SIMULATED DIABETES TESTING EXPERIMENT USING ARTIFICIALLY MANIPULATED LIVESTOCK BLOOD

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ABBREVIATED TITLE Simulated Diabetes Testing Experiment Using Livestock Blood

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ABSTRACT:

Due to the societal prevalence of diabetes and lack of simple, but also realistic, endocrine physiology experiments, a laboratory protocol was developed to increase student knowledge of the disease and diabetic testing process. This protocol provides a hands-on, student-centered activity; is inexpensive; can be performed in under an hour using commercially available glucose meters; and maintains realism by using blood rather than artificial plasma. Ringer's solution and glucose were added to bovine blood to create six test samples representing three hypothetical “patients” (pre-prandial and postprandial, normal; pre-prandial and postprandial, pre-diabetic; pre-prandial and postprandial, diabetic). Students in Human Anatomy and Physiology II at the University of Mississippi during the Spring 2014 semester “field-tested” the experiment and were surveyed for their pre- and post-experiment knowledge of diabetes as well as subjective responses to the experiment. Throughout the laboratory sessions, blood glucose readings were consistently within projected ranges for the three hypothetical patients. Upon completion of the experimental protocol, the majority of students reported a self-assessed increase in knowledge concerning diabetes (164 of 209) and enjoyed the laboratory exercise (190 of 209). Bovine blood was used in the experiment to increase realism, and 194 of the students believed that this experiment was realistic given the constraints of laboratory safety, expense, and ease of testing. Due to student results and survey answers, we concluded that this laboratory exercise was successful. Suggested modifications to the protocol are included for use in advanced, inquiry-based laboratory exercises.

INTRODUCTION

Objectives and Overview

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 (12). 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 to create preprandial and postprandial blood samples for three hypothetical patients. 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 viscosity. It is our aim that students become more knowledgeable about diabetes and diabetes testing using this modified blood.

Background

Diabetes mellitus refers to a metabolic disorder “resulting from defects in insulin secretion, defects in insulin action, or both” (15). 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 (15). 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" (25). Blood glucose levels are one mechanism used in diagnosing diabetes and typical values for each condition are shown in Table 1.

Table 1: Blood glucose concentrations used for the 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

*Values greater than 200 mg/dL are termed random blood glucose.

Approximately 25.8 million people in the United States have diabetes including 7 million that have not been formally diagnosed (25). An estimated 25.6 million Americans over the age of 19 have the disease while 10.9 million of them are ages 65 and older, accounting for 11.3% and 26.9% of these age groups, respectively (25). Type 2 is the most prevalent form of diabetes, and 90% of individuals diagnosed with the disease have this form (4, 25). Only 5-10% of individuals are diagnosed with the more severe Type 1 diabetes and 2-3% of patients with gestational diabetes (4). Only 215,000 Americans younger than 19 years of age have diabetes, and most have Type 1 (25).

Type 1 diabetes, also known as juvenile-onset diabetes, is a condition in which the pancreas does not produce a sufficient amount of insulin and can result from an autoimmune disorder that attacks and destroys the insulin producing beta cells within the pancreas (4).

Individuals with Type 1 diabetes can experience acute symptoms such as excessive thirst (polydipsia) and frequent urination (polyuria) (15). 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 (15). 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 (15).

Type 2 diabetes is also known as maturity-onset diabetes and results from reduced insulin production or a decrease in insulin utilization (15). 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 (15). 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, polydipsia, polyphagia, and recurring infections especially on the feet (4). While Type 2 diabetes is the most prevalent form of the disease, it is preventable and can be managed with lifestyle changes (33). 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).

Diabetes can result in multiple, chronic complications such as micro vascular conditions such as retinopathy, neuropathy, nephropathy, and Peripheral Arterial Disease. 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). Among those diagnosed with diabetes, 40 to 45 percent have developed some form of diabetic retinopathy, but individuals with Type 1 diabetes are more likely to develop non-proliferative retinopathy (26). Patients are more likely to develop retinopathy the longer they have diabetes especially if the condition is not adequately controlled (5).

Individuals with diabetes are also 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 and 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 is the seventh leading cause of death listed on death certificates in America (25). However, most patients with diabetes do not typically die directly because of diabetes with 70% of patients dying 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 (13). In the same time period, 57,677 white Americans were affected and 12,548 African Americans were affected each year (13). In 2012, 246,000 deaths were attributable to diabetes of which 73,000 were directly caused by the disease (3, 18).

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 (22). 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). It is estimated that undiagnosed diabetes costs the nation $18 billion, or $2,864 per adult (37). 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 using data from the National Health Interview Survey, it was projected that by 2025 20 million Americans would have diabetes; however, this number was surpassed in 2009 (7). It is projected that the number of adults in the world with diabetes will rise to 300 million by 2025 (22). 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) (21).

Treatment of diabetes targets symptom prevention, restoration of carbohydrate metabolism, and complication minimization. Individuals with Type 1 diabetes are generally treated with insulin injections (15). 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 (15).

Individuals who treat the disease with insulin therapy often experience hypoglycemia, a condition that can result in confusion, hunger, sweating, palpitations, and death if blood glucose concentrations remain too low (15).

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, 15). 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 because it promotes better glucose uptake and improves blood glucose concentrations (4, 15). To measure his or her blood glucose throughout each day, the patient will need a blood glucose meter that uses a small drop of blood to 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).

Because of the individual and collective costs and the risks of diabetes, college students with and without the disease should be educated about the disease (29). Most college students live sedentary lives and have a poor diet during their undergraduate years; in fact, up to 23.3% of college students do not exercise, 14.3% exercise once a week, and only 16.8% exercise twice a week (29). A study performed in 2011 showed that student knowledge of the disease is low (e.g., students incorrectly defined diabetes and could not name the risk factors of the disease) (29). 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 (14). 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 (14). 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 (14). 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 encouragement to participate in skipping meals, eating away from home, and snacking (14).

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 (35); virtual simulation using PowerPhys ® (1); virtual simulation using Physiology Interactive Lab Simulations ® to explore endocrine function (24); exercises requiring torso models, compound microscopes, prepared slides and a cat or a pig for dissection (36); insulin shock using fish (16); and self-testing one's glucose tolerance (28). 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.

Because student safety is of the upmost importance, instructors must ensure that students avoid exposure to human biological fluids. Human blood can contain blood borne pathogens, and using needles or lancets can potentially expose students to these pathogens (27). 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 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. Hotchkiss (20) developed a simple diabetes testing protocol using artificial plasma (i.e., glucose water in varying concentrations) but this artificial plasma neither resembles real plasma in appearance or viscosity or the blood that is commonly tested by diabetics.

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.

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 modifications will be discussed later 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: Before doing this 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 in less than one hour. On average, students took 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 the experiment:

1. *Ringer's solution* (Table 2) will be used as a medium for adding glucose to the blood to simulate blood samples from the three hypothetical patients. 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 Ringer's solution:
    - Chemicals
        - sodium chloride, crystals; potassium chloride, crystals; calcium chloride, anhydrous granules; sodium bicarbonate, powder; and glucose, anhydrous flakes
    - Distilled water[1]
    - 1 liter container
    - A stir plate and a magnetic stir bar will be needed in order to mix the glucose-enhanced Ringer’s solution thoroughly.
    - An electronic balance

Table 2: Chemicals 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

[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 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 glucose-enhanced Ringer’s solution and blood samples. It will also be needed to remix the samples on the day of the experiment to ensure the mixture is homogenous. Because hematocrit values can adversely affect glucose readings, it is imperative that the blood is mixed thoroughly.

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

1. *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.
2. *One glucose meter* is needed to measure the blood glucose concentrations of the samples provided. We purchased ours from Ward's Science; however, one can possibly get meters and strips donated from a company since the exercise is educational.
3. *Six glucose meter test strips* are needed. 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.

4. *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 during testing.

Instructions

One week before the laboratory. One week before the laboratory exercise, the instructor or laboratory manager should prepare 1 liter of glucose-enhanced 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.

“Patient” samples are prepared according to the following procedure:

1. The instructor will add glucose-enhanced Ringer's solution to the defibrinated bovine

blood according to the ratios found in Tables 3 and 4, pre-prandial and postprandial values, respectively.

2. 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.
3. After preparing solutions, each sample should be tested and values 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 mg/dL of those listed in Tables 3 and 4 in order to ensure precision and accuracy.
4. 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 Defibrinated Bovine 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 Defibrinated Bovine 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 six pre-prandial and postprandial vials of blood for each patient from the laboratory instructor as well as one glucose meter and six glucose meter test strips. For each sample, the student should insert one meter test strip into the glucose meter. Using a disposable, plastic transfer pipette, a drop of blood should be placed on the tip of the meter strip and results recorded in a table provided in the laboratory handout. Used meter strips should be disposed of appropriately. These steps are repeated for each blood sample.

The complete laboratory protocol given to the students including background readings, pre-lab focus questions, step-by-step instructions, data table, and post-lab discussion questions is presented in the Appendix.

Troubleshooting

In order to provide 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 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 (19). Allowing students to use different brand meters may result in slight differences between students' reported values and values the instructor has recorded. Variation between brands of meters may be as much as 50-70 mg/dL (19).

Due to the sensitivity and complexity of the glucose meter and test strips, degradation of the meter and strips can also result in variation. The expiration date of the meter strips should be checked since old strips can lead to variation. Strip and meter exposure to humidity, vibration, heat, or dirt can either increase of decrease the values measured by the glucose meters (31, 32). 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 (32). There is an inverse relationship between exposure to extreme temperatures and the value of readings in the test strips (31). 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% (31). 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 (32). 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 (32). 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 (32). 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 $\pm 2 - 3\ \mathrm{mg/dL}$.

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.

Human Subjects

This laboratory experiment was designed for inclusion into a lower-division, Human Anatomy and Physiology laboratory curriculum and to be performed with other endocrinology laboratory exercises. Subjects that will perform this experiment will be Human Anatomy and Physiology students most of whom are interested in health professions (e.g., nursing, allied-health, and exercise science). This experiment was "field-tested" with Human Anatomy and Physiology II students at the University of Mississippi during the Spring 2014 semester. Students completed two assessment surveys (pre and post lab) to determine their prior knowledge of diabetes as well as the success of the experiment. A five-point Likert scale was utilized for all subjective response survey questions (17, 34). In order to correlate student responses before and after the exercise, an anonymous seat numbering system was used. Subjective response survey data were analyzed using a Chi-square Goodness-of-Fit test and Microsoft Excel® with the level of significance was set at $\alpha \leq 0.05$ (30) with the a priori hypothesis of no difference in number of responses between categories for each question. Critical values of X^2 with 4 degrees of freedom are 9.49 at $\alpha \leq 0.05$ and 18.46 at $\alpha \leq 0.001$. The experimental protocol examining student response data 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.

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.

Table 5: Mean and Standard Deviation of Pre-prandial and Postprandial Blood Glucose Values Recorded by Human A&P Students during the Spring 2014 semester.

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

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.

Prior to beginning the experiment, students were given a pre-laboratory survey 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 (Table 6). In the pre-lab survey, 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 representing a significant departure from a priori hypotheses (X^2 = 179.21). Students also felt that it was Important (94) or Very Important (57) for a person to know their own blood glucose levels (X^2 = 126.45). Eighty-four and 71 students felt it was Important or Very Important, respectively, for a person to be able to interpret their own blood glucose readings (X^2 = 122.16). 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 (X^2 = 147.64). Ninety-seven and 95 students Strongly Agree and Agreed, respectively, that they felt more knowledgeable about diabetes testing after the experiment (X^2 = 236.35).

Table 6: Pre-laboratory and post laboratory survey questions for Human A&P students during the Spring 2014 semester. Categorical responses for each question are shown in italics with the number of student responses in parentheses. Total number of students responding = 209.

Pre-laboratory Survey
Which is the most accurate manner in which to measure glucose levels within the body? *Blood testing (178)* *Urine testing (2)* *Each is equally accurate (29)*
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 to? *A diet high in carbohydrates (5)* *An average American diet (2)* *A healthier diet that the average American diet (202)*
I believe that I am sufficiently knowledgeable about the risks and effects of diabetes. *Strongly Agree (32) Agree (115) Neutral (41) Disagree (18) Strongly Disagree (3)*
How important to you is it to have realism in the lab? *Very Unimportant (8) Unimportant (0) Neutral (32) Important (104) Very Important (65)*
How important is it to know your own blood glucose readings? *Very Unimportant (7) Unimportant (8) Neutral (43) Important (94) Very Important (57)*

How important is it to be able to interpret these glucose readings? *Very Unimportant (8) Unimportant (5) Neutral (41) Important (84) Very Important (71)*
Post-laboratory Survey
Given the constraints in the laboratory (i.e., using simulated blood), the laboratory exercise was realistic. *Strongly Agree (88) Agree (106) Neutral (13) Disagree (2) Strongly Disagree (0)*
I feel more knowledgeable about diabetes testing. *Strongly Agree (97) Agree (95) Neutral (15) Disagree (2) Strongly Disagree (0)*
I understand the disease diabetes better than I did before this laboratory exercise. *Strongly Agree (80) Agree (84) Neutral (38) Disagree (6) Strongly Disagree (1)*
Overall, I enjoyed this laboratory exercise. *Strongly Agree (100) Agree (91) Neutral (18) Disagree (0) Strongly Disagree (0)*
How would you rate the overall level of difficulty? *Very Easy (65) Easy (99) Neutral (44) Difficult (1) Very Difficult (0)*

An anonymous numbering system was used in order to determine the overall increase in each student's knowledge. For a self-reported gain in knowledge (e.g., 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. A decrease in knowledge is never a goal of a learning exercise, but this drop is potentially attributable to students over-estimating their knowledge prior to the experiment. According to the Dunning-Kruger effect, students "will dramatically overestimate their ability and performance relative to objective criteria" prior to actual performance (23).

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
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).

Sample answers to pre-lab and post-lab questions are included in the protocol provided in the Appendix and are identified by italics.

Inquiry Applications

This laboratory exercise is a methods-level application but can be expanded for facilitated inquiry applications by using the following discussion questions. Sample answers are provided in italics.

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 by incorporating hypothesis development and case-based learning. 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. Students could be asked to present and defend their conclusions to the entire class as a discussion exercise.

Additional Information

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" ($X^2 = 234.97$). 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.”

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, felt that is Important or Very Important to have realism present in the lab ($X^2 = 176.02$), and 194 of the students believed that this experiment was realistic given the constraints (i.e., using simulated blood; $X^2 = 248.02$). Most of the students classified this experiment as Easy or Very Easy, and 44 gave neutral responses ($X^2 = 172.07$).

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.

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APPENDIX

Simulated Diabetes Testing: Instructor Resources

Materials and Chemicals* Needed to Prepare for Experiment

Sodium Chloride, Crystals

Potassium Chloride, Crystals

Calcium Chloride, Anhydrous Granules

Sodium Bicarbonate, Powder

Glucose, Anhydrous Flakes

Distilled Water

1-Liter Container

Defibrinated Bovine Blood

Transfer Pipettes

Electronic Balance

Stir Plate

6 Vials Per Group

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. Mammalian Ringer's Solution

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

*Lab grade quality

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 glucose enriched 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.

5. Samples can be tested immediately or refrigerated up to two weeks prior to testing.

Simulated Diabetes Testing: Student Protocol

Diabetes mellitus[2] refers to a metabolic disorder resulting from defects in insulin secretion, insulin action, or both and affects more than 240 million people worldwide. According to the American Diabetes Association, over eight percent of the American population has diabetes. Insulin is a hormone that facilitates glucose uptake in body cells and 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, is a condition in which the pancreas does not produce sufficient amounts of insulin. Type 1 diabetes is most often diagnosed in children and young adults and can result from an autoimmune disorder that attacks and destroys the insulin producing beta cells within the pancreas. Individuals with Type 1 diabetes can experience acute symptoms such as excessive thirst and frequent

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[2] Background 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

urination (i.e., 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. Individuals with severe cases 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.

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 relies on regular testing of blood glucose levels and targets symptom prevention, carbohydrate metabolism restoration, and complication minimization. Individuals with Type 1 diabetes are treated with insulin injections. Type 2 diabetics can be treated with diet, oral medication, or insulin injections depending on the severity of the disease. Individuals who treat their diabetes with insulin therapy often experience hypoglycemia. Hypoglycemia results from a decrease in blood glucose concentration. The condition can result in confusion, hunger, sweating, or death if concentrations remain too low.

Individuals with diabetes must follow strict guidelines when treating their disease. 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 (i.e., 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.

For both Type 1 and Type 2 diabetics, regular blood testing is used to determine how well blood glucose concentrations are being controlled as well as how diet, exercise, and medicine affect one's diabetes. By using a small drop of blood and a compatible blood glucose meter, a patient can determine the glucose concentration in his or her blood. Blood glucose measurements should be taken before eating and/or two hours after a meal. These readings are referred to pre-prandial and post-prandial glucose concentrations, respectively, with typical values for healthy and affected individuals shown in Table 1. Reliable readings are possible with home testing if care is taken to avoid the following situations that can result in inaccurate readings: a dirty meter, an outdated test strip, an un-calibrated meter, a meter or test strip above or below room temperature, or an inadequate amount of blood.

Table 1. Pre-prandial and post-prandial blood glucose concentrations in healthy and diabetic individuals.

	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

Individuals with Type 1 diabetes must be very vigilant when treating the disease by checking his or her blood glucose before insulin injections and 2-3 hours after each meal. Patients must use 2-3 insulin injections each day because the pancreas no longer produces insulin. 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 elevated 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.

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:

a) 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.]*

b) 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

Patient Case Studies

Below are three case studies for Patients 1, 2, and 3. These 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.

<u>Procedure</u>

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 small drop of blood on the tip of the meter strip. Once a reading is obtained from the glucose meter, record the results in the table provided in your laboratory report.
5. Dispose of the used meter strip appropriately.
6. Repeat steps 3-5 for each remaining sample of blood.

Simulated Diabetes Testing: Laboratory Report

Group Members:____________________ ______________________

____________________ ______________________

Course: ________________ Date: ___________________

Data Table

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

Post-Lab 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.]