STRATEGIES FOR TREATMENT OR PREVENTION OF DIABETES USING A CHEMICAL THAT MIMICS EXERCISE

DelShawn Fowler
Faculty Mentor: Jonathan Fisher, Ph.D.
Saint Louis University; Harris-Stowe State University

Abstract
The purpose of this experimental research is to identify a few objectives or aims that could potentially develop strategies for treatment or prevention of diabetes, with exercise being one of the concentrations for treatment. The aims will be treating muscle tissues with chemicals that mimic the effects of a chemical that mimics the metabolic stress of exercise (AICAR) and examining effects on proteins involved in control of metabolism, including AS160, NADPH oxidase (NOX), serum/glucocorticoid-regulated kinase 1 (SGK1),AKT, protein kinase C(PKC), and glycogen synthase. Studying these proteins will help to understand if they have a connection to diabetes. Conducting this experiment will assist in distinguishing whether the activation of each proteins is caused by the exercise-mimicking chemical. This experimental study will be conducted to test the hypothesis that a drug mimicking exercise could be used to potentially lessen the effects diabetes by activating or deactivating proteins that are responsible for controlling metabolism including the response to insulin. We found that AICAR increased glycogen synthase activity and also increased AS160 phosphorylation.

Introduction

Diabetes is a disease that occurs when your blood glucose is too high. It can also be referred to as high blood sugar, which is a main source of energy created when you consume food. Insulin is important when dealing with diabetes because it serves as a hormone made by the pancreas. A function of insulin is helping to get glucose from food into your cells for ATP or energy. In many cases your body may not make enough insulin or your cells will not respond properly to insulin.. With this in mind glucose will then stay in your blood and does not reach your cells. This means that there is an irregular regulation of blood glucose, such as diabetes (Anon, 2018).

Diabetes can increase the risk of cardiovascular diseases (heart disease), which is the leading cause of death in the U.S. This is becoming a great problem in the United States because if the number of people with heart disease increases then the cost of health care will also increase, making it much harder for many low income households to afford it. Between 2010 and 2030, the total direct medical costs of heart disease is projected to triple, from $273 billion to $818 billion (Khavjou, 2011). As of 2014, more than 29 million people in the United States had diabetes, according to a report released by the Centers for Disease Control and Prevention. Without weight loss and moderate physical activity, 15 percent to 30 percent of people with prediabetes will develop type 2 diabetes within five years of their life.

The normal regulation of glucose is very important because it is necessary for cellular respiration which is needed for all body cells to develop and give energy. The body obtains glucose from the breakdown of foods and drinks consumed containing carbohydrates. Glucose is taken up by cells

immediately after meals, and for later use it can also be stored in the liver and muscles as glycogen. Hormones such as insulin and glucagon regulate both the storage and the utilization of glucose as it is required by the body's cells. Pancreatic cells can sense blood glucose levels, and afterwards the pancreatic cells secrete glucagon or insulin to maintain normal blood glucose levels. The level for blood glucose sugar concentration is maintained between 70 mg/dL and 110 mg/dL. If blood glucose concentration rises above this range, insulin will be released. This will help to stimulate the body's cells to remove glucose from the blood. If the opposite happens, glucagon is released stimulating the body's cells to release glucose into the blood (17.9 The Endocrine Pancreas, 1999-2018).

Many patients with diabetes suffer from obesity, making it very difficult to exercise. Exercise is important because it aids in the prevention of insulin resistance, which is the main cause of diabetes. Previous research on rats with normal insulin sensitivity revealed that regular exercise increased insulin-stimulated glucose uptake. This means that exercise helps to decrease blood glucose concentrations and also the insulin requirement for people with diabetes. Procedures were conducted and determined that exercise would affect the rate of glycogen accumulation in skeletal muscle. Exercise stimulates glucose clearance from the bloodstream (17.9 The Endocrine Pancreas, 1999-2018).

In other words, insulin is a hormone that causes blood sugar (glucose) to be stored in muscle, and diabetes basically is the abnormal regulation of blood glucose concentrations caused by resistance to insulin. Studies show that the muscle is the largest storage site for blood sugar, which is why muscle is the most important tissue for glucose storage. Exercise is very important for patients with diabetes because it makes insulin work better, causing sugar uptake into muscles to improve (known as insulin sensitivity). It is not entirely known how exercise causes insulin sensitivity, thus causing my mentor and I to develop an experiment to understand this process a bit more (Fisher, 2008).

Proteins are long chemical building blocks of amino acids that are used for cell growth and development. In this case, the proteins of interest are specifically responsible for controlling metabolism (chemical reactions that take place inside the body) including the response to insulin. The proteins of interest are regulated by phosphorylation which works to modify proteins making them more active or less. To phosphorylate a protein means that amino acids in proteins are being altered by adding a phosphate group to the protein, causing a change in the protein's structure or function (Franz, 2018).

We will treat cells with AICAR to see if this will lead to modification of proteins of interest. The presence of AICAR is used to mimic metabolic stress. AICAR will help to mimic the effects of exercise, causing an increase in insulin-stimulated glucose uptake or increased insulin sensitivity. By examining effects of AICAR on protein phosphorylation, our aim is to uncover processes that could help to prevent or lower high blood glucose. In the long run this prevention will benefit in creating a cure or lessen the effects of diabetes in obese people.

Our hypothesis is that AICAR will lead to modification of proteins of interest shown in figure 1, which would suggest a role of these proteins in causing sensitivity to insulin. The figure shows that AICAR causes

modification of these particular proteins. When this is done each protein could increase or activate insulin sensitivity. Our long-term goal is to determine whether use each protein could work with the help of AICAR to treat type 2 diabetes or lessen the effects that it has on patients. When insulin sensitivity increases, the blood glucose will be regulated properly and will stop the buildup of cells in the bloodstream.

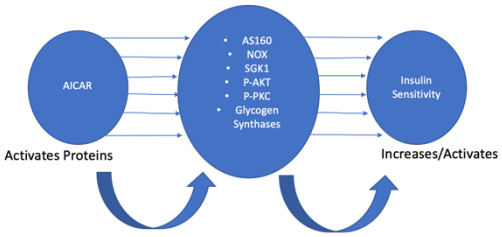

Figure 1. Hypothesized role of the exercise mimetic AICAR in causing insulin sensitivity. (This picture basically summarizes the research that is the focus of this study. It shows how AICAR will turn each protein off or activate it causing an increase in insulin sensitivity for muscle cells.)

Methodology

To start this research experiment, we used western blots to examine specific proteins after treatment of muscle cells with AICAR. Western blots are used for protein analysis because it produces qualitative data about the protein of interest. This action is known as protein phosphorylation which is a process that can modify proteins making them more active or less. To phosphorylate a protein means that amino acids in the protein are being altered or modified. The term “blotting" refers to the transfer of biological samples from a gel to a membrane which later helps to detect complex protein mixtures. In the most basic terms, western blots are used to detect the presence of a specific protein in a complex mixture extracted from cells or tissue (Scofield, 2009).

Furthermore, We also used a microplate reader for the glycogen synthase assay. This reader works to detect biological, chemical or physical reactions of samples. With this reader, a 96 well plate is used with 100-200 microliters of a solution, allowing the reader to measure each reaction with either absorbance, luminescence, or fluorescence. Microplate readers are usually used for protein and cell growth assays. With this in mind, we measured the density of glycogen synthase which shows that the reaction was altered or modified (Crutchfield, 2001).

Lastly, we cultured mice tissue to cure the cells with different treatments. Cell culturing is a technique in which cells are removed from an organism and placed in a fluid medium. The cells are grown in a plate with growth medium as cell food, and are kept inside an incubator so that they are not contaminated. With cell culturing, it is possible to control the growth rate of cells; cells can also be manipulated by introducing nucleic acids to a cell. The samples from the mice tissue are also used as samples in western blotting from the cell culturing process (Philippeos, 2012). Collection of muscle tissue from mice was approved by the Saint Louis University Institutional Animal Care and Use Committee.

Results

With the information presented, Figure 2 shows the density of the control group and the protein combined with AICAR. In figure two below, the results show that AICAR has no effect on this protein because on average the control (C)and AICAR-treated (A) samples are similar. With this protein in mind, we now know that SGK1 does not

help to decrease insulin resistance because the control group and test group have the same density level on the membrane(density is the mass of the protein that is detected).

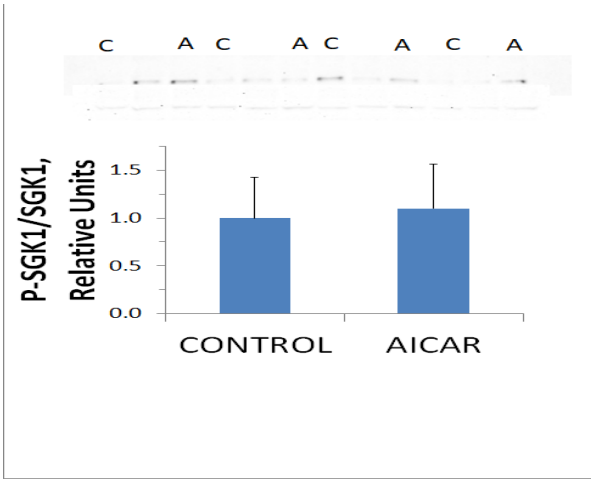

Figure 2: Phospho-SGK1 Data

Figure 3 also shows AICAR does not affect NOX activity. Thus, we can assume that this protein will have no benefit in assisting to decrease insulin resistance in muscle cells. Image 1, shows accumulation of a product of the enzymatic activity of NOX proteins.

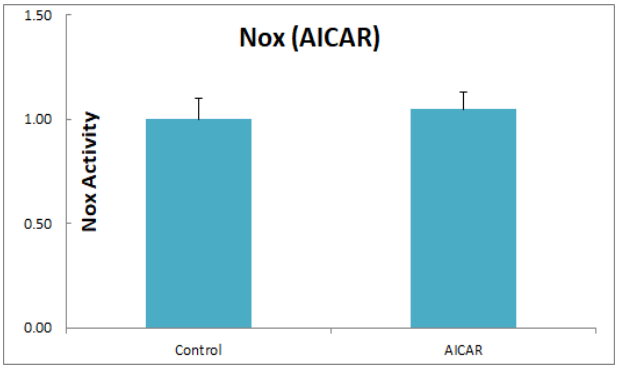

Figure3: NOX Activity

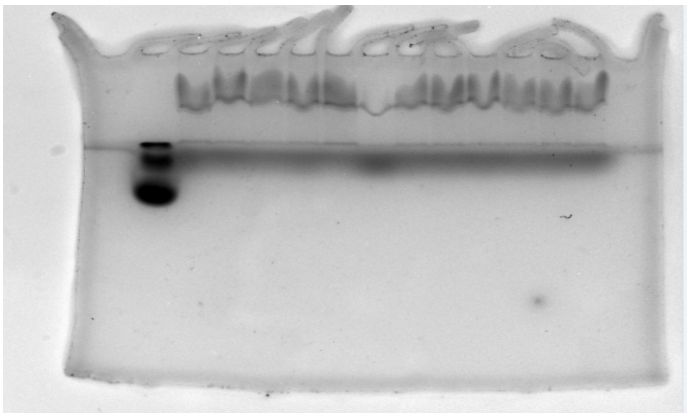

Image 1: Enzymatic activity of NOX proteins

Figure 4 shows that the AICAR has an effect on glycogen synthase, meaning that it could help to increase the sensitivity to insulin. This will be noted to use in further research.

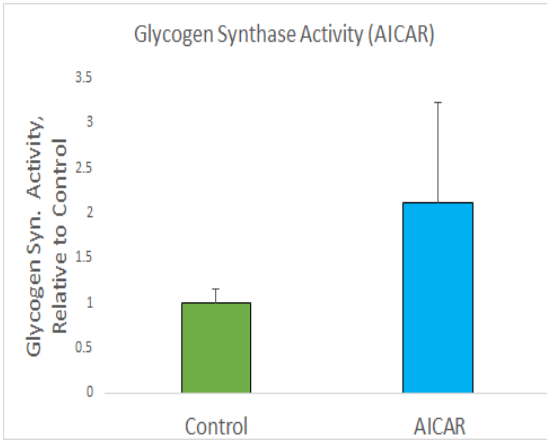

Figure 4: Glycogen Synthase Activity Data

Image 2 shows a membrane that has transferred proteins on it that were phosphorylated after treatment of cells with AICAR. Figure 5 shows that this protein certainly had a reaction to AICAR meaning that it could help to activate insulin sensitivity.

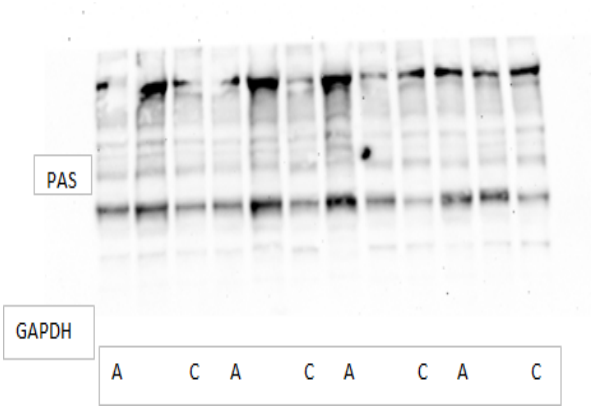

Image 2: Phospho-AS160

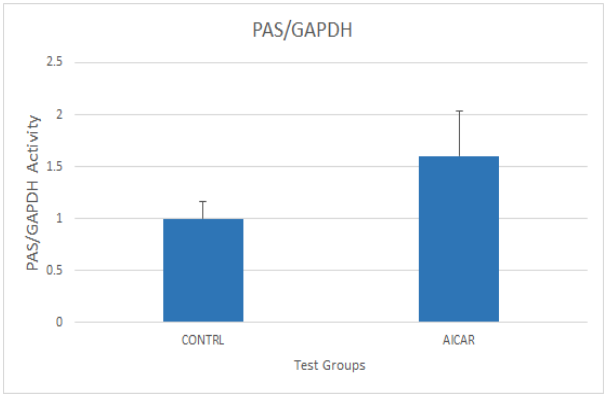

Figure 5: Phospho-AS160

Figure 6 shows that AICAR has no effect on phosphorylation of Akt. The control group has darker bands than the bands for the AICAR-treated group. The amount of GAPDH protein was measured as a loading control.

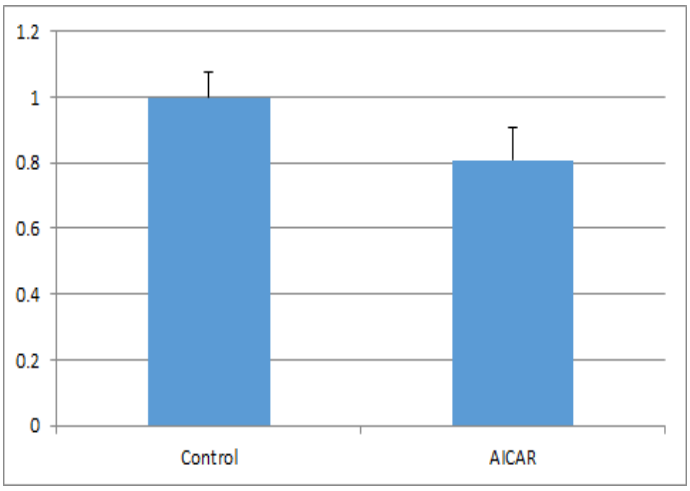

Figure 6: Phospho-AKT

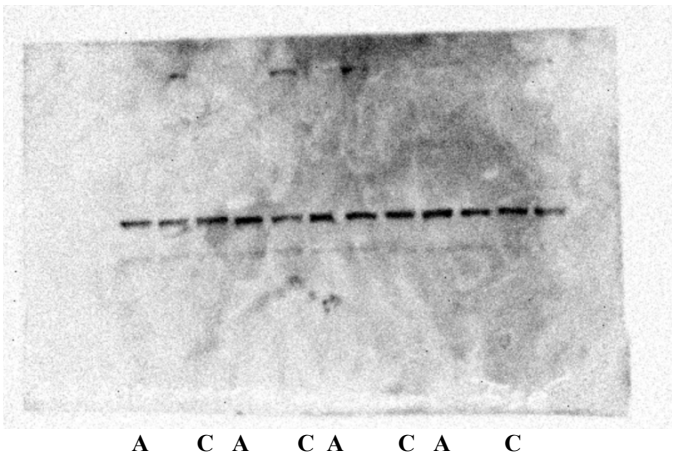

Image 3

Figure 7 shows the presence of AICAR has no change on the amount of Phospho-PKC protein. On average, the control group and test group has the same density as shown in the membrane. We can infer that this protein is not involved in causation of insulin sensitivity to increase.

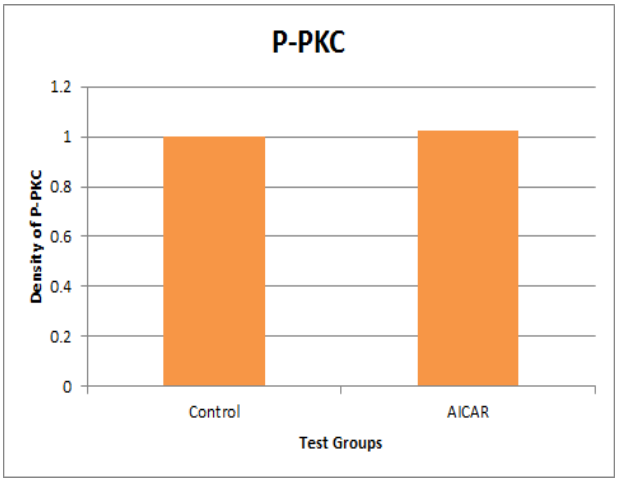

Figure 7

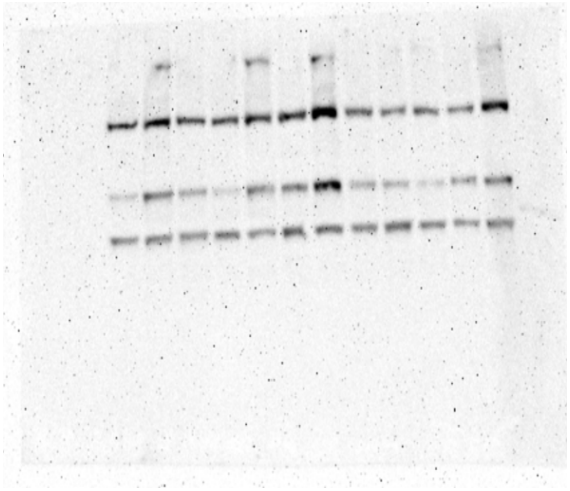

Image 4 A C A C A C A C

Conclusion

As stated above, our hypothesis is that treating cells with AICAR would affect candidates for control of insulin sensitivity. Our data shows that 2 out of 6 proteins are responsive to AICAR which is great because four unresponsive proteins were eliminated as candidates for control of insulin sensitivity in response to AICAR. To conclude, we found that AS160 and glycogen synthase are the two targets to focus on for further research. They show a higher reaction than any of the other proteins after the AICAR treatment. Furthermore, with more search down the line, we could focus more on these two proteins to figure out if they are in connection with decreasing insulin resistance. This discovery will help to solve the mystery of how exercise causes insulin to facilitate (make easier or possible) glucose uptake.

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