Dimethylbiguanide's Effect on Pyruvate Carboxylase

Vrishank Sai Bikkumalla

University of Northern Colorado

Frontiers of Science Institute

Abstract

With type II diabetes growing at an alarming rate, cures for the disease are developing each day. Despite this, it is unclear at times how some drugs work to inhibit the upregulated processes that take place in type II diabetes patients, specifically, the process of gluconeogenesis. It was hypothesized that an enzyme known as pyruvate carboxylase would be inhibited by dimethylbiguanide. Through the use of a diode array spectrophotometer, dimethylbiguanide concentrations of 250 μM, 1000 μM, 2500 μM, and 5000 μM were tested with statistical analysis, which included an ANOVA and a Duncan Waller post hoc test.. A ^{13}C-NMR was also conducted with combined compounds of pyruvate/dimethylbiguanide, oxalacetate/dimethylbiguanide, and biotin/dimethylbiguanide. Based on the results, significant data was found within the data set at 1000 μM, 2500 μM, and 5000 μM and an interaction between biotin and metformin occurred in the NMR trials. Both methods indicate support for dimethylbiguanide inhibition of pyruvate carboxylase, thereby leading to the inhibition of gluconeogenesis. Further studies could possibly lead to supporting results, further supporting pyruvate carboxylase inhibition by dimethyl biguanide, or the opposite could possibly occur.

Keywords: dimethylbiguanide, pyruvate carboxylase, type II diabetes, gluconeogenesis

Table of Contents

Introduction

During the reign of the third dynasty of Egypt in 1552 BCE, physician Hesy-Ra recorded the first symptoms for the disease diabetes mellitus. Half a millennia later, in 2012, 9.3% of the American populace had this disease. At seventh place leading causes of death in the United States, 234051 Americans died from the disease in 2010 (American Diabetes Association[ADA], 2017). Mostly affecting hispanic, native, and african americans, 90% of diabetes patients have a type of diabetes known as type II diabetes, which is also known as adult onset diabetes (ADA, 2017). Symptoms of the disease include dehydration, frequent and excessive micturition, polyphagia, weight loss, and blurred vision (United States National Library of Medicine[NLM], 2017a).

Type II diabetes mellitus is defined through the non genetic, inappropriate increase of glucose in the body (National Institute of Health[NIH], 2017). This non normal increase of glucose occurs due to the the glucose regulating hormone, insulin, inability to bind to hepatic insulin receptors to regulate the glucose creating process known as gluconeogenesis (NIH). This inability for insulin to bind is known as insulin resistance (National Institute of Diabetes and Digestive and Kidney Diseases[NIDDKD]). Increased glucose and insulin resistance leads to the failure of a successful negative feedback loop to regulate blood glucose, leading to a simultaneous increase of the insulin hormone from the pancreas, as the pancreas has no knowledge that insulin resistance is occurring (NIDDKD). On top of this, the gluconeogenesis process is escalated in type II diabetes patients. Furthermore, the inability of insulin to bind to hepatic insulin receptors leads to the failure of glycogenesis, which is the storing of glucose in

the liver. However, this process is not relevant to the experiment conducted (University of California San Francisco[UCSF]a).

Due to the populace affected by type II diabetes, the treatment options for type II diabetes mellitus are developing exponentially each day, including drugs such as glipizide, glimepiride, and rosiglitazone (UCSFb). However, one drug stands out as an affordable medication and is the first prescribed medication for type II diabetes mellitus due to its low cost and genericness (He, Fredic, & Windisfor, 2015). Dimethylbiguanide, with the more common name of metformin, is an anti diabetic drug that was introduced in the United States during the mid 1990's (NLM, 2017b). Despite its known properties in fighting type II diabetes and inhibiting gluconeogenesis, which assists in the lowering of glucose levels, major discourse surrounds the pathways how dimethylbiguanide inhibits gluconeogenesis. (NLM, 2017b).

The experiment presented focuses on the role of dimethylbiguanide in gluconeogenesis, focusing on the role of dimethylbiguanide on different enzymes and molecules contributing to the formation of glucose in the liver. Specifically, this experiment will focus on the enzyme pyruvate carboxylase, as this study hypothesized that dimethylbiguanide inhibits gluconeogenesis through inhibition of the pyruvate carboxylase enzyme. As more properties of dimethylbiguanide are being discovered each day, a question arises: Does the drug dimethylbiguanide inhibit gluconeogenesis through an interaction with pyruvate carboxylase?

Review of Literature

Metformin Background

In the early 20th century, early European physicians used a plant called *Galega officinalis* as an early medicine to treat diabetes. The plant was known to have blood sugar lowering properties and had the anti diabetic molecule known as guanidine, leading to its name, dimethylbiguanide (He et al, 2015). Later, in the 1920's the drug was synthesized and was modified to a biguanide, lowering the toxic effects of its sister, guanidine. First used where it was discovered of Europe, dimethylbiguanide was approved by the Federal Drug Administration in 1994 (He et al, 2015). The drug's popularity grew and 150 million people depend on the drug each year worldwide (He et al, 2015). It is the most common and a popular anti diabetic drug on the market (New York Times[NYT], 2013). Furthermore, research has found that the drug has anticancer, and anti fatty liver disease properties. (He et al, 2015; NLMb).

Anticancer properties

It has been shown that dimethylbiguanide has increased the survival rates in patients with cancer (Quinn, Kitagawa, Memmott, Gills, Dennis, 2013). Despite this, these anticancer properties have yet to be elucidated. However, it is thought that dimethylbiguanide inhibits cancer through the mTORC1 pathway. The pathway inhibited is responsible for the growth and reproduction of the cell. Inhibition of the pathway leads to apoptosis (Quinn, Kitagawa, Memmott, Gills, Dennis, 2013). Many other hypotheses exist regarding this cancer inhibition and is still debated today.

Pharmokinetics

The anti diabetic medication, dimethylbiguanide, is a widely prescribed drug to combat type II diabetes, due to its cost and availability (He et al, 2015). 40% to 60% of a dimethylbiguanide sample consumed has an active effect in the body and is completely absorbed within six hours after ingestion (AJ, 1996). After digestion, dimethylbiguanide is transported to the portal vein of the liver. Excess dimethylbiguanide is excreted through the kidneys. Dimethylbiguanide has a plasma elimination between 4 and 8.7 hours. Little data has been obtained regarding the metabolic effects of dimethylbiguanide in bloodstream (AJ, 1996). Side effects of dimethylbiguanide include diarrhea, stomach pain, constipation, heartburn, and constipation, however, in severe cases, lactic acidosis can occur, leading to severe cramping (NLM, 2017c).

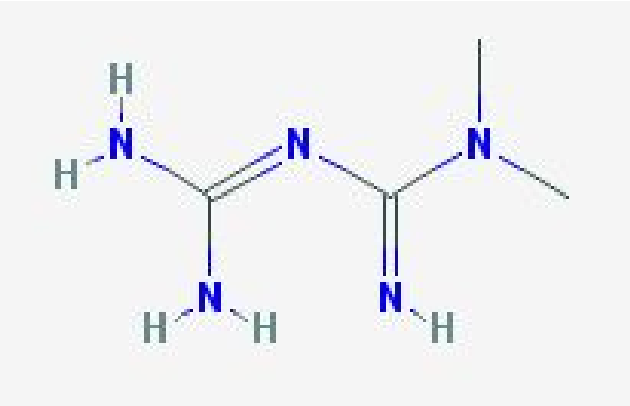

Figure 1: Many forms of dimethylbiguanide exist, however, the chemical structure of the dimethylbiguanide named as metformin has the chemical formula of $C_4H_{11}N_5$ (PubChem)

Gluconeogenesis

Due to the fact that high levels of glucose are common in type II diabetes patients, the inhibition and process of gluconeogenesis is vital for type II patients. Occurring in the mitochondria of hepatic cells, gluconeogenesis enlists the creation of glucose, which is necessary for cellular energy (UCSFa). In patients with type II diabetes, the gluconeogenesis process is increased inappropriately, leading to high levels of glucose. On top of this, insulin resistance does not allow for the glycogenesis process to occur. Under anaerobic conditions, gluconeogenesis uses the main reactant of lactate, however, under aerobic conditions, pyruvate is used (Miles, n.d). Dimethylbiguanide has been observed to inhibit the process of hepatic gluconeogenesis, however major discourse surrounds how dimethylbiguanide inhibits gluconeogenesis (He et al, 2015).

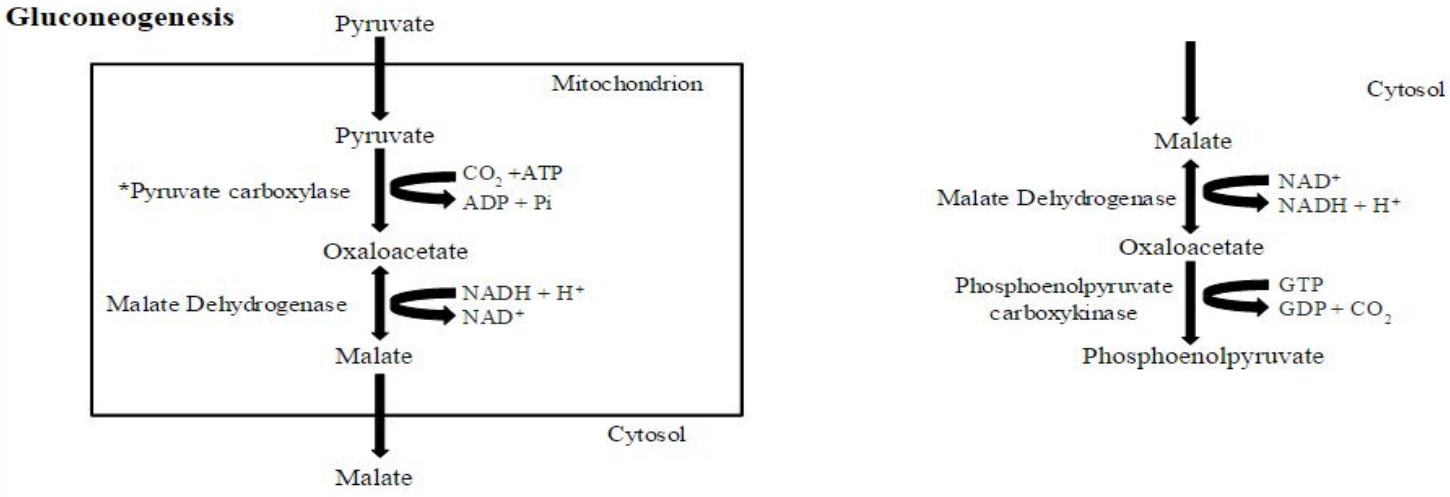

Figure 2: The process of gluconeogenesis occurs in the mitochondria and when in the malate creation state, it is continued outside the cytosol (Kiran, 2015)

LKB1/AMPK

A proposed theory for how dimethylbiguanide works is through the liver kinase B1 (LKB1) in the 5' adenosine monophosphate-activated protein kinase (AMPK) pathway. The AMPK is a kinase, meaning it is a group of enzymes that donates a phosphate group to activate or deactivate an enzyme or some other kinase (Cheng, Qi, Paudel, & Zhu, 2011;Figure 3) For example, the AMPK pathway is activated by the liver kinase through the donation of a phosphate group. A study conducted in 2010 found dimethylbiguanide worked independently from the LKB1/AMPK pathway (Foretz, Hébrard, Leclerc, Zarrinpashneh, Soty, Mithieux, & Sakamoto, 2010). Previous studies have hypothesized that dimethylbiguanide inhibits hepatic gluconeogenesis through signaling of the LKB1, therefore, affecting the AMPK pathway (Zhou, Myers, Li, Chen, Shen, Fenyk-Melody, Wu, Ventre, Doebber, Fujii, Musi, Hirshman, Goodyear, Moller, 2013). In the study, two groups of mice were injected intraperitoneally, meaning into a cavity in the body, with dimethylbiguanide, with one group not having LKB1 activated, and the other not having AMPK. Dimethylbiguanide still suppressed gluconeogenesis in both these groups, leading to the statement that dimethylbiguanide does not work through the LKB1/AMPK pathway. However, the mice used in the study were not under the diabetic conditions of type II diabetic patients. The study suggests that dimethylbiguanide inhibits the gluconeogenesis pathway in alternative ways.

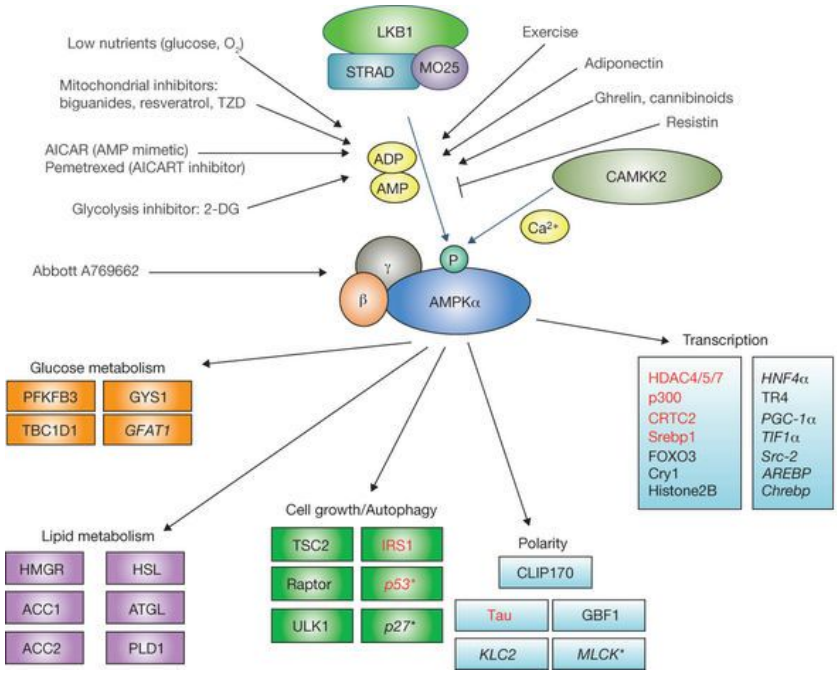

Figure 3: Representation of factors AMPK pathway is able to control. (Mihaylova, 2011)

Mitochondrial Glycerophosphate Dehydrogenase

Mitochondrial glycerophosphate dehydrogenase (MGD) is an enzyme responsible for the reoxidation of electron carriers in the mitochondria (figure 4). In this scenario, MGD is responsible for the reoxidation of NAD+, a co-enzyme used for gluconeogenesis, which is necessary for gluconeogenesis. This transport chain transports electrons from donors to acceptors through a redox reaction. However, inhibition of the enzyme would affect gluconeogenesis. A study conducted in 2014 suggested that gluconeogenesis was inhibited due to the suppression of MGD from dimethylbiguanide (Madiraju, Erion, Rahimi, Zhang, Braddock, Albright, and Shulman, 2014). The inhibition of MGD had been shown to reduce the pool of another coenzyme named NADH, and NAD+. This stopped the flow of redox reaction dependant

substrates, leading to a failure of gluconeogenesis. Although shown to support dimethylbiguanide's suppression of MGD, AMPK activation and suppression of the energy releasing cycle, Kreb, was observed during the knockdown of MGD during the study, meaning lowered glucose levels were present in the mice used for the study. Furthermore, the mice in the study were not under the conditions of what type II diabetes patients are when ingesting dimethylbiguanide. Dimethylbiguanide was injected into the mice directly into the bloodstream through catheters, which is not how a majority of type II diabetes patients take the drug. There is also no mention of if the mice are hyperglycemic and are under similar conditions to type II diabetes patients.

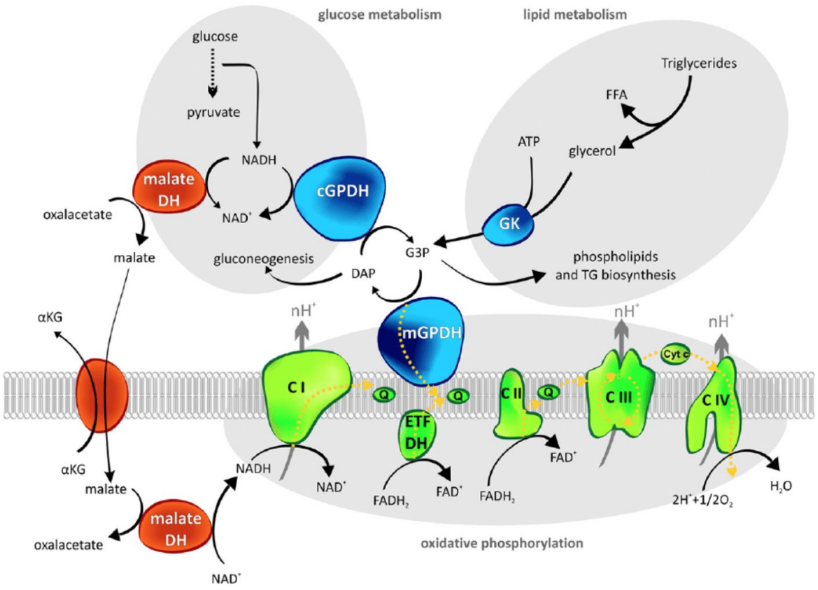

Figure 4: How MGD acts in the mitochondria and its effect on gluconeogenesis (Mráček, 2013)

SIRT1 and GCN5

SIRT1 and GCN5 are thought to be the enzymes responsible for gluconeogenic gene expression. Through the regulation and moderation of other enzymes, SIRT1 and GCN5 have the

ability to be indirectly responsible for the control and inhibition of gluconeogenesis. In a study conducted in 2010, a group of mice was treated with dimethylbiguanide and it was shown that higher levels of SIRT1 and GCN5 proteins were present in the mice treated with the drug, compared to a group of mice who had not been treated with the drug (Caton et al, 2010). This increase led to a decrease in gene expression and lower glucose and insulin levels. Furthermore, when inhibited, SIR1 had blocked the effects that dimethylbiguanide had on gluconeogenesis. Despite these results which show how dimethylbiguanide inhibits gluconeogenesis, a relative amount of dimethylbiguanide to average type II diabetes patients was not used for the study. These high amounts of dimethylbiguanide could have led to abnormal effects on the body by dimethylbiguanide.

PEPCK

Phosphoenolpyruvate carboxykinase (PEPCK) is enzyme kinase responsible for the final conversion between oxaloacetate to phosphoenolpyruvate, which occurs in the cytosol. It was determined that PEPCK has a significantly large influence over gluconeogenesis. In fact, the expression of PEPCK increase by a 2.9 multiple is various types of diabetes (Veneziale, Donofrio & Nishimura, 1983). Despite this, a study argued against the large influence of PEPCK in gluconeogenesis. A study conducted in 2009 found no significant correlation between PEPCK mRNA and plasma glucose levels in hyperglycemic mice (Samuel et al, 2009). On top of this, a metabolic control factor of 0.18 was seen in the experiment, suggesting PEPCK has a weak level control of gluconeogenesis. This shortens the list for possible theories on what is inhibited by dimethylbiguanide and how it works.

PC

Pyruvate Carboxylase (PC) is an enzyme responsible for the conversion of pyruvate to oxaloacetate, as seen in figure 2. Activated when oxaloacetate levels are low, a coenzyme to PC acetyl CoA, undergoes the Krebs cycle, resulting in an increase in acetyl CoA (Rensselaer Polytechnic University). This increase allows for the activation of PC to produce more oxaloacetate. PC has a prosthetic group, allowing for its function, named biotin (Attwood, 1995). Little is known about dimethylbiguanide's inhibition of PC, leading to one of the main reasons why experimentation was conducted.

Purpose

It has been a challenge to figure out how dimethylbiguanide reacts with parts of gluconeogenesis for its inhibition. Numerous studies have been done regarding the inhibition of gluconeogenesis through dimethylbiguanide, however, many of these studies do not mimic the conditions which type II diabetes patients are under and their intake of dimethylbiguanide. For example, many studies use non hyperglycemic rats, excessive concentrations or amounts of metformin, and catheters to allow for the entry of dimethylbiguanide into the body. High amounts of dimethylbiguanide can be toxic to the body and it is of the utmost importance to test and simulate the conditions of type II diabetes patients and how they ingest dimethylbiguanide for experimentation (He et al, 2015). Experimentation close to what the conditions of what type II diabetes patients are under will be done. Furthermore, little has been researched regarding dimethylbiguanide's interaction with pyruvate carboxylase.

Methods and Materials

The experimentation conducted was performed at the University of Northern Colorado Department of Chemistry and Biochemistry, Greeley, including lab space, chemicals and equipment.

Reagents for Pyruvate Carboxylase Assay

Step One: Reagent A

A 135 mM triethanolamine buffer with 7 mM magnesium sulfate, 9 mM pyruvic acid, and 0.15% (w/v) bovine serum albumin is necessary for the reaction to occur will be amalgamated with reagents C and E and a potential hydrogen of 8 was reached, while simultaneously being 30°C. The potential hydrogen was adjusted through a one molarity solution of hydrochloric acid. Reagent A was prepared in deionized water.

Step Two: Reagent B

A 0.3 mM acetyl coenzyme a solution was prepared in deionized water and was prepared proceeding the beginning of experimentation.

Step Three: Reagent C

For every 1 milliliter of reagent B, 30 units of the malic dehydrogenase enzyme solution and an amount of deionized water that is double the amount of reagent B was added into the solution.

Step Four: Reagent D

A 100 mM triethanolamine buffer with 30 mM adenosine 5'-triphosphate and 450 mM sodium bicarbonate was prepared for Reagent D. A potential hydrogen of 8 should be reached at 30°C. The potential hydrogen was adjusted with 1 molarity sodium bicarbonate.

Step Five: Reagent E

A 2.6 mM β-nicotinamide adenine dinucleotide solution was prepared in deionized water.

Step Six: Reagent F

A 50 mM tris HCl buffer with 50% (v/v) glycerol, 2 mM magnesium acetate, and 1 mM ethylenediaminetetraacetic acid was prepared for Reagent F. The solution was prepared in deionized water. The potential hydrogen of the solution was adjusted to 7.4 at 30°C with 1 molarity hydrochloric acid. Reagent F was amalgamated with reagent G.

Step Seven: Reagent G

A pyruvate carboxylase solution was prepared in deionized water, containing 32.5 units/mL. The solution was prepared proceeding experimentation.

Diode Array Spectrophotometry for Pyruvate Carboxylase Assay

The A+C+E reagent cocktail, a concentration of dimethylbiguanide (dependent on trial number), the pyruvate carboxylase solution, the acetyl coenzyme A solution, the malic dehydrogenase solution, and the adenosine triphosphate solution were all added into a 1 millimeter cuvette, which was placed into the diode array spectrophotometry A blank test was first conducted using the spectrophotometer, which was later followed more other trials. Light shined through the array at 1.4 seconds, 7 seconds, and every 5 seconds after, until a time of one minute had been reached. 20 trials were conducted, excluding the blank test. A constant

temperature of 30.0°C was maintained during the the conducting of the experiment. Six trials were conducted with a dimethylbiguanide concentration of 250 μM/L, 500 μM/L, 2500 μM, and 0 μM/L, while three trials were conducted at 1000 μM/L, 5000 μM/L.

The absorbance rates of NADH that were received from the diode array spectrophotometer were converted to enzymatic activity rates through Beer-Lambert's Law, which states that the absorbance of light is directly proportionate to the thickness of where the light is conveyed through, multiplied by the absorbent ($A = \varepsilon lc$; where ε is the the molar attenuation coefficient (6220), l is the length of the medium of where light is being conveyed through, and c is concentration of the solution).

Statistical Analysis

The rates were then inputted into SAS (Cary, NC) for analysis. Enzymatic activity rates from the trials were ran through a one-way analysis of variance test (ANOVA). A Duncan Waller's post hoc test was then later conducted.

^{13}C-Nuclear Magnetic Resonance (NMR)

^{13}C-NMRs for dimethylbiguanide, biotin, a biotin/ dimethylbiguanide solution, oxaloacetate, a oxaloacetate/ dimethylbiguanide solution, pyruvate, and a dimethylbiguanide/ pyruvate solution have been done for analysis. Each combined compound was compared to its two parent compounds for changes in peaks and/or if any similarities were present.

^{13}C-NMR for Combined Compounds

All singular compounds have been analyzed through the 13C-NMR with a 99.8% D_2O solvent (Sigma-Aldrich, MO). Combined compounds have been specifically prepared based on the required process to allow for the ^{13}C-NMR process to proceed correctly.

Equal volumes of dimethylbiguanide and pyruvate, biotin, or oxaloacetate have been combined and have been heated to 30-36°C for 10 minutes. The final amalgamated sampled was mixed with D_2O. For each sample, 12,000-15,000 scans were done overnight. A Bruker Avance I 360 MHz FT-Nuclear Magnetic Resonance Machine was used for the experiment and was kept at an ambient temperature.

Results

Absorbance of Diode Array

Absorbance was collected from the diode array and was graphically mapped using Excel 2011. It was consistently showed that the control group had a greater decline than other trials performed, with a few exceptions.

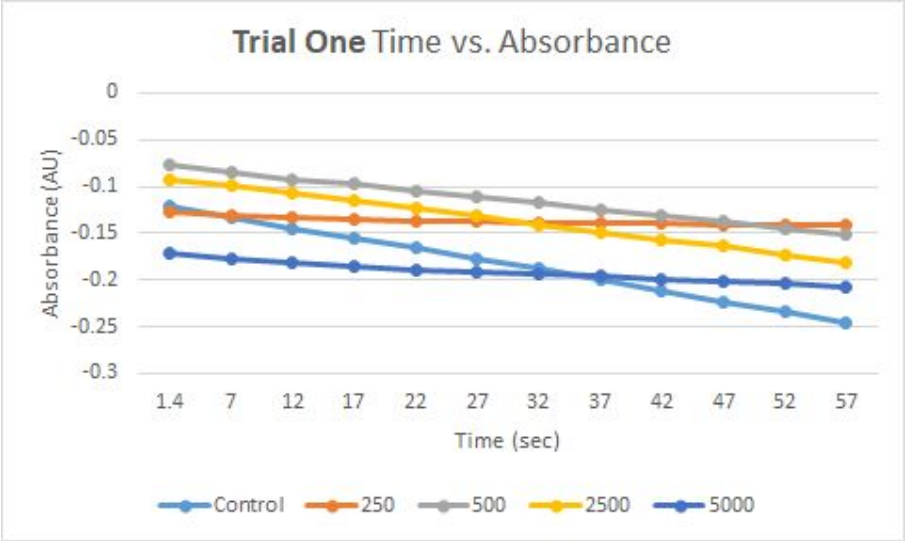

Figure 5: A graphical representation of the first trial of time of reaction and absorbance of NADH.

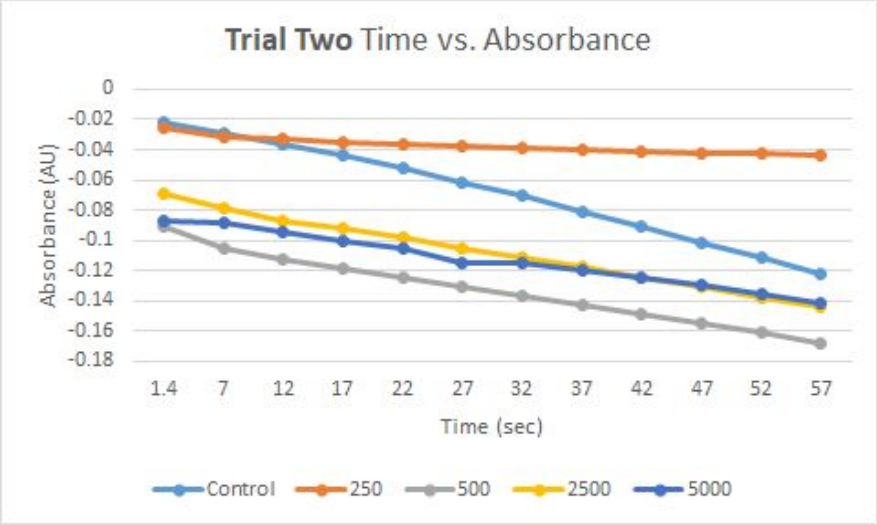

Figure 6: A graphical representation of the second trial of time of reaction and absorbance of NADH.

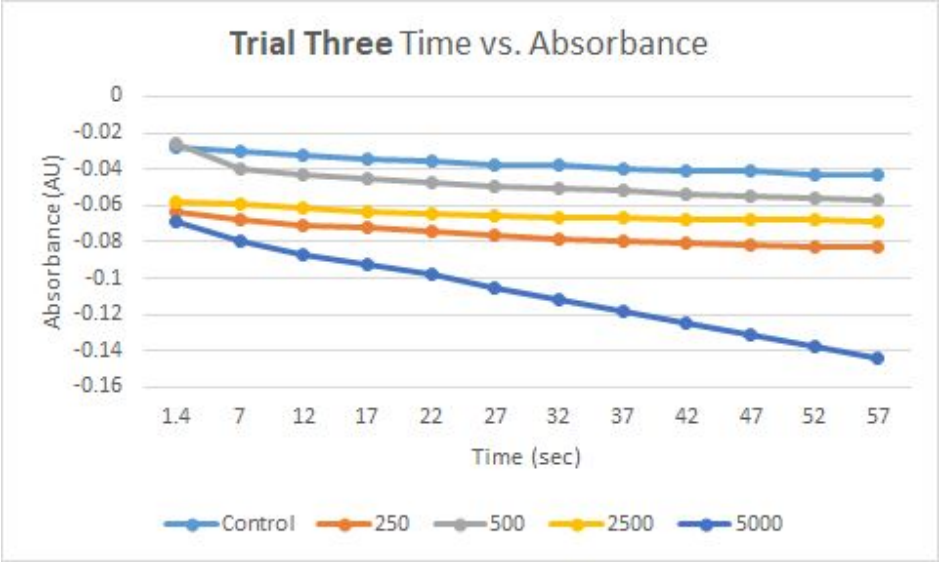

Figure 7: A graphical representation of the third trial of time of reaction and absorbance of NADH.

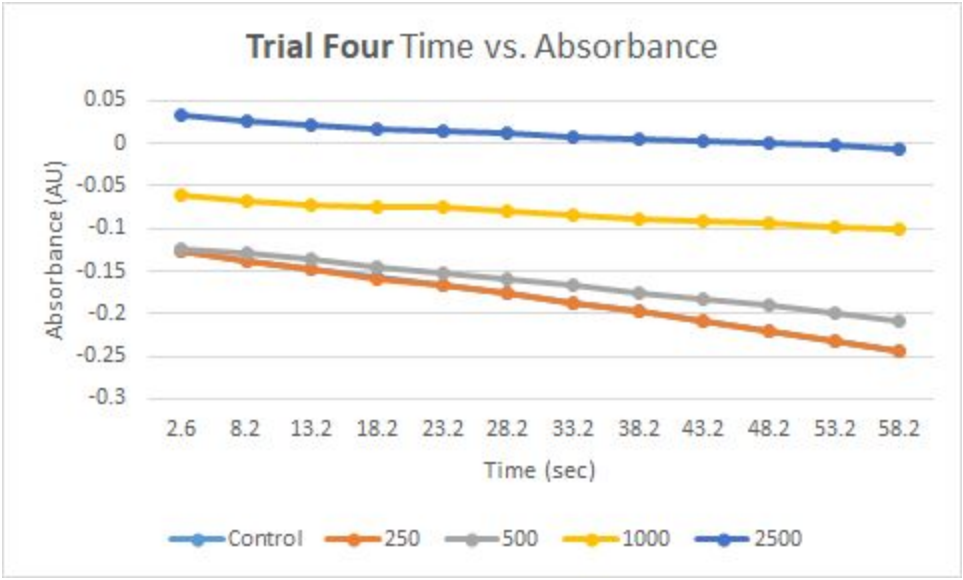

Figure 8: A graphical representation of the fourth trial of time of reaction and absorbance of NADH.

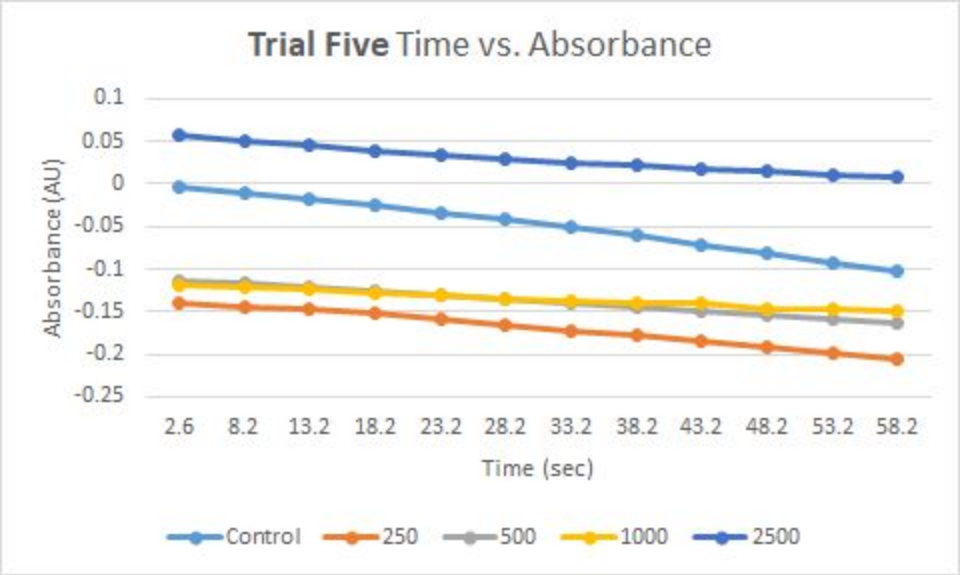

Figure IX: A graphical representation of the fifth trial of time of reaction and absorbance of NADH.

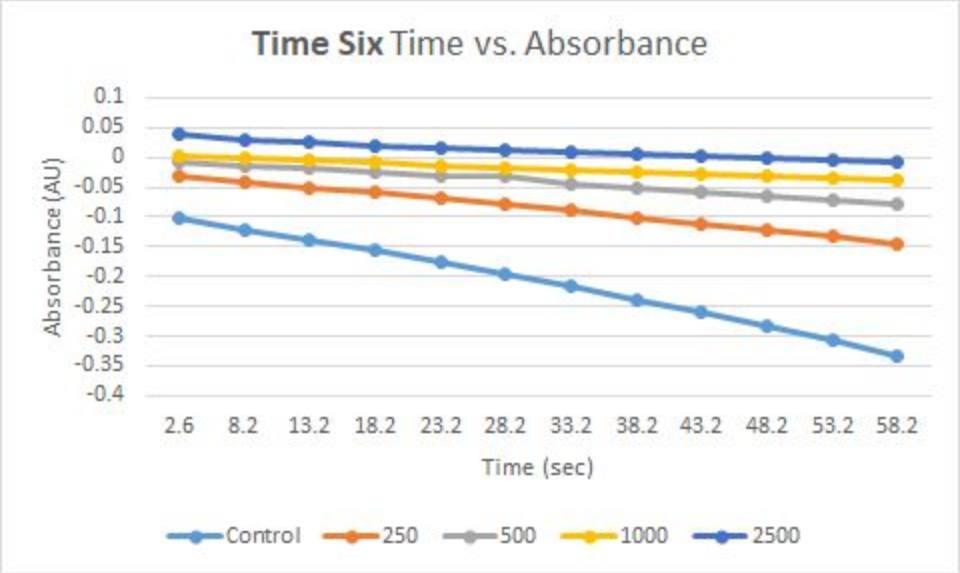

Figure X: A graphical representation of the sixth trial of time of reaction and absorbance of NADH.

Statistical Analysis

ANOVA

An ANOVA was performed on the concentrations of NADH collected.

F	2.66
DF	5
α	0.05
P	0.0473

Table 1: Representation of F, DF, α and P values gathered from experimentation using SAS 9.4 (Cary, NC).

It can be seen be seen from the ANOVA that the p value is less than the α value, meaning significance was found in the data set.

Duncan Waller's Post Hoc

Means with the same letter are not significantly different.				
Waller Grouping		Mean	N	concentration
	A	3.34941E-7	6	0000Rate
	A			
B	A	1.79528E-7	6	500Rate
B	A			
B	A	1.66131E-7	6	250Rate
B				
B		1.1254E-7	3	1000Rate
B				
B		1.09861E-7	6	2500Rate
B				
B		1.07181E-7	3	5000Rate

Table II *Representation of significant and insignificant values in data set from SAS (Cary, NC).*

As seen in the Duncan Waller (table two), the significantly different values found in experimentation were at dimethylbiguanide concentrations of 1000 µM, 2500 µM and 5000 µM, compared to the control.

^{13}C-NMR

A ^{13}C-NMR was performed with all solutions, using the Bruker software. It was noticed that a carbonyl group had disappeared in the Dimethylbiguanide and Biotin solution.

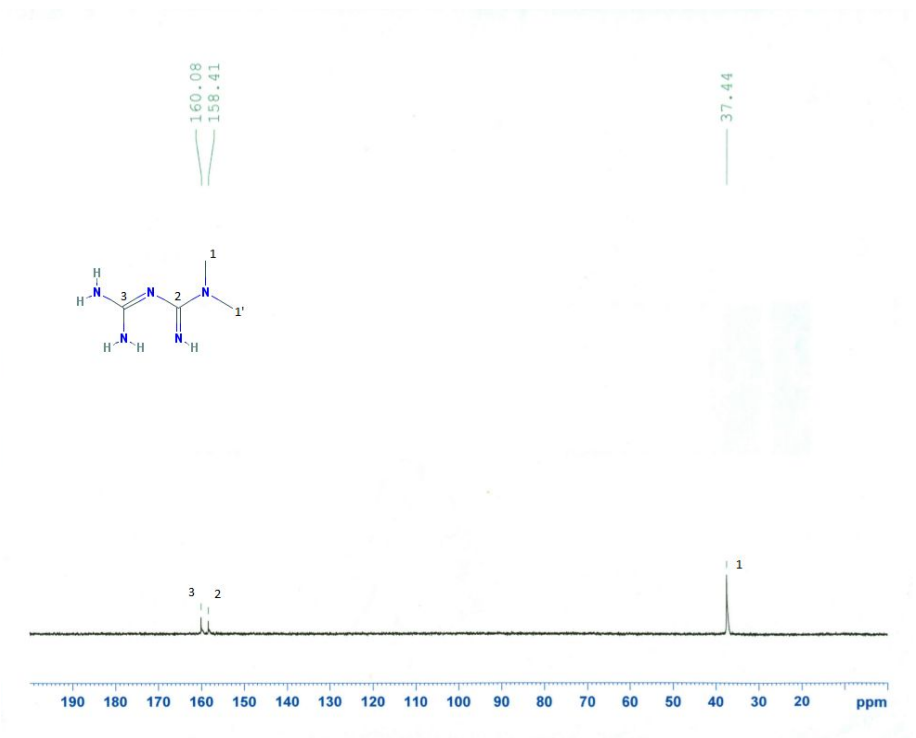

Figure Eleven: Trial One of ^{13}C-NMR of Dimethylbiguanide

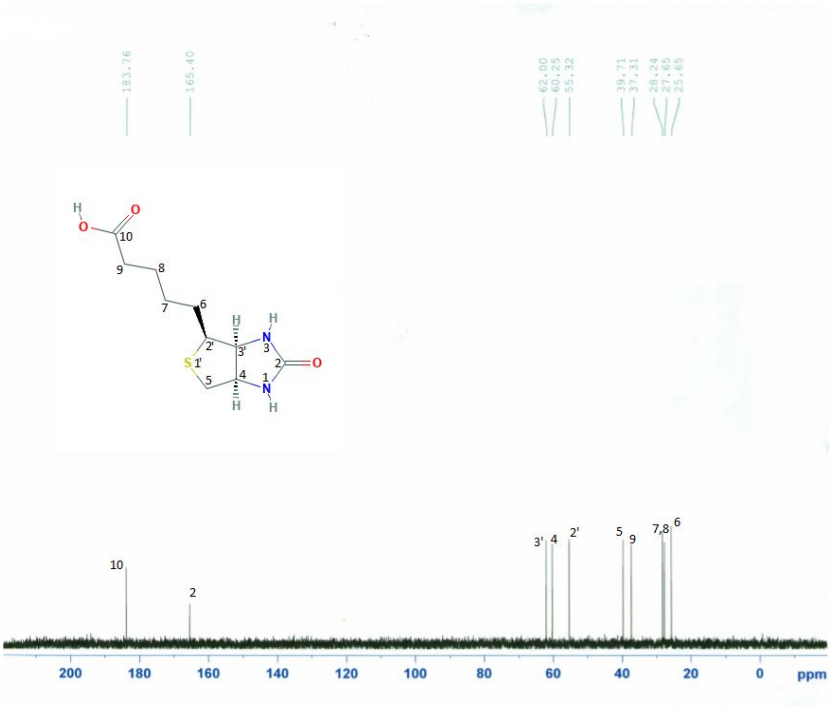

Figure Twelve: Trial One ^{13}C-NMR of Biotin

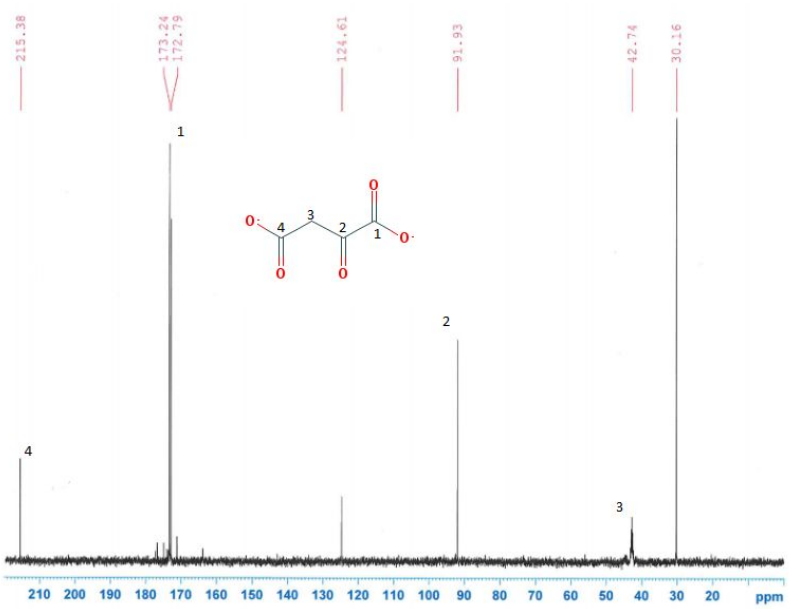

Figure Thirteen: Trial One ^{13}C-NMR of Oxalacetate

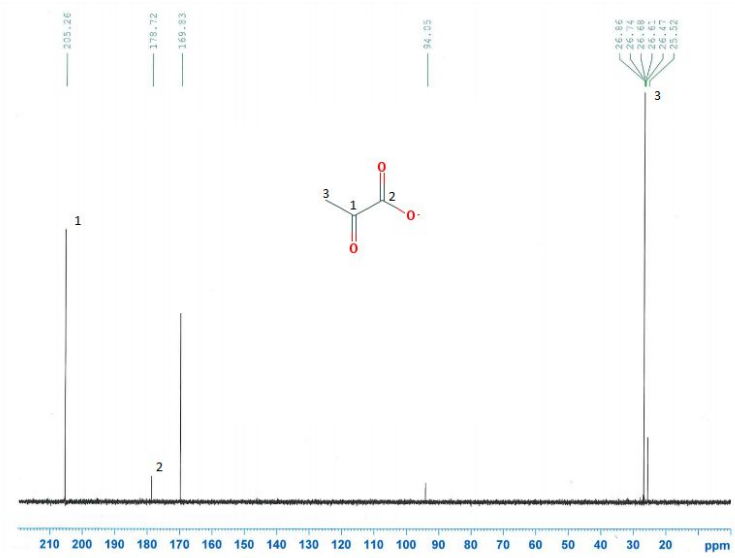

Figure Fourteen: Trial One ^{13}C-NMR of Pyruvate

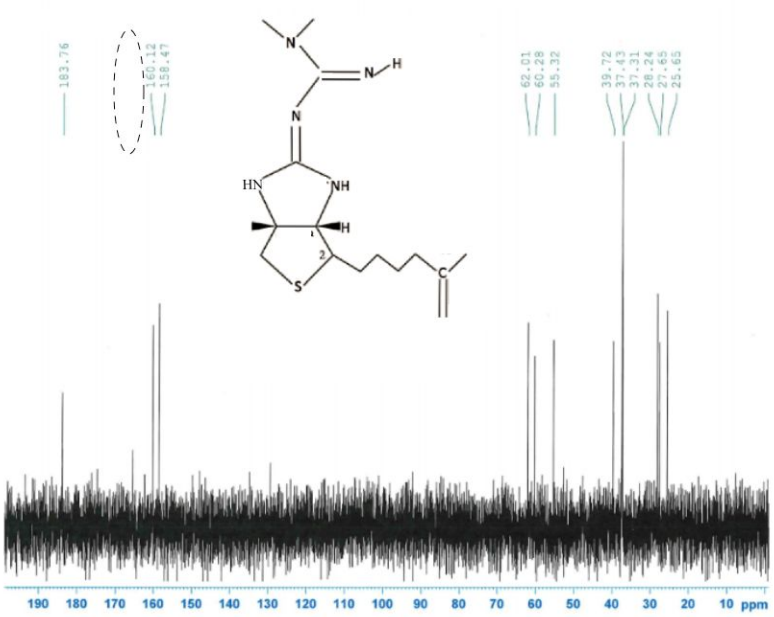

Figure Fifteen: Trial One ^{13}C-NMR of Dimethylbiguanide-Biotin

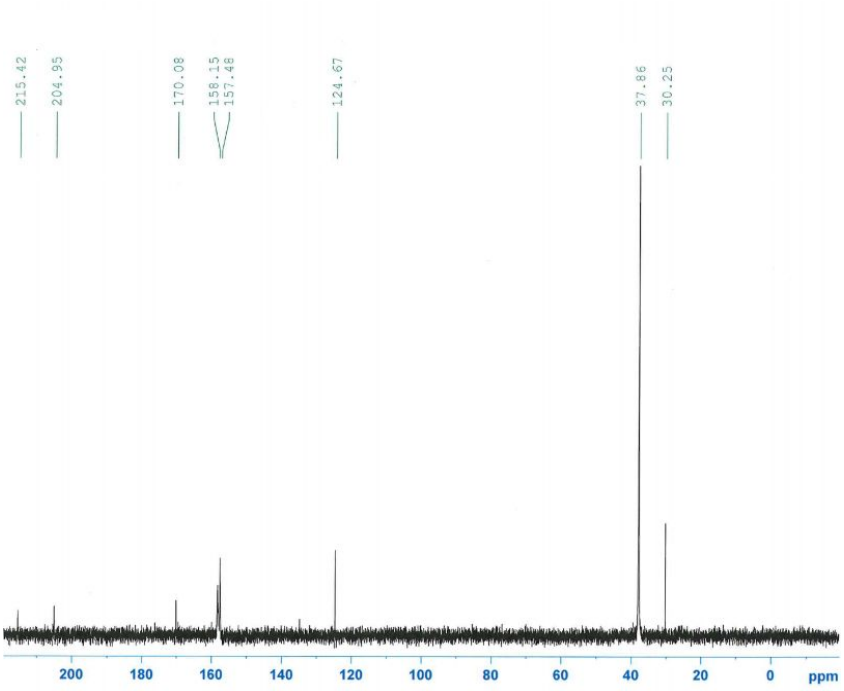

Figure Sixteen: Trial One ^{13}C-NMR of Dimethylbiguanide-Oxalacetate

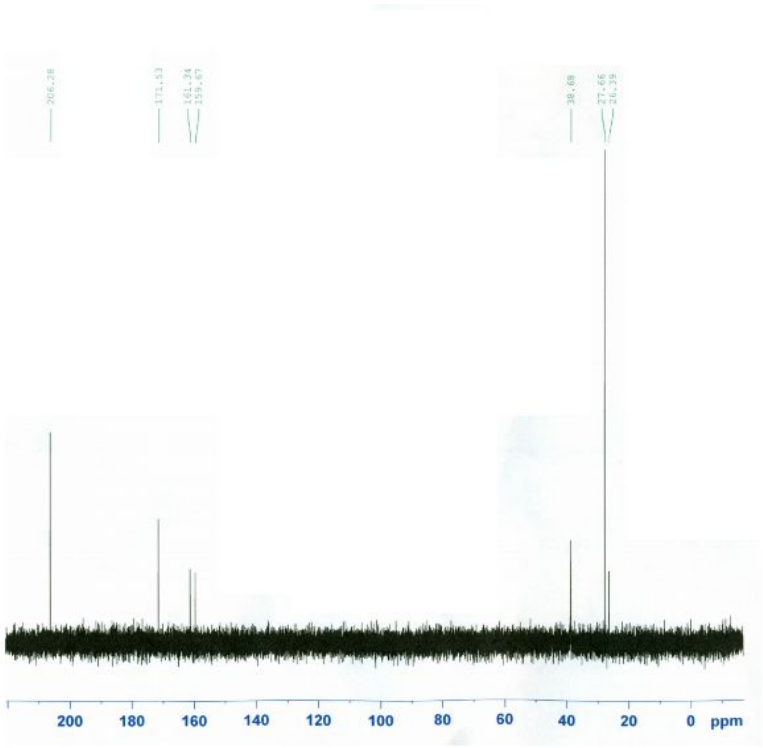

Figure Seventeen: Trial One ^{13}C-NMR of Dimethylbiguanide-Pyruvate

The computer did not recognize a carbonyl peak in the dimethylbiguande and biotin solution, while the other mixed solutions had a carbonyl group.

Discussion

There was a statistically significant difference between groups as determined by one-way ANOVA (table 1). A Duncan Waller post-hoc test revealed that the rate of reaction slowed down significantly between the control and 1000µM, 2500µM, and 5000µM of dimethylbiguanide. This supports the hypothesis of dimethylbiguanide inhibition of gluconeogenesis through the inhibition or interaction between pyruvate carboxylase. The ^{13}C-NMR data supported the diode array experimentation, as dimethylbiguanide interacted with biotin, further supporting dimethylbiguanide inhibition of gluconeogenesis through pyruvate carboxylase.

Limitations

Anomalous data was, however, present in experimentation. It was seen after analyzation of data that in trial three of the diode array methods, the 5000 μM concentration of dimethylbiguanide slope was at a greater decline than the control group (trial three), meaning that the reaction was faster compared to the control group, where dimethylbiguanide was not added. The anomalous data point in the diode array might have occurred for many various reasons. The enzymes used have a storage temperature of -20^0C. They were not stored at this temperature for the time of experimentation but stored on ice, leading to the possible denaturation of enzyme. This means dimethylbiguanide, in some cases, did not have the ability to inhibit the pyruvate carboxylase enzyme, leading to a greater decline in the reaction, rather than if dimethylbiguanide had the ability to inhibit the enzyme. Although unlikely, amounts of contaminants could have affected reagents in the cuvette, leading to this greater decline in slope for the 5000 μM concentration in trial three. More anomalous included the binding of dimethylbiguanide to pyruvate and oxalacetate. It was also proposed that dimethylbiguanide would bind with oxalacetate and pyruvate through a transamination reaction through the carbonyl group on these molecules. However, this only occurred with biotin.

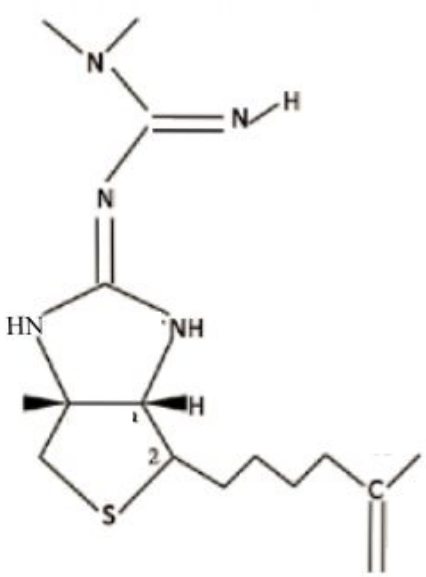

Figure 14: Proposed molecule of biotin and metformin

There are many reasons why the reaction did not occur with pyruvate and oxaloacetate, however, it is most likely due to the unlikely reaction between these two compounds. It is unlikely outside factors was the reason why oxalacetate and pyruvate did not bind with dimethylbiguanide and was most likely due to the right factors pyruvate and oxaloacetate lack for a reaction to take place.

The significant data of dimethylbiguanide inhibiting pyruvate carboxylase challenges the prevalent theory of the AMPK pathway. However, experimentation conducted challenges other studies previously conducted for other enzymes. Due to the errors in data seen in the experiment, further trials could allow for replication or a change in the results seen in the experiment.

Understanding how dimethylbiguanide inhibits gluconeogenesis can lead to an understanding of how to reduce the reaction rate of gluconeogenesis. This could lead to the synthesis of other antidiabetic drugs that interact with pyruvate carboxylase to slow down the

reaction rate of gluconeogenesis. The experimentation conducted could also lead to in vivo studies and observe if the same results are obtained from experimentation.

Conclusion

Understanding the inhibition of gluconeogenesis through dimethylbiguanide is important to understanding the gluconeogenic process itself. Through supporting the results that dimethylbiguanide inhibits gluconeogenesis through pyruvate carboxylase, possibly through the biotin prosthetic group. Overall, a better understanding of gluconeogenesis has been reached, a possible way to inhibit gluconeogenesis has been found**.** This could possibly improve type II diabetes patients' outcomes, as a method of shutting down gluconeogenesis was seen in the experimentation conducted. The experimentation conducted leads to many other directions, regarding dimethylbiguanide's inhibition of gluconeogenesis and type II diabetes.

Future Directions

An in vitro study could be conducted to observe if the same results are repeated from experimentation. Given that dimethylbiguanide has other implications (He et al; Quinn et al:), an investigation into the the effectiveness of dimethylbiguanide's other uses could also be done, leading to different possible treatment options for those diseases, including cancer, and PCOS. Investigations into the other possible enzymes inhibited by dimethylbiguanide could also be done. Additional research is also important to allow for the discovery of dimethylbiguanide's many properties, including the synthesis of other biguanides leading to better treatment outcomes for diabetes patients. Additional research is vital to understand the properties of

dimethylbiguanide further, and additional research should be conducted to discover these properties.

References

American Diabetes Association. (5 April 2017). *Statistics About Diabetes.* Retrieved from http://www.diabetes.org/diabetes-basics/statistics/

Diabetes Canada. (n.d). *History of Diabetes.* Retrieved from http://www.diabetes.ca/about-diabetes/history-of-diabetes

United States National Library of Medicine. (5 July 2017). *Diabetes Type 2.* Retrieved from https://medlineplus.gov/diabetestype2.html

University of California San Francisco. (n.d). *Table of Medications*. Retreived from https://dtc.ucsf.edu/types-of-diabetes/type2/treatment-of-type-2-diabetes/medications-and-therapies/type-2-non-insulin-therapies/table-of-medications/

He, L. E, Fredic. Windisford. (3, February, 2015). *Metformin Action: Concentrations Matter. Cell Metabolism,* 21(2), 159-162. doi: 10.1016/j.cmet.2015.01.003

United States National Library of Medicine. (2 June 2017). *DRUG RECORD: METFORMIN.* Retrieved from https://livertox.nih.gov/Metformin.htm

National Institute of Health. (5 July 2017). *Diabetes Type 2.* Retrieved from https://medlineplus.gov/ency/article/000313.htm

National Institute of Diabetes and Digestive and Kidney Diseases. (n.d). Prediabetes & Insulin Resistance. Retrieved from https://www.niddk.nih.gov/health-information/diabetes/overview/what-is-diabetes/prediabetes-insulin-resistance

University of California, San Francisco. (n.d). *The Liver & Blood Sugar*. Retreived from https://dtc.ucsf.edu/types-of-diabetes/type2/understanding-type-2-diabetes/how-the-body-processes-sugar/the-liver-blood-sugar/

Foretz, M. Hébrard, S. Leclerc, J. Zarrinpashneh, E. Soty, M. Mithieux, G. Sakamoto, K. Andreelli, F. Viollet, B. (23 June 2010). *Metformin inhibits hepatic gluconeogenesis in mice independently of the LKB1/AMPK pathway via a decrease in hepatic energy state. The Journal of Clinical Investigation,* 120(7):2355–2369. doi: 10.1172/JCI40671

United States National Library of Medicine. (15 April 2017). *Metformin.* Retrieved from https://medlineplus.gov/druginfo/meds/a696005.html

AJ, S. (May 1996). *Clinical pharmacokinetics of metformin*. *Clinical Pharmacokinetics,* 30(5):359-71

Cao, J. Meng, S. Chang, E. Beckwith-Fickas, K. Xiong, L. Cole, R. Radovick, S. Wondisford, S. E, R. He, L. (2014 July 25). *Antidiabetic Drug Metformin Suppresses Gluconeogenesis: Low Concentrations of Metformin Suppress Glucose Production in Hepatocytes through AMP-activated Protein Kinase (AMPK). The Journal of Biological Chemistry,* 289: 20435-20446. doi:10.1074/jbc.M114.567271

Baur, J. Birnbaum, M. (5 August 2014). *Control of Gluconeogenesis by Metformin: Does Redox Trump Energy Charge?. Cell Metabolism,* 20(2): 197-199. doi:10.1016/j.cmet.2014.07.013

Madiraju, A. K., Erion, D. M., Rahimi, Y., Zhang, X., Braddock, D. T., Albright, R. A., . Shulman, G. I. (2014). Metformin suppresses gluconeogenesis by inhibiting

mitochondrial glycerophosphate dehydrogenase. *Nature, 510*(7506), 542-6. Retrieved from http://www.nature.com/nature/journal/v510/n7506/full/nature13270.html

Caton, P., Nayuni, N., Kieswich, J., Khan, N., Yaqoob, M., & Corder, R. (2010). Metformin suppresses hepatic gluconeogenesis through induction of SIRT1 and GCN5. *Journal of Endocrinology, 205*(1), 97-106. doi:10.1677/JOE-09-0345

Rensselaer Polytechnic University. (n.d). *Gluconeogenesis; Regulation of Glycolysis & Gluconeogenesis.* Retreived from https://www.rpi.edu/dept/bcbp/molbiochem/MBWeb/mb1/part2/gluconeo.htm

Mihaylova, M. M., & Shaw, R. J. (2011). The AMP-activated protein kinase (AMPK) signaling pathway coordinates cell growth, autophagy, & metabolism. *Nature Cell Biology*, *13*(9), 1016–1023. doi: 10.1038/ncb2329

Samuel, V. T., Beddow, S. A., Iwasaki, T., Zhang, X., Chu, X., Still, C. D., . . . Shulman, G. I. (2009). Fasting hyperglycemia is not associated with increased expression of PEPCK or G6Pc in patients with type 2 diabetes. *Proceedings of the National Academy of Sciences of the United States of America, 106*(29), 12121-12126. doi:10.1073/pnas.0812547106

Ravi, K. (2015 May 30). *Gluconeogenesis for medical school* [PowerPoint slides]. Retrieved July 3, 2017, from SlideShare website: http://www.slideshare.net /ravikiran35977897/gluconeogenesis-for-medical-school

New York Times. (25 June 2017). *Type 2 Diabetes.* Retreived from http://www.nytimes.com/health/guides/disease/type-2-diabetes/medications.html

Cheng, H. Qi, R. Paudel, H. Zhu, H. (2011). Regulation and Function of Protein Kinases and Phosphatases. *Enzyme Research, 2011*, 1-3. doi:10.1073/pnas.0812547106

Attwood, P. (1995). The structure and the mechanism of action of pyruvate carboxylase. *The International Journal of Biochemistry & Cell Biology, 27(3), 231-249.*

Quinn, B. Kitagawa, H. Memmott, M. Gills, J. Dennis, P. (2013 Sep). Repositioning metformin for cancer prevention and treatment. *Trends in Endocrinology and Metabolism, 24*(9), 469-80. doi: 10.1016/j.tem.2013.05.004

Mihaylova, M. Shaw, R. (2 Sep 2011). The AMPK signalling pathway coordinates cell growth, autophagy and metabolism. *Nature Cell Biology. 13*(2011), 1016–1023. doi: 10.1038/ncb2329

Mráček, T. . Drahota, Z. Houštěk, J. (March 2013). The function and the role of the mitochondrial glycerol-3-phosphate dehydrogenase in mammalian tissues. *Biochimica et Biophysica Acta (BBA) - Bioenergetics. 1827*(3), 401-410. doi: 10.1016/j.bbabio.2012.11.014

Miles, B. (n.d). *Integration of Metabolism*. Retreived from https://www.tamu.edu/faculty/bmiles/lectures/integration.pdf

Zhou, G. Myers, R. Li, Y. Chen, Y. Shen, X. Fenyk-Melody, J…Moller, D. (2001 Oct 15). Role of AMP-activated protein kinase in mechanism of metformin action. *The Journal of Clinical Investigation, 108*(8), 1167–1174. doi: 10.1172/JCI13505

Lashen, H. (Jun 2010). Role of metformin in the management of polycystic ovary syndrome. *Therapeutic advances in endocrinology and metabolism, 1*(3), 117–128. doi: 10.1177/2042018810380215

Veneziale, C. M., Donofrio, J. C., & Nishimura, H. (1983). The concentration of P-enolpyruvate carboxykinase protein in murine tissues in diabetes of chemical and genetic origin. *Journal of Biological Chemistry, 258*(23), 14257. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/6643479

Acknowledgments

Special thanks to FSI, the University of Northern Colorado and the Department of Chemistry and Biochemistry, Guadalupe Andrea Solis, Matt DeSelm, Lori Ball, Dr. Michael Mosher, Dr. Patrick Burns, and Dr. Richard Hyslop. Through their support and help, a better understanding of dimethylbiguanide and its inhibition in gluconeogenesis has been reached. Research would also not be possible without the support of the Rollie R. Kelley foundation, Union Pacific, and Xcel Energy.