EFFECT OF ORAL LIPIDS ON BLOOD GLUCOSE

A Senior Project

presented to

the Faculty of Biomedical and General Engineering Department

California Polytechnic State University,

San Luis Obispo

In Partial Fulfillment

of the Requirements for the Degree

Bachelor of Science in Biomedical Engineering

by

Tyler R. Knisley

June, 2013

PROJECT INFORMATION

TITLE:	EFFECT OF ORAL LIPIDS ON BLOOD GLUCOSE
AUTHOR:	Tyler R. Knisley
DATE SUBMITTED:	June, 2013
ADVISOR:	Dr. Trevor R. Cardinal

ABSTRACT

Effect of Oral Lipids on Blood Glucose

Tyler R. Knisley

Diabetes mellitus is a disease in which insulin hormone is ineffective or no longer produced. Multiple pathophysiologies can cause either insulin dependent diabetes mellitus (IDDM) or insulin independent diabetes mellitus (IIDM), which can lead to chronic hyperglycemia and an array of short and long term health risks. Prior research has shown that IDDM patients have dyslipidemia, and experiments infusing lipids directly into the blood result in heightened insulin resistance. The hypothesis that dyslipidemia increases insulin resistance was tested by performing oral glucose tolerance tests across three treatment conditions. Participants were nondiabetic college-aged students. Data from a control group was gathered and analyzed against a lipid and carbohydrate treatment. The lipid group showed the greatest increase in glucose level at any single time period, but the carbohydrate group remained steadily elevated throughout the three hour testing window. Analysis of the data showed the only significant difference existed between the carbohydrate and dextrose treatments, meaning the original hypothesis could not be supported. For future testing, it is recommended that each test group contain all the same subjects and a longer fasting period is given after the carbohydrate treatment is consumed. The research and experiments were condensed into a lab protocol so students enrolled in BMED 460 may further extend their knowledge of diabetes and help lead the future of treatment and device innovation.

Keywords: Insulin Independent Diabetes Mellitus, Dyslipidemia, Hyperglycemia,

Insulin Resistance

ACKNOWLEDGMENTS

A special thanks to Dr. Trevor R. Cardinal for advising this research, and to the Hannah-Forbes Scholarship for their aid in funding this project.

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

Chapter 1. Introduction

Diabetes mellitus affects over 25 million Americans and its prevalence is increasing as people are living longer [1]. Over one quarter of these cases are found in senior patients older than 65, and across all age groups, it is estimated that over 7 million affected individuals have yet to be diagnosed [1]. There are two common types of diabetes: insulin dependent diabetes mellitus (IDDM), also known as type 1, and insulin independent diabetes mellitus (IIDM), also known as type 2. Insulin is a hormone that activates a signaling cascade which allows for the transport of glucose molecules from the blood stream and interstitium to myofibers and adipocytes. These tissues may then utilize the glucose for cell growth and survival. Both forms of diabetes result in improper glucose transport and hyperglycemia (elevated blood sugar levels). Over time, this chronic hyperglycemia causes major health complications, including kidney failure, lower limb amputation, blindness, stroke, and heart disease [1]. Overall, diabetes is the seventh-highest cause of death in the United States.

Background

There are three forms of diabetes, IDDM, IIDM, and gestational, each with differing etiologies and pathophysiologies. IDDM, also known as "Juvenile Diabetes," occurs when patients are no longer able to produce insulin. Scientists believe this is caused by a combination of genetics and environmental factors, specifically a viral infection [2]. These factors trigger an autoimmune disease that slowly but permanently attack the insulin-producing β-cells of the pancreas [3]. Once they are destroyed, the pancreas produces minimal to zero new insulin for the rest of the patient's life. This

absence of insulin prevents glucose from being properly transported to muscle and adipose tissue, and can cause hyperglycemia. IDDM may develop at any age, but is most often diagnosed in children or young adults [4].

IIDM is the most common form of diabetes, and coupled with the growing obesity problem in America, is evolving into an "epidemic." In 2010, the Center for Disease Control and Prevention estimated that over 79 million Americans had prediabetes [1]. While a diagnosis of insulin resistance (prediabetes) does not guarantee these patients will develop IIDM, it indicates their blood glucose is consistently higher than normal and if left untreated, the condition is likely to progress [5].

The fat, liver, and muscle cells of IIDM patients do not properly respond to insulin. Due to this, sugar remains in the blood stream and is not properly utilized by these specific cells [6]. IIDM is often called "Insulin Resistant Diabetes" because of this. This buildup of sugars, or hyperglycemia, slowly increases the risk of additional complications occurring. Risks of developing IIDM depend on family history, excess body weight, and dietary habits involving high lipid intake.

The least common and most temporary type is gestational diabetes, which can occur around week 20 of pregnancy [7]. This is thought to be caused by the increased levels of estrogen, cortisol, and human placental lactogen, which can inhibit insulin from initiating glucose transport [8]. Gestational diabetes is often addressed without the need for medication or insulin shots. The most important concern is monitoring fetal health by measuring the fetal body size and heart rate to ensure it is developing correctly [7]. Women who become pregnant at older ages and who are overweight before conception

are at higher risks of developing this condition. However, unless the mother was diabetic before her pregnancy, this disorder normally disappears after parturition.

Insulin and Glucose Signaling Pathways

The etiology of diabetes can be explained by the interactions between insulin and glucose molecules, which are shown in **Figure 1**. Insulin is a hormone produced by the endocrine portion of the pancreas, and its release is stimulated by postprandial hyperglycemia. The endocrine portion is composed of millions of cell clusters called Islets of Langerhans. Over 75% of these cell clusters are β-cells, which are responsible for producing insulin molecules and then releasing them from the pancreatic islets [9]. Insulin's main metabolic function is activating the signaling cascade that causes glucose transport to skeletal myofibers and adipocytes, as well as reducing gluconeogenesis in the liver [10].

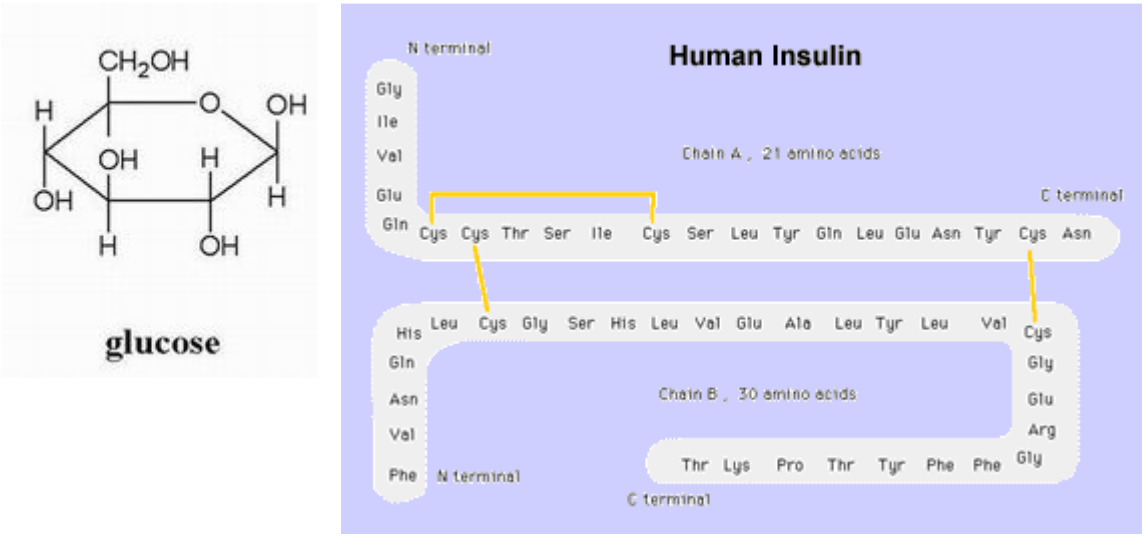

Figure 1: Diagrams of a simple glucose ($C_6H_{12}O_6$) and complex insulin molecule [11, 12].

Glucose stimulates insulin secretion by entering a β-cell via a GLUT-2 transporter [9]. Inside the β-cell it is phosphorylated by a glucokinase into glucose-6-phosphate (G6P). Additional glucose increases the ATP:ADP ratio and closes ATP-sensitive potassium channels. The closing of these channels causes the β-cell membrane to depolarize, opening calcium-ion channels. This pathway, shown in **Figure 2**, causes exocytosis of insulin secretory granules from the β-cell into the bloodstream [9].

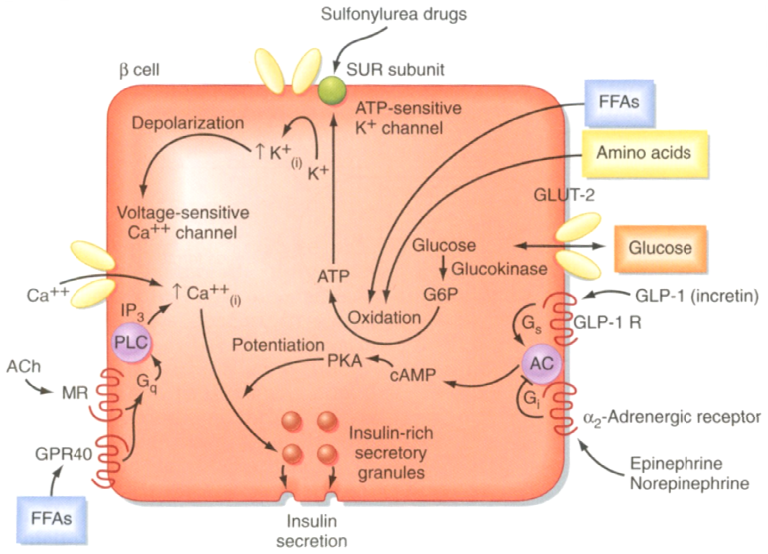

Figure 2: Signaling pathway for insulin secretion. Glucose enters the β-cell via the GLUT-2 transporter. This process eventually leads to insulin cells being secreted into the body [9].

Once insulin is secreted, it stimulates glucose transport from the blood to myofibers and adipocytes. This pathway is initiated when insulin binds to a specific type of receptor tyrosine kinase (RTK) known as an insulin receptor, which is found in almost all cells of the body [13]. The β subunits of this RTK phosphorylate tyrosine residues, which in turn recruit insulin receptor substrates (IRS). The IRS adaptor proteins aid

intracellular-signaling molecules and help specific proteins bind to appropriate receptors [14]. The IRS recruit phosphoinositide-3-kinase to the cell membrane, where it phosphorylates phosphatidylinositol 4,5-bisphosphate (PIP2) into PIP3. This step activates a protein kinase B-dependent pathway, which increases the metabolic effects of insulin and also causes GLUT-4 glucose transporter proteins to be inserted into the cell membranes of myofibers and adipocytes.

This pathway can be antagonized by hyperlipidemia. Lipids are broken down into diacylglycerol, which physiologically activate protein kinase C (PKC) to phosphorylate IRS on serine residues. This activation of serine, instead of tyrosine, phosphorylation occurs upstream in the insulin signaling pathway and stops the signal from advancing, contributing to insulin resistance.

Understanding these pathways is crucial to analyzing additional health risks and creating both therapies and better diabetes management devices.

Health Risks

While IDDM and IIDM lead to many of the same health problems, IIDM is much more prevalent, accounting for up to 90% of all diabetics [10]. Because poor health and dieting have a strong association with these patients, it is necessary to understand how these factors, especially dyslipidemia, impact the body's signaling of insulin and the relations of IIDM to insulin resistance.

Acutely, hyperglycemia is the buildup of glucose in the blood, which means cells that rely on this glucose for energy sources are not getting it properly transported to them. As a result, extra lipids are broken down into 2-carbon molecules, which are able to

undergo cellular respiration and produce ATP [15]. This shift towards lipid metabolism produces an increase in acetyl coenzyme A (acetyl-CoA), which is derived from fatty acids. The additional acetyl-CoA that the mitochondria cannot metabolize is converted into toxins called ketones. If this extreme hyperglycemic condition is not reversed, diabetic ketoacidosis may occur which can put the patient into a diabetic coma [16].

Similar to ketoacidosis, diabetic hyperosmolar syndrome occurs when insulin levels are slightly deficient, but lipolysis and ketogenesis are avoided [17]. While insulin is present, it is not functioning properly, which inhibits the body from using either glucose or fat for energy metabolism [16]. A hyperglycemic environment will still exist, and even after the kidneys filter the blood, some excess glucose will be present in the preurine. This decreases water reabsorption and will cause patients to urinate more frequently [18]. If untreated, this syndrome may also lead to a diabetic coma and critical dehydration levels.

These acute conditions can often be avoided with frequent blood-glucose monitoring. Slight levels of transient hyperglycemia will occur naturally, but testing will allow for earlier detection and quicker treatment. However, lengthened periods of hyperglycemia over many years can cause chronic conditions such as cardiovascular disease, retinopathy, nephropathy, neuropathy, and non-healing ulcers. Additionally, IIDM is often associated with metabolic syndrome, a condition caused by diets with elevated cholesterol and triglyceride levels [19]. Metabolic syndrome is diagnosed when any three of the following three conditions are present: excess adiposity in the patient's midsection, dyslipidemia, decreased high-density lipoprotein (HDL) levels, hypertension, and fasting hyperglycemia [19].

While metabolic syndrome has varying pathophysiologies, the primary effects of chronic hyperglycemia are due to endothelial dysfunction. Diabetic patients are shown to have increased production of reactive oxygen species and decreased bioavailability of endothelial nitric oxide [20]. As vascular superoxide (O_2^-) reacts with the lessened levels of nitric oxide, it produces peroxynitrite ($ONOO^-$) which inhibits receptors and enzymes that remove free radicals [20]. Excess free radicals contribute to the vasoconstriction that occurs in adverse cardiovascular diseases including atherosclerosis. The American Heart Association attributes cardiovascular disease as the cause of death in at least 65% of diabetic patients, and finds these same people to be two to four times more likely to develop heart disease or have a stroke than non-diabetic adults [21].

Chronic hyperglycemia can also affect vision. Diabetic retinopathy, or a disorder concerning the retina, is the most common condition [22]. Nonproliferative retinopathy occurs when inflammation occludes the capillaries in the back of the eye. Vision loss is rare at this stage, and often no treatment is necessary as long as the patient keeps better control of their blood glucose fluctuations [23]. This condition can progress to proliferative retinopathy when a large number of capillaries are occluded, stimulating angiogenesis, or new capillary growth in the retina. Due to their immaturity, blood may leak from newly formed capillaries, causing vitreous hemorrhage or scar tissue to form [22]. Retinopathy at this stage may result in partial or total vision loss that may be permanent.

Another common effect of diabetes is nephropathy. The nephron is the functional unit of the kidney that is responsible for filtering blood. Chronic hyperglycemia causes the kidneys to filter more blood, which damages them and makes the nephrons less

efficient over time. Waste from the kidneys begins to leak and albumin is passed into the urine [24]. Diabetic nephropathy is the leading cause of kidney failure in the United States [25]. Because early-stage symptoms are often not shown, routine blood tests should be conducted so specialists may diagnosis if a patient is at risk.

A final health risk is dyslipidemia, or increased levels of lipids and lipoproteins in the blood. This can be caused by chronic hyperglycemia and insulin resistance over time. Insulin resistance causes high triglyceride levels and increases production of very low-density lipoproteins (VLDL) and low-density lipoproteins (LDL), the pro-atherogenic forms of cholesterol. Average levels of the anti-atherogenic cholesterol, HDL, are decreased as they are rapidly depleted by transporting the excess LDL to the liver [26]. Excess LDL can pass through the endothelial layer and become oxidized and endocytosed by macrophages, which transforms them into foam cells, causing atherosclerosis and intimal thickening via hyperplasia, which can restrict blood circulation [9].

These health risks, coupled with the number of existing and future patients, necessitate more research be done to better understand and treat diabetes. The goal of this research is to develop a laboratory protocol for BMED 460 students at Cal Poly, San Luis Obispo. This protocol will lead students through a physiology experiment in which they will determine the consistency of an oral glucose tolerance test (OGTT), and test the hypothesis that lipid consumption increases insulin resistance. Upon completion of this experiment, students will be able to describe how insulin signaling becomes impaired when diacylglycerol levels rise and ultimately how IRS phosphorylation becomes dysregulated.

Chapter 2. Methods

Participants were five college-aged, non-diabetic students with no known health conditions. Due to the length of each trial run, not every participant was involved in every treatment.

Oral Glucose Tolerance Test

Oral glucose tolerance tests were performed similar to the clinical procedure outlined by the National Health and Nutrition Examination Survey [27]. Participants fasted for approximately nine hours before drinking a solution containing seventy-five grams of glucose (Azer Scientific). In a hospital, the patient's blood glucose level would be tested via venipuncture two hours after the glucose treatment. For this experiment, however, readings were taken in thirty minute intervals for three hours using a OneTouch Ultra glucose meter where the subject would prick their finger with a lancet device and use this blood for the measurement.

Lipid and Carbohydrate Treatments

After fasting between eight and ten hours, participants of the lipid treatment consumed 16 ounces of 70% lean / 30% fat cooked ground beef, which included 136 g of fat and 360 mg of cholesterol, or 208% and 120% of the respective daily recommended values. Subjects fasted for three additional hours to allow for gastric clearance, at which time the OGTT was performed as before [28].

Subjects in the carbohydrate treatment fasted for eight to ten hours, and then consumed pasta made with white flour. This meal totaled 168 g of carbohydrates, or 56%

of the daily recommended value. The white flour acts as a source of simple carbohydrates that will be transported to myofibers and hepatocytes to be stored as glycogen. After consuming the pasta, subjects were not allowed to eat for one hour to give the stomach time to void, after which the OGTT was performed as per to the two previous treatments [29].

Statistical Analysis

The data is presented as mean ± standard. A one-way ANOVA test was performed test the hypothesis that one of the treatments impaired glucose transport, followed by a Tukey post hoc test.

Chapter 3. Results

To create a representative curve, an OGTT with no additional treatments was performed. Following glucose consumption, participants tested their blood sugar every thirty minutes for three hours. Average values of the four trials are shown in **Figure 3**.

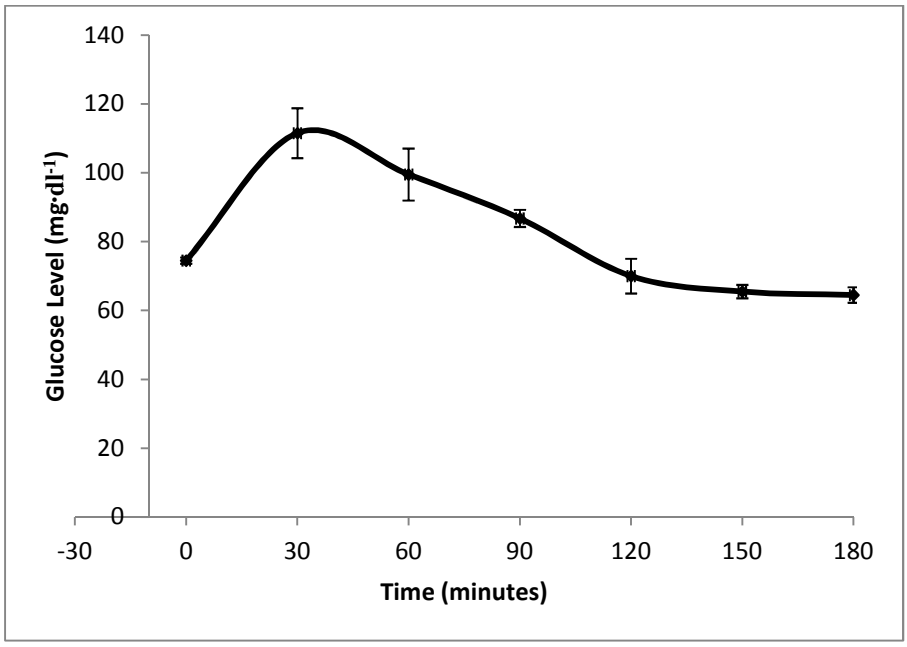

Figure 3: Average glucose levels plotted against time.

As **Figure 3** shows, a quick increase in glucose levels occurs during the first hour, after which, levels steadily decline to the original baseline reading.

Once the OGTT's consistency was established, the hypothesis that an increase of lipids would increase insulin resistance was tested. The carbohydrate treatment was included as a control for how insulin responds to an influx of carbohydrates with no additional variables affecting insulin resistance. The average values from the lipid and carbohydrate treatments are combined in **Figure 4**.

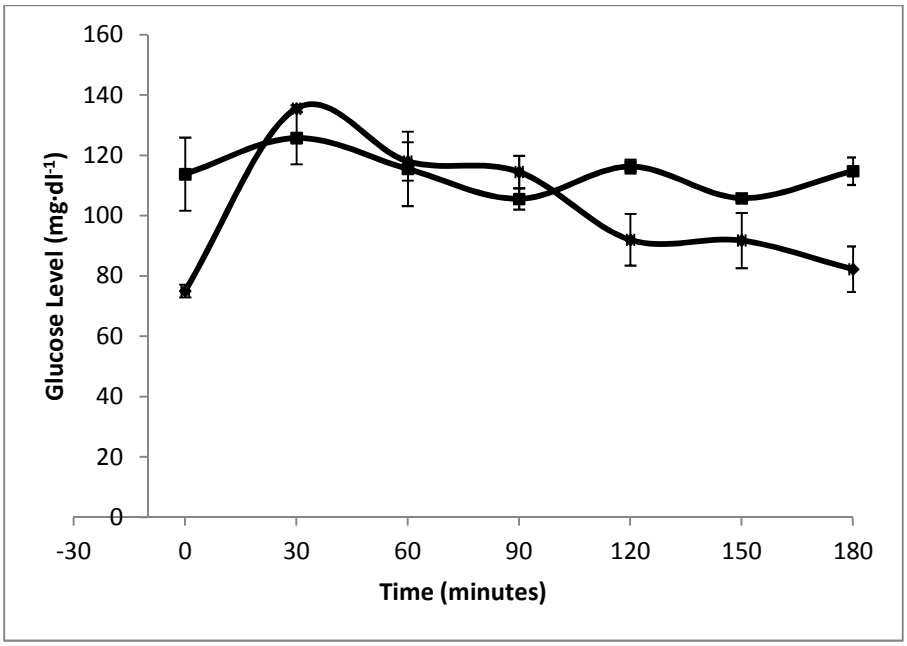

Figure 4: Average glucose levels of the lipid (diamond) and carbohydrate (square) treatments plotted against time.

The lipid values have the highest average glucose value at thirty minutes. They also seem to have two slight "plateaus," and at the final monitored time point, return close to the initial baseline reading. The blood glucose levels following the carbohydrate treatment were considerably higher and oscillated around 115 $mg \cdot dl^{-1}$ for the duration of the trial.

Statistical analysis was then performed to determine if the null hypothesis needed to be rejected. A single factor ANOVA test calculated a p-value of 0.00675, showing that a significant difference existed between at least two of the treatment groups. The complete results of this test can be found in Table I located in Appendix A. A Tukey test provided further analysis, and showed a significant difference existed between the carbohydrate and dextrose treatments. No differences were found when comparing the lipid test to either of the other treatments.

Chapter 4. Discussion

Diabetes is being diagnosed with greater frequency in the United States. IIDM is affecting over 25 million Americans, and is extending into younger age groups. Hyperglycemia and dyslipidemia associated with this disease can cause fatal health effects if left untreated over time. Previous studies, such as the one performed by The Howard Hughes Medical Institute and Yale University School of Medicine, have studied how free fatty acids effect the transportation of glucose to muscle tissue [26]. The objective of this senior project was similar in that it analyzed the effects of dyslipidemia on insulin resistance by performing OGTTs.

In order to analyze the effect of excess lipids, an OGTT had to be performed with no additional treatments to show its consistency, **Figure 3**. The blood sugar levels peaked at thirty minutes, and then steadily declined back to the baseline value at around two hours. In a clinical setting, readings greater than 200 $mg \cdot dl^{-1}$ after two hours deem the patient diabetic, although the test will be ran identically on a following day to ensure correct results. If this reading falls between 140-199 $mg \cdot dl^{-1}$, the patient is considered to be prediabetic and at serious risk of developing diabetes even if no other symptoms are present [27].

With this consistency determined, the hypothesis that excess lipid levels increase insulin resistance could be tested by comparing the results of an OGTT after a lipid-rich and carbohydrate-rich meal was consumed.

The OGTT following ingestion of carbohydrates, **Figure 4**, resulted in fairly horizontally linear values, neither showing a definitive peak nor declining to a normal fasting glucose level. This may have been due to the fact that once insulin secretion is

stimulated, it takes only minutes for insulin to be secreted from β-cells, where it can then activate glucose transport in myofibers and adipocytes. When this glucose stimulation proceeds for longer than ten minutes, the insulin secretion quickly drops off and then slowly increases again over the next hour [9], **Figure 5**.

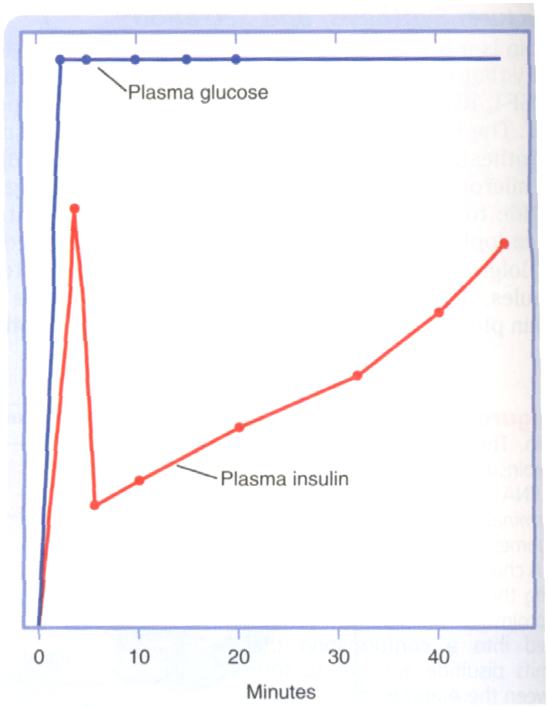

Figure 5: Plasma insulin levels when exposed to constant elevated glucose. This graph illustrates the initial insulin spike, followed by a drop and slower, steady increase over time. This process occurs when a constant treatment of glucose is being delivered to the system [9].

It takes about 10-15 minutes to consume the carbohydrate meal, and an additional hour of fasting is allowed for gastric clearance. During this period, the early phase of insulin release decreases, and the slower, longer lasting late phase of insulin release is activated [9]. This late phase steadily increases for the hour in which subjects fasted before the OGTT. This means insulin was consistently being secreted, which led to the fairly constant blood-glucose levels that were observed.

The OGTT following the lipid treatment showed the highest average value of 135 $mg \cdot dl^{-1}$ at the thirty minute mark. It also had two "plateaus," and while it eventually returned to the baseline reading, it took longer than the dextrose treatment to do so. A possible explanation for this is that gastric emptying of a high-fat meal is decreased and levels of glucagon-like peptide-1 (GLP-1) are increased [30]. One effect of slower gastric emptying is that it takes longer for glucose to enter the stomach and circulate through the body, which means that less insulin is released. For this senior project, this fact was accounted for by the three hour wait time between consuming the lipid-rich meal and performing the OGTT. The increase in GLP-1 may have factored into the decreasing glucose levels of the lipid treatment as it neared the three hour testing mark. GLP-1, which is secreted from L cells in the intestine, stimulates insulin secretion and inhibits glucagon secretion; both of which lower blood glucose levels [30]. The increase of GLP-1 was unaccounted for and may explain why no significant difference in blood sugar levels existed between the lipid and other two treatments.

Another explanation for statistical differences not existing between the lipid and carbohydrate treatments may have been that gastric emptying was not complete before the OGTT of the carbohydrate treatment. Although subjects waited an hour after eating, future tests should incorporate an additional thirty minutes of fasting. This adjustment will hopefully keep the carbohydrate blood sugar levels fairly constant, but within a lower range that may produce results significantly different from the lipids. If this occurred, it would support the hypothesis that lipid-rich meals increase insulin resistance through the phosphorylation of IRS onto serine amino acids instead of tyrosine amino acids.

Relevance to BMED 460

This study may be used to supplement the BMED 460 curriculum. While the insulin pathways are already included in this course, these results provide additional information on factors that affect insulin resistance and their compounding health effects if diabetes is left untreated. If a lab is implemented, it will give students hands-on insight to what diabetic patients must live through on a daily basis. With this disease being so prevalent, this experience may stimulate students to develop treatments and innovate existing medical devices.

Conclusion

This study was not able to support the hypothesis that lipid-rich meals increase insulin resistance. However, there was evidence to suggest that some resistance was occurring, and if this experiment was performed again, a few changes to the experimental design can be suggested. Mainly, extend the post-carbohydrate consumption fasting period to ninety minutes, and include a larger sample size of nondiabetic subjects.

References

1. National Diabetes Information Clearinghouse. (2011, February). National Diabetes Statistics, 2011. *NIDDK*, NIH Publication No. 11-3892. Retrieved from http://diabetes.niddk.nih.gov/dm/pubs/statistics/#fast.

2. Umpierrez, G.E., Murphy, M.B., & Kitabchi, A.E. (2002, January) Diabetic Ketoacidosis and Hypderglycemic Hyperosmolar Syndrome. *American Diabetes Association*. Retrieved from http://spectrum.diabetesjournals.org/content/15/1/28.full.

3. National Diabetes Information Clearinghouse. (2012, July). Diagnosis of Diabetes and Prediabetes. *NIDDK*. NIH Publication No. 12-4642. Retrieved from http://diabetes.niddk.nih.gov/dm/pubs/diagnosis/.

4. A.D.A.M. Medical Encyclopedia. (2012, August). Gestational diabetes. *U.S. National Library of Medicine*. Retrieved from http://www.ncbi.nlm.nih.gov/pubmedhealth/PMH0001898/.

5. A.D.A.M. Medical Encyclopedia. (2011, June). Type 2 diabetes. *U.S. National Library of Medicine*. Retrieved from http://www.ncbi.nlm.nih.gov/pubmedhealth/PMH0001356/.

6. Eckman, A. (2011, June). Type 1 diabetes. *U.S. National Library of Medicine*. Retrieved from http://www.nlm.nih.gov/medlineplus/ency/article/000305.htm.

7. Tamarkin, D. (2011). Lipids. *Springfield Technical Community College*. Retrieved from http://faculty.stcc.edu/AandP/AP/AP2pages/Units24to26/nutrition/lipids.htm.

8. Hose, J.E. (2012). *Lecture presented for the BMED 450 course*. San Luis Obispo, CA.

9. Wexner Medical Center. (n.d.). Gestational Diabetes. *The Ohio State University*. Retrieved from http://medicalcenter.osu.edu/patientcare/healthcare_services/diabetes_endocrine/about_diabetes/forms_of_diabetes/gestational_diabetes/Pages/index.aspx.

10. Koeppen, B.M. & Stanton, B.A. (2010). *Berne and Levy Physiology, 6e*. Philadelphia, PA: Mosby Elsevier.

11. Williford, C. (2002). Assignment #2: My Favorite Protein: Insulin. *Davidson College*. Retrieved from http://www.bio.davidson.edu/Courses/Molbio/MolStudents/spring2003/Williford/assignment2_home.htm.

12. Department of Molecular & Cell Biology. (n.d.). Endocrine Pancreas. *University of California, Berkeley*. Retrieved from http://mcb.berkeley.edu/courses/mcb135e/pancreas.html.

13. Reference.MD. (2012, June). Adaptor Proteins, Signal Transducing. *NLM PubMED*. Retrieved from http://www.reference.md/files/D048/mD048868.html.

14. National Health and Nutrition Examination Survey. (2007, January). Oral Glucose Tolerance Test (OGTT) Procedures Manual. *Centers for Disease Control and Prevention*. Retrieved from https://docs.google.com/viewer?a=v&q=cache:3RzZTNiDD54J:www.cdc.gov/nchs/data/nhanes/nhanes_07_08/manual_ogtt.pdf+what+is+an+oral+glucose+tolerance+test&hl=en&gl=us&pid=bl&srcid=ADGEESjLCiWi-VNUeKA5mo6cCgeez04vsugGeq5o0UGa8xlU9tGb9LxJYOrRlenl-5ODICU6s7PVmqtIjAxgOXFeKUxNBHhh0jGTgW_IDfzWSdKvcNuU6r_VQ03DZRGnQuv74VV7ZAzb&sig=AHIEtbQOInVQtlzCNbz6cw-RoHj1U6mZlw.

15. Mayo Clinic staff. (2012, June). Hyperglycemia in diabetes. *Mayo Clinic*, DSO1168. Retrieved from http://www.mayoclinic.com/health/hyperglycemia/DS01168/DSECTION=complications.

16. Division of Diabetes Translation. (2012, November). Diabetes Public Health Resource. *Centers for Disease Control and Prevention*. Retrieved from http://www.cdc.gov/diabetes/consumer/problems.htm.

17. National Space Biomedical Research Institute. (n.d.). The Formation of Urine. *National Space Biomedical Research Institute*. Retrieved from http://www.nsbri.org/humanphysspace/focus4/ep-urine.html.

18. Camici, G.G., Schiavoni, M., Francia, P., Machschmid, M., Martin-Padura, I., Hersberger, M., Tanner, F.C., Pelicci, P.G., Volpe, M., Anversa, P., Luscher, T.F., & Cosentino, F. (2007 March). Genetic deletion of $p66^{Shc}$ adaptor protein prevents hyperglycemia-induced endothelial dysfunction and oxidative stress. *Proceedings of the National Academy of Sciences of the United States of America*. Retrieved from http://www.ncbi.nlm.nih.gov/pmc/aricles/PMC1829289/.

19. American Heart Association. (2013, January). Cardiovascular Disease & Diabetes. *American Heart Association*. Retrieved from http://www.heart.org/HEARTORG/Conditions/Diabetes/WhyDiabetesMatters/Cardiovascular-Disease-Diabetes_UCM_313865_Article.jsp.

20. American Diabetes Association. (n.d.). Kidney Disease (Nephropathy). *American Diabetes Association*. Retrieved from http://www.diabetes.org/living-with-diabetes/complications/kidney-disease-nephropathy.html.

21. American Diabetes Association. (n.d.). Living With Diabetes. *American Diabetes Association*. Retrieved from http://www.diabetes.org/living-with-diabetes/complications/eye-complications/.

22. National Eye Institue. (2012, June). Facts About Diabetic Retinopathy. *National Institutes of Health*. Retrieved from http://www.nei.nih.gov/health/diabetic/retinopathy.asp.

23. MedlinePlus. (2013, January). Diabetic Kidney Problems. *U.S. National Library of Medicine*. Retrieved from http://www.nlm.nih.gov/medlineplus/diabetickidneyproblems.html.

24. Roizman, T. (2011, May). Insulin Resistance & Deyslipidemia. *The Livestrong Foundation*. Retrieved from http://www.livestrong.com/article/440851-insulin-resistance-dyslipidemia/.

25. National Kidney Foundation. (2013). Dialysis. *National Kidney Foundation*. Retrieved from http://www.kidney.org/atoz/content/dialysisinfo.cfm.

26. Dresner, A., Laurent, D., Marcucci, M., Griffin, M.E., Dufour, S., Cline, G., Slezak, L.A., Andersen, D.K., Hundal, R.S., Rothman, D.L., Petersen, K.F., Shulman, G.I. (1998, November). Effects of free fatty acids on glucose transport and IRS-1 associated phosphatidylinositol 3-kinase activity. *Yale University of Medicine*. Retrieved from https://connect.calpoly.edu/service/home/~/Shulman%2C%20Dresner-%20effects%20of%20FFA%20on%20glc%20trans%20%26%20IRS-1%20assoc%20PI3K%20activ%20(1999)%20JCI.pdf?auth=co&loc=en_US&id=17686&part=

27. Miller, J. (2011, July). How Long Does a Fatty Meal Take to Digest? *LiveStrong Foundation*. Retrieved from http://www.livestrong.com/article/498950-how-long-does-a-fatty-meal-take-to-digest/.

28. Howard, S. (2013). What to Eat Before and After a Workout. *University of Portland*. Retrieved from http://www.up.edu/recservices/print.aspx?cid=9880&pid=2490.

29. Bloom, S.R., Brynes, A.E., Dhillo, W.S., Frost, G.S., & McBurney, M. I. (2002, April). The effects of fiber enrichment of pasta and fat content on gastric emptying, GLP-1, glucose, and insulin responses to a meal. *European Journal of Clinical Nutrition*. Retrieved from http://www.nature.com/ejcn/journal/v57/n2/full/1601520a.html.

30. University of Maryland Medical Center. (2009, May). Diabetes – type 1 – Causes. *University of Maryland*. Retrieved from http://www.umm.edu/patiented/articles/what_causes_type_1_diabetes_000009_2.htm

Appendix A – Statistical Data

Table I: Results for Single Factor ANOVA Test

```
Source  DF    SS    MS     F      P
Factor   2  3695  1848  6.69  0.007
Error   18  4970   276
Total   20  8665

S = 16.62   R-Sq = 42.65%   R-Sq(adj) = 36.27%


                                 Individual 95% CIs For Mean Based on
                                 Pooled StDev
Level          N    Mean  StDev  ----+---------+---------+---------+-----
Dextrose       7   81.75  18.15  (--------*-------)
Carbohydrates  7  113.91   6.92                    (--------*--------)
Lipids         7  101.86  21.23            (--------*--------)
                                 ----+---------+---------+---------+-----
                                    75        90       105       120

Pooled StDev = 16.62
```

Table II: Results for Tukey Test

```
Grouping Information Using Tukey Method

               N    Mean  Grouping
Carbohydrates  7  113.91  A
Lipids         7  101.86  A B
Dextrose       7   81.75    B

Means that do not share a letter are significantly different.


Dextrose subtracted from:
               Lower  Center  Upper  ----+---------+---------+---------+-----
Carbohydrates   9.49   32.16  54.83                  (--------*--------)
Lipids         -2.56   20.11  42.78            (--------*--------)
                                     ----+---------+---------+---------+-----
                                       -25         0        25        50
Carbohydrates subtracted from:
               Lower  Center  Upper   ----+---------+---------+---------+-----
Lipids        -34.72 -12.04  10.63    (--------*--------)
                                      ----+---------+---------+---------+-----
                                        -25         0        25        50
```

Appendix B – Student Protocol

OGTT and Insulin Signaling Pathway

In this experiment, you will explore type 2 diabetes and the insulin signaling pathway on a macroscopic level by performing an Oral Glucose Tolerance Test (OGTT).

Background

Diabetes affects over 8% of the American population. Directly, it is the seventh leading cause of death in the country. Indirectly, it is a primary factor of kidney failure, lower limb amputation, and blindness, along with playing a role in hypertension and numerous cardiovascular complications. It has become an epidemic and affects people of all ages. Type 2 diabetes, in particular, is the most common and often avoidable form of this disease. Type 2 diabetics experience insulin resistance which results in sustained elevated blood sugar levels.

This experiment will provide a review of the insulin signaling pathway to show how elevated glucose levels promote insulin secretion, along with the mechanism insulin uses to metabolize this excess glucose and help keep the body in homeostasis. Students will then get the chance to undergo an OGTT. This is the same process hospitals use to screen patients who are believed to be at risk of having or developing diabetes.

The hormone insulin is a primary factor in regulating blood sugar levels for all humans. Insulin is produced by β-cells located in the endocrine portion of the pancreas, but what signals it to be released into the blood stream? Glucose triggers the first signaling pathway which is responsible for regulating insulin secretion (**Figure 1**). As the glucose travels through the blood, it uses a GLUT-2 transporter in the liver to enter a β-cell. Once here, it uses a glucokinase ("glucose sensor") to phosphorylate G6P. More glucose means more available energy, which increases the ATP:ADP ratio and closes ATP-sensitive potassium channels. Making these channels unavailable causes the β-cell membrane to depolarize and upregulate calcium-ion channels. The main effect of this is the exocytosis of insulin secretory granules from the β-cell out into the bloodstream.

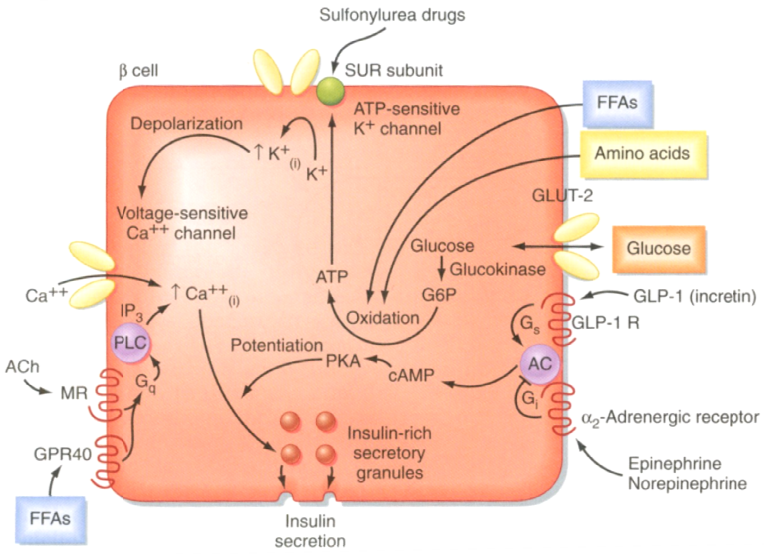

Figure 1: Cellular pathway depicting the events from glucose entering the β-cell to insulin secretion.

The goal of the OGTT is to expose the body to a large amount of glucose in a short period of time. The body then produces and secretes insulin to naturally metabolize this excess sugar. This will be quantitatively measured using a glucose monitoring device at specified time intervals. Naturally, there will be an initial spike in glucose levels. The key reading will occur two hours after consumption. If a patient's blood sugar is above 140 $mg \cdot dl^{-1}$ at this point, they may be considered to have prediabetes and further testing will need to be performed.

Ultimately, this lab is trying to give engineering students first-hand exposure to some of the routines diabetic patients have to commit to on a daily basis. With this experience, more and more improvements to monitoring devices and regulatory drugs can be made. Ultimately this will lead to fewer medical complications and hopefully fewer instances of the disease in the first place.

Required Equipment

- Lancet Device
- Lancets
- Glucose Monitor
- Glucose Monitor Test Strips (at least 7 per person)
- Glucose Solution (1 per person)
- Sanitizing Alcohol Wipes

Procedure

Exercise: Oral Glucose Tolerance Test

1. Change the lancet. To do this, unscrew the top of the lancet device. Using a new lancet, "cap" the old one already in the mechanism. The old one can be pulled straight out and the new one inserted. To disconnect the two, move the old one in a circular motion until it becomes loose and separates. Finally, screw the top of the lancet device back on.
   ⚠ **Note: To prevent the possible spread of bloodborne pathogens, always swap the lancet out for a new one.**

2. Insert a test strip into the glucose meter. A number should appear, followed by a flashing symbol which indicates it is ready to read a blood sample (**Figure 2)**.

Figure 2: This flashing symbol indicates that the meter is ready to receive a blood sample.

3. Choose which finger will be pricked (They should be rotated for each test, and never use the thumb), and clean the skin with an alcohol wipe. Wait a few seconds for the skin surface to dry.

4. Pull back the slider on the lancet device to "arm" it. Hold it against the tip of the finger, preferably on the side, and press in on the white button. The lancet will quickly project outward and produce a small drop of blood (**Figure 3)**. Note: Additional blood may need to be squeezed out to adequately fill the test trip. If squeezing does not work, the depth at which the lancet projects may be adjusted using the knob at the top of the lancet device.

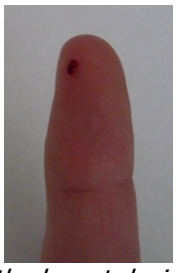

Figure 3: A drop of blood after using the lancet device on the side and tip of finger.

5. Slowly feed the drop of blood into the test strip. Once they come in contact with one another, the test strip should act like a vacuum and "pull" the blood in.

6. The glucose meter will count down 5 seconds and then display a result. This number is the blood sugar level measured in units of $mg \cdot dl^{-1}$ (**Figure 4)**. If the display shows

"Er#," this generally means not enough blood was gathered and an accurate reading could not be made. Remove the test strip and repeat the above steps to record an accurate measurement.

Figure 4: If all the previous steps are performed correctly, an accurate reading will look like this.

7. This first value is the Baseline reading before any treatment has been applied and occurs at "Time = 0 minutes." After recording this first value, students will drink the solution of 75 mg of glucose. Blood sugar will then be tested every 30 minutes following steps 1-6 with the data being recorded in a table similar to this for each student:

Time (min)	**Glucose Level ($mg \cdot dl^{-1}$)**
0	
30	
60	
90	
120	
150	
180	

8. Because this experiment involves blood, make sure all surfaces remain clean and all used materials are disposed of properly.

Analysis

1. Traditionally, a doctor will require the patient to fast for 8-10 hours before the OGTT is done. Why is this important? If a meal is consumed two hours before the test, what may be some effects it has on the results?

2. For the group's data, plot the average values at each testing time point in Excel (Scatter plot with smooth lines and markers). At what time is the glucose level the highest? The lowest?

3. Here is the data for four patients that consumed a carbohydrate-rich meal one hour before an OGTT:

Time	Trial #1	Trial #2	Trial #3	Trial #4
0	110	151	83	111
30	125	136	98	144
60	121	130	74	137
90	105	99	101	117
120	115	117	110	123
150	107	104	107	105
180	104	115	111	129

Do statistical differences exist at any time points between data from this test and what your group recorded? What statistical test(s) did you use and why?

Figure Preparation

To complete the data analysis, create two figures to display your data. The first figure will be the Excel plot of your group's averaged data. The second figure will be an Excel plot of both your average collected data and the average carbohydrate treatment data given in Problem 3. If statistical significances exist, be sure to make note of them on the graph.

All bar charts must include a white background, standard error bars, no gridlines, axes labels, legend (if necessary) within chart area, figure caption, no figure title, and statistical significance indicated.

Laboratory Report

To complete the laboratory, you will write a laboratory report (less than 2 pages) that includes introduction, methods, results, and discussion sections.

In the introduction section, you explain why a particular injury or disease-state is important to study and how recruitment, summation, max force, and/or fatigue rate can be used to assess the injury or disease state.

The methods should briefly describe how the data were collected, such that an experienced researcher could repeat your experiments from the methods section alone.

The results should include your figures and objective conclusions of the data (i.e. what).

The discussion section should include subjective interpretation(s) of the data (i.e. why), explanations for any technical/methodological difficulties, and suggestions for future applications of the utilized technique.

The report must also include a title, the team name, and all team members' names in the upper right-hand corner.