that they know what diabetes is, what the risks factors are, and how treatment affects the body).

Inquiry Applications

Question 1. Using the blood glucose values recorded and the table provided, diagnose each patient.

Patient 1 does not have diabetes. Patients 2 and 3 have pre-diabetes and diabetes, respectively.

Question 2. Why do doctors recommend exercise for their patients with Type 2 diabetes?

Why does exercise help those with the disease?

Exercise promotes the use of glucose, and reduces the amount of glucose present in the blood stream.

Question 3. A hypothetical patient is a responsible diabetic who exercises regularly, eats healthy, and takes his medicine properly. The patient checks his blood glucose in the morning after he awakens. Why is his blood glucose concentration higher in the morning

than any other time of the day?

The liver produces glucose into the blood during the night. The patient's body cannot purge the glucose from the blood stream because his pancreas does not produce enough insulin to take up this excess glucose. Thus, the individual will have more glucose present in his bloodstream until he takes an insulin injection.

Question 4. A patient has been recently diagnosed with diabetes. The patient uses insulin injections in order to control her diabetes. This patient has experienced a slight weight gain. What are some possible causes of such weight gain?

Before the patient used insulin injections, her body did not use the glucose in her blood efficiently. Now the patient is using the glucose she takes in from her meals. Also, insulin can cause a decrease in blood glucose and result in hypoglycemia. Patients treat hypoglycemia with an increased food intake and thus ingest more calories.

Question 5. A patient with diabetes becomes shaky and light-headed multiple times a week. What condition are these symptoms characteristic of and what are some possible causes of this condition?

Individuals who treat the disease with insulin therapy often experience hypoglycemia. Hypoglycemia results from a decrease in blood glucose concentration. The condition can result in confusion, hunger, sweating, palpitations, and death if concentrations remain too low.

Wider Educational Applications

As discussed earlier, this exercise can be modified for upper-level science students. The experiment can be modified to incorporate hypothesis testing. Based on the glucose concentrations of the “patients”, students could predict if the individual has or

had a chance of developing retinopathy, neuropathy, nephropathy, and/or other risk factors associated with diabetes. Based on their knowledge of diabetes, students could be asked to predict a healthy diet for each of the “patients” based on their blood glucose concentrations.

Additional Information

Prior to beginning the experiment, students were given a questionnaire to ascertain their knowledge of diabetes and diabetes testing before the exercise. Upon completion of the exercise, students were given a post-laboratory survey in order to determine the effectiveness of the laboratory exercise. For the surveys, the Likert scale was utilized (Strongly Agree, Agree, Neutral, Disagree, Strongly Disagree) (38). Pre-laboratory and post-laboratory survey questions are given in Table 6. In order to correlate student responses before and after the exercise, an anonymous seat numbering system was used.

Overall, students enjoyed the laboratory exercise. Of 209 students completing the experiment, 190 of them agreed or strongly agreed to the following statement: “Overall, I enjoyed this laboratory exercise”. Comments evaluating the experiment include the following:

“It was an interesting experiment overall.”

“It was fun to do something hands-on dealing with one of the world's most prevalent diseases.”

“I like that the lab was applied to a real life situation.”

Table 6: Pre-laboratory and Post Laboratory Survey Questions

Pre-laboratory Survey
Which is the most accurate manner in which to measure glucose levels within the body?
An individual has been diagnosed with diabetes. His doctor advises him to maintain a diet that is conducive with his treatment. What sort of diet does the doctor refer?
I believe that I am sufficiently knowledgeable about the risks and effects of diabetes.
How important to you is it to have realism in the lab?
How important is it to know your own blood glucose readings?
How important is it to be able to interpret these glucose readings?
Post-laboratory Survey
Given the constraints in the laboratory (i.e., using simulated blood), the laboratory exercise was realistic.
I feel more knowledgeable about diabetes testing.
I understand the disease diabetes better than I did before this laboratory exercise.
Overall, I enjoyed this laboratory exercise.
How would you rate the overall level of difficulty?

This experiment was helpful because the methods are used in real life in the clinical field.”

“Fun lab, very interesting!”

“Wish we would have more labs like this that seem to relate to clinical medicine setting since most students take A&P are going into the medical field anyway.”

“Loved it!”

“That was FUN!”

The majority of students, 169 of 209, believe that is important to have realism present in the lab, and 194 of the students believed that this experiment was realistic given the constraints (i.e., using simulated blood). Most of the students classified this experiment as easy or very easy, and 44 gave neutral responses. Prior to the experiment, students were asked to self-evaluate their knowledge of diabetes. Of the students, 115 agreed and 32 strongly agreed that they were sufficiently knowledgeable about the risks and effects of diabetes. After the experiment, students were asked to self-interpret their increase in understanding of the disease diabetes. The majority of students agreed, 84, or strongly agreed, 80, that they understood the disease better after the experiment.

A number system was devised in order to determine the overall increase in student knowledge. For a self-reported gain in knowledge (Agree to Strongly Agree), a +1 was assigned to that student. If a student reported a Neutral response when asked if he/she was sufficiently knowledgeable about the risks and effects of diabetes and then Strongly Disagreed that he/she understood the disease diabetes better than he/she did before the

laboratory exercise the student was assigned the number -2. Sixty-four students had a calculated value of 0 and saw no increase in knowledge while 74 students reported a gain of 1. Nineteen and eight students reported a gain of 2 and 3, respectively. Only one student saw a gain of 4. At the end of the experiment, forty-three students had a calculated negative value indicating a drop in their self-assessed knowledge of diabetes. According to the Dunning-Kruger effect students “will dramatically overestimate their ability and performance relative to objective criteria” (18). As a result, this drop is likely attributable to an over-estimation of their knowledge prior to the experiment.

Conclusion

As diabetes becomes increasingly prevalent in the United States, it is imperative that young adults become educated about the effects and risk factors of the disease. Using this laboratory exercise will inform students about the disease and the diabetes testing process. This laboratory exercise provides a physical, hands-on student activity that highlights pancreatic endocrinology and the results of pancreatic malfunction. The laboratory exercise is relatively inexpensive and can be performed in under an hour. When tested, the blood samples glucose readings were consistently within the desired ranges. Upon its completion most students report an increase in knowledge concerning diabetes. An overwhelming majority of the students enjoyed the laboratory exercise.

List of References

1. **Allen C, Harper V.** *Laboratory Manual for Anatomy and Physiology*. Hoboken, NJ: Wiley and Sons, 2011, p. 398.
2. **American Diabetes Association (2014).** Complications [Online]. <http://www.diabetes.org/living-with-diabetes/complications/?loc=lwd-slabnav> [2014].
3. **American Diabetes Association.** (March 2013). Economic costs of diabetes in the U.S. In 2012 [Online]. *Diabetes Care*. <http://care.diabetesjournals.org/content/early/2013/03/05/dc12-2625.full.pdf+html> [2013].
4. **American Diabetes Association.** Diabetes Basics [Online]. <http://www.diabetes.org/diabetes-basics/?loc=db-slabnav> [April 2013].
5. **American Optometric Association.** Diabetic Retinopathy [Online]. <http://www.aoa.org/patients-and-public/eye-and-vision-problems/glossary-of-eye-and-vision-conditions/diabetic-retinopathy> (Dec. 2013).
6. **Blanchard T, Lyson T.** Food availability and food deserts in the nonmetropolitan south. *Southern Rural Development Center* 12:1-7, 2006.
7. **Boyle J, HoneyCutt A, Narayan K, Hoerger T, Geiss, L, Chen H, Thompson T.** Projection of diabetes burden through 2050. *Diabetes Care*. 11: 1936-40, 2001.
8. **Brandle M, Zhou H, Smith B, Marriott D, Burke R, Tabaei B, Brown M, Herman W.** The direct medical cost of Type 2 diabetes. *Diabetes Care*. 8: 2300-2304, 2003.
9. **Centers for Disease Control (2013).** Prevalence of diabetes [Online]. Centers for Disease Control. <http://www.cdc.gov/diabetes/statistics/prev/national/figpersons.htm> [2013].
10. **Centers for Disease Control and Prevention (Dec. 2013).** Healthy eating for a healthy weight [Online]. http://www.cdc.gov/healthyweight/healthy_eating/ [Jan. 2014].
11. **Centers for Disease Control and Prevention (Jan. 2014).** 2011 National Diabetes Fact Sheet [Online]. <http://www.cdc.gov/diabetes/pubs/estimates11.htm#12> [Dec 2013].
12. **Centers for Disease Control and Prevention (Oct. 2013).** Diabetes interactive atlas [Online]. <http://www.cdc.gov/diabetes/atlas/> [Jan. 2014].
13. **Coleman-Jensen A, Nord M, Singh A.** Household food security in the United States in 2012. *United States Department of Agriculture*. 155: 1-33, 2013.
14. **Congressional Diabetes Caucus.** Facts and figures: diabetes in the United States [Online]. <http://www.house.gov/degette/diabetes/facts.shtml> [9 January 2014].
15. **Data-Planet: A Universe of Data (2014).** Diabetes [Online]. <http://0-data-planet.conquestsystems.com.umiss.lib.olemiss.edu/statistical/Main.jsp;jsessionid=>

- B6B06E56D9EF79160CC03F35F0503967?dataplanet=true [2013].
16. **Deshpande S, Basil M, Basil D.** Factors influencing healthy eating habits among college students: an application of the health belief model. *Health Marketing Quarterly*. 26:145-164, 2009.
 17. **Diabetes Mellitus.** *Black's Medical Dictionary, 42nd Edition*. London: A&C Black, 2010. *Credo Reference*. 9 Mar. 2010.
 18. **Kruger, Justin; David Dunning (1999).** "Unskilled and Unaware of It: How Difficulties in Recognizing One's Own Incompetence Lead to Inflated Self-Assessments". *Journal of Personality and Social Psychology* 77 (6): 1121–34.
 19. **Fox S.** *A Laboratory Guide to Human Physiology Concepts and Clinical Applications*. New York: McGraw Hill, 2004, p. 350-351.
 20. **Gonzalez, A.** Vulnerable populations and diabetes. *Diabetes Spectrum*. 25: 6-7, 2012.
 21. **Hellman R.** Glycemic variability in the use of point-of-care glucose meters. *Diabetes Spectrum*. 3: 135-140, 2012.
 22. **Huang E, Basu A, O'Grady M, Capretta J.** Projecting the future diabetes population size and related costs for the U.S.. *Diabetes Care*. 12: 2225-2229, 2009.
 23. **King H, Aubert R, Herman W.** Global Burden of Diabetes, 1995-2025. *Diabetes Care*. 9: 1414-31, 1998.
 24. **Lopez A, Seligman H.** Clinical management of food-insecure individuals with diabetes. *Diabetes Spectrum* 25: 14-18, 2012.
 25. **Martin T.** *Laboratory Manual for Human Anatomy and Physiology*. New York: McGraw Hill, 2013, p. 395-397.
 26. **McDaniels C.** (Nov. 2013). Oxford sustains a changing food culture [Online]. The Daily Mississippian Online, University of Mississippi. <http://thedmonline.com/oxford-sustains-a-changing-food-culture/> [Nov. 2013].
 27. **Mississippi State Department of Health (Sept. 2012).** Diabetes in Mississippi. [Online]. http://msdh.ms.gov/msdhsite/_static/43,0,296.html [2013].
 28. **National Diabetes Education Program (2011).** The facts about diabetes: a leading cause of death in the U.S. [Online]. <http://ndep.nih.gov/resources/MediaDetail.aspx?ResId=334> [2013].
 29. **National Eye Institute (June 2012).** Facts about diabetic retinopathy [Online]. <http://www.nei.nih.gov/health/diabetic/retinopathy.asp> [Dec. 2013].
 30. **Occupational Safety and Health Administration.** Blood borne pathogens and needle stick prevention [Online]. <https://www.osha.gov/SLTC/bloodbornepathogens/> [Dec. 2013].
 31. **Pflanzer R.** *Experimental and Applied Physiology Laboratory Manual*. New York: McGraw Hill, 2007, p. 348-356.
 32. **Reyes-Velazquez W, Hoffman E.** Toward reducing the diabetes pandemic: college students' perspectives of Type 2 diabetes. *Diabetes Spectrum*. 3:161-168, 2011.
 33. **Tang Z, Lee J, Louie R, Kost G.** Effects of different hematocrit levels on glucose measurements with handheld meters for point-of-care testing. *Arch*

- Pathol Lab Med.* 124: 1135-1140, 2000
34. **The University of Mississippi Food Bank.** About us [Online]. <http://dos.orgsync.com/org/umfoodbank/About> [Feb. 2014].
 35. **Tonyushkina K, Nichols J.** Glucose meters: a review of technical challenges to obtaining accurate results. *Journal of Diabetes Science and Technology.* 4: 971-980, 2009.
 36. **Tuomilehto J, Lindstrom J, Erikson JG, Valle TT, Hamalainen H, Ilanne-Parikka P.** Lifestyle changes can prevent the development of diabetes mellitus. *CMAJ* 164: 1885, 2001.
 37. **United Health Foundation (2013).** America's health rankings: Mississippi [Online]. <http://www.americashealthrankings.org/ALL/Diabetes/2013> [2014].
 38. **Vagias, Wade M. (2006).** *Likert-type scale response anchors.* Clemson International Institute for Tourism & Research Development, Department of Parks, Recreation and Tourism Management. Clemson University.
 39. **Wise E.** *Anatomy and Physiology the Unity of Form and Function.* New York: McGraw Hill, 2012, p. 375-376.
 40. **Wood M.** *Laboratory Manual for Anatomy and Physiology.* Boston: Pearson, 2013, p. 367-368.
 41. **Zhang Y, Dall T, Mann S, Chen Y, Martin J, Moore V, Baldwin A, Reidel V, Quick W.** The economic costs of undiagnosed diabetes. *Population Health Management.* 2: 95-101, 2009.

Appendix

Diabetes Simulation Protocol

Materials and Chemicals* Needed to Prepare for Experiment

Sodium Chloride, Crystals	Defibrinated Bovine Blood
Potassium Chloride, Crystals	Transfer Pipettes
Calcium Chloride, Anhydrous Granules	Electronic Balance
Sodium Bicarbonate, Powder	Stir Plate
Glucose, Anhydrous Flakes	6 Vials Per Group
Distilled Water	
1-Liter Container	

Materials Needed Per Student Group

Glucose Meter
Six Glucose Meter Test Strips (compatible with the glucose meter)
Transfer Pipettes
Sample Vials of Pre-prandial and Postprandial Blood for Patients 1, 2, and 3

1. Prepare 1 liter of Ringer's Solution.

*Lab grade quality

Mammalian Ringer's Solution

Distilled Water	1.00 liter
Sodium Chloride	9.00 grams
Potassium Chloride	0.42 grams
Calcium Chloride	0.24 grams
Sodium Bicarbonate	0.20 grams

2. Add 2.0 grams of anhydrous glucose to the Ringer's Solution. Stir the mixture thoroughly. For best results, stir for 15 minutes or more. Test the glucose concentration of the Ringer's Solution before adding it to the blood. The glucose reading should be approximately 200 mg/dL.
3. Using defibrinated bovine blood, prepare the following blood solutions. After preparing the solutions, test each sample. The readings may vary slightly from those listed in the table.

	Pre-prandial Ringer's Solution with Glucose to Blood Ratio	Expected Glucose Readings
Patient 1 - Normal Glucose Concentration	1.0: 2.5	71 mg/dL
Patient 2 - Pre-Diabetic	0.6: 1.0	123 mg/dL
Patient 3 - Diabetic	1.6: 1.0	191 mg/dL

	Postprandial Ringer's Solution with Glucose to Blood Ratio	Expected Glucose Readings
Patient 1- Normal Glucose Concentration	0.7: 1.0	125 mg/dL
Patient 2 - Pre-Diabetic	1.0: 1.0	145 mg/dL
Patient 3 - Diabetic	3.0: 1.0	216 mg/dL

4. Place the blood solutions in vials. Label each vial with patient number and pre-prandial or postprandial depending on which ratio was used.

Diabetes Mellitus¹

Diabetes mellitus refers to a metabolic disorder resulting from defects in insulin secretion, defects in insulin action, or both. Insulin is a hormone that facilitates glucose uptake in the cells of the body. Insulin is released by beta cells in the pancreas when an individual consumes a meal. Without insulin, the concentration of glucose in the blood increases greatly, and the body experiences abnormalities in carbohydrate, fat, and protein metabolism. There are three major forms of diabetes: Type 1, Type 2, and gestational diabetes. Type 2 is the most prevalent form of diabetes, and 90% of individuals diagnosed with the disease have this form. Only 5-10% of individuals are diagnosed with the more severe Type 1 diabetes. It is estimated that patients with gestational diabetes only account for 2-3% of patients with diabetes.

Type 1 diabetes, also known as juvenile-onset diabetes, describes a condition in which the pancreas does not produce a sufficient amount of insulin. Type 1 diabetes can result from an autoimmune disorder that attacks and destroys the insulin producing beta cells within the pancreas. Type 1 diabetes is most often seen in children and young adults. Individuals with Type 1 diabetes can experience acute symptoms such as excessive thirst and frequent urination, or polyuria. Frequent urination results due to the body's attempt to purge the body of the excess glucose in the blood stream. Other symptoms include weight loss and abdominal pain.

¹ Information obtained from the following sources:
"Diabetes Mellitus." *Black's Medical Dictionary, 42nd Edition*. London: A&C Black, 2010. *Credo Reference*. 9 Mar. 2010.
American Diabetes Association

Individuals with severe cases of diabetes may develop ketoacidosis as a result of conversion of fats and proteins into ketones. This conversion could lead to a coma and even death if left untreated. As a result, insulin therapy is essential for individuals who suffer from Type 1 diabetes.

Type 2 diabetes is also known as maturity onset diabetes. This form of diabetes results from reduced insulin production or decreases in insulin utilization. As a result, the blood glucose concentrations rise. Type 2 diabetes is most often seen in patients who are older, obese, have a family history of diabetes, or do not exercise.

Individuals with Type 2 diabetes rarely experience weight loss or ketosis. Patients with Type 2 diabetes may have the disease for many years and experience no symptoms. However, those with more severe cases may experience polyuria, thirst, and recurring infections especially on the feet.

Treatment of diabetes targets symptom prevention, carbohydrate metabolism restoration, and complication minimization. The concentration of glucose in the blood is used to give an indication of blood glucose control. Individuals with Type 1 diabetes are treated with insulin injections. On the other hand, Type 2 diabetes can be treated with diet, oral medication, or insulin injections depending on the severity of the disease. Individuals who treat the disease with insulin therapy often experience hypoglycemia. Hypoglycemia results from a decrease in blood glucose concentration. The condition can result in confusion, hunger, sweating, and death if concentrations remain too low.

Individuals with diabetes must follow strict guidelines when treating their disease. For both Type 1 and Type 2 diabetes patients, blood testing is used to determine how

well blood glucose concentrations are being controlled. A healthy diet of vegetables, fruits, whole grains, and lean meats is necessary in order to control diabetes. Such a diet helps maintain a stable glucose concentration within the bloodstream because these foods have a low glycemic index, the increase in blood glucose two hours after a meal. Meals should be spaced evenly throughout the day, and it is imperative that patients do not skip meals. Healthy weight loss can significantly help those with diabetes. Weight loss promotes better glucose uptake and improves blood glucose concentrations.

If it is necessary for the patient to measure his or her blood glucose throughout the day, they patient will need a blood glucose meter. By using a small drop of blood, the meter can determine the glucose concentration in one's blood. Checking blood glucose concentrations can help determine how diet, exercise, and medicine affect one's diabetes. Blood glucose should be measured before eating or two hours after a meal. These measurements are referred to pre-prandial and postprandial glucose concentrations, respectively.

It has been found that blood glucose lab testing is more accurate than at home testing using a glucose meter. However, such variation is due to the person using the meter and not the meter itself. For instance, the following situations can result in inaccurate blood glucose readings: a dirty meter, an outdated test strip, an uncalibrated meter, a meter or test strip above or below room temperature, or an inadequate amount of blood.

Individuals with Type 1 diabetes must be very vigilant when treating the disease. Because the pancreas no longer produces insulin, patients must use 2-3 insulin

injections each day. One must check his or her blood glucose before insulin injections and 2-3 hours after a meal. The amount of insulin in each injection is adjusted depending on the patient's physical activities and diet.

Recently, more individuals have been developing pre-diabetes which results in high blood glucose concentrations. However, these concentrations are not high enough to be considered diabetes. Those with pre-diabetes are more likely to develop Type 2 diabetes. In fact, those with Type 2 diabetes almost always have had pre-diabetes in the past. Individuals who are overweight are more likely to develop pre-diabetes. Those with pre-diabetes do not usually present symptoms.

Reports show that diabetes affects more than 240 million people worldwide and is one of the world's most serious health problems. According to the American Diabetes Association, over eight percent of the American population has diabetes. Thus, it is imperative that individuals are aware of the symptoms in order to catch the disease early or prevent it entirely.

Blood Glucose Concentrations

	Pre-prandial	Postprandial
Normal Glucose Concentration	<100 mg/dL	<140 mg/dL
Pre-Diabetic	100 mg/dL – 126 mg/dL	140 mg/dL – 200 mg/dL
Diabetes	>126 mg/dL	>200 mg/dL
Hypoglycemia	<70 mg/dL	<70 mg/dL

Pre-Lab Questions

1. What is diabetes mellitus?

Diabetes mellitus refers to a metabolic disorder resulting from defects in insulin secretion, insulin action, or both.

2. Define the following:

1. *Type 1 Diabetes - Type 1 diabetes, also known as juvenile-onset diabetes, describes a condition in which the pancreas does not produce a sufficient amount of insulin.*

2. *Type 2 Diabetes - Type 2 diabetes is also known as maturity onset diabetes. This form of diabetes results from reduced insulin utilization and insulin resistance.*

3. What are the most common symptoms of diabetes?

Individuals with diabetes experience acute symptoms such as excessive thirst and frequent urination, polyuria. Other symptoms include weight loss and abdominal pain and recurring infections.

4. What is hypoglycemia?

Hypoglycemia results from a decrease in blood glucose concentration. The condition can result in confusion, hunger, sweating, palpitations and death if concentrations remain too low.

5. Why do postprandial and pre-prandial blood glucose concentrations differ?

Blood glucose should be measured before or two hours after a meal. These measurements are referred to pre-prandial and postprandial glucose concentrations, respectively. Postprandial blood glucose concentrations will be higher

due to the recent intake of glucose.

Materials Needed Per Group

Glucose Meter

Glucose Meter Test Strips (compatible with the glucose meter)

Transfer Pipettes

Sample Vials of Pre-prandial and Postprandial Blood for Patients 1, 2, and 3

Case Studies

Below are three case studies. The following patients were seen by a doctor and a summary of each patient's visit was recorded.

Patient 1 - A 21 year old woman has experienced unexplainable weight loss and is concerned about her health. The woman has had no previous health problems. However, her family does have a history of diabetes.

Patient 2 - A 50-year-old man complains of a lack of energy. This patient is overweight and does not exercise; his diet mostly consists of unhealthy processed foods. His family has a history of Type 2 diabetes.

Patient 3 - A 12-year-old girl of average size complains of frequent urination and excessive thirst. She lacks energy and has experienced unexplainable weight loss. The patient's parents decided their child should see the doctor when she complained of severe stomach pain.

Procedure

1. Obtain the postprandial and pre-prandial vials of blood for each patient. You should have a total of six vials of blood.
2. Obtain a glucose meter and six glucose meter test strips.
3. Insert one meter test strip into the glucose meter.

4. Using a pipette, place a drop of blood on the tip of the meter strip and record the results in the given table.
5. Dispose of the used meter strip appropriately.
6. Repeat steps 3-5 for the remaining samples of blood.
7. Record hematocrit values obtained earlier in lab.

Group Members: _____

Course: _____

Date: _____

Data Table

Patients' Glucose Concentrations			
Patient	Pre-prandial (mg/dL)	Postprandial (mg/dL)	Diagnosis
Patient 1			
Patient 2			
Patient 3			

Questions

1. Why do doctors recommend exercise for their patients with Type 2 diabetes? Why does exercise help those with the disease?

Exercise promotes the use of glucose, and reduces the amount of glucose present in the blood stream.

2. A hypothetical patient is a responsible diabetic who exercises regularly, eats healthy, and takes his medicine properly. The patient checks his blood glucose in the morning after he awakens. Why is his blood glucose concentration higher in the morning than any other time of the day?

The liver releases glucose into the blood during the night. The patient's body cannot purge the glucose from the blood stream because his pancreas does not produce enough insulin to take up this excess glucose. Thus, the individual will have more glucose present in his bloodstream until he takes an insulin injection.

3. A patient has been recently diagnosed with diabetes. The patient uses insulin injections in order to control her diabetes. This patient has experienced a slight weight gain. What are some possible reasons of such weight gain?

Before the patient used insulin injections, her body did not use the glucose in her blood efficiently. Now the patient is using the glucose she takes in from her meals. Also, insulin can cause a decrease in blood glucose and result in hypoglycemia. Patients treat hypoglycemia with an increased food intake and thus ingest more calories.

4. A patient with diabetes becomes shaky and light-headed multiple times a week. What condition are these symptoms characteristic of? What are some possible

causes of this condition?

Individuals who treat the disease with insulin therapy often experience hypoglycemia. Hypoglycemia results from a decrease in blood glucose concentration. The condition can result in confusion, hunger, sweating, and death if concentrations remain too low.