Investigating Glucose Effectiveness in Rats

By

Ali K. Nelson

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Approved: _______________________________ Date: 5/27/98
Thesis Director Signature
Nancy Peterson

Approved: _______________________________ Date: 5/27/98
Second Reader Signature
Thomas Williams
Diabetes and Its History

Throughout history, people have struggled to understand how the body obtains its nutrients to continue life. Many people were interested in a particular disease in which, despite the amount of food ingested, the victim would eventually starve to death. Four thousand years ago the Egyptians are known to have chronicled the disease (Harris 1946). Almost two thousand years ago the Greeks named this disease “diabetes” recognizing the rapid passing of water through the victim (Harris 1946). This unquenchable thirst and constant urination is still one recognizable symptom of diabetes. The high concentration of glucose in the blood causes the victim to drink water as the body attempts to regulate the level by releasing glucose into the urine. This was first recognized by the Romans who noted that the urine of diabetics had a sweet taste and that bees were attracted to it (Harris 1946). They then added the mellitus, which is Latin for “sweet as honey”, to the name of the disease (Harris 1946). Prior to the discovery of insulin, treatment for diabetes mellitus consisted of starvation diets. People with diabetes were restricted to diets that consisted mainly of fats and protein but avoided carbohydrates (Harris 1946). By doing this, they were able to slow the deterioration of their bodies. People exposed to this treatment were able to extend their lives an average of five to six years (Harris 1946).

Often physiological systems are regulated by several different hormones. After much research, scientists determined that this is also the case with glucose homeostasis. Frederick Banting and Charles Best made a great deal of progress in this field in the summer of 1921. Initially, all that was known about the pancreas and its ability to regulate glucose levels was that without the pancreas glucose levels would increase (Harris 1946). Banting and Best set out to isolate the unknown substance that regulated glucose levels
Trypsin is a proteolytic enzyme produced by the pancreas for digestion of proteins. In order to isolate the unknown hormone, they had to stop the production of trypsin (Harris 1946). They did by ligating the ducts of the pancreas with silk in an attempt to cause the pancreas to atrophy and no longer be able to produce trypsin but still be able to produce the mystery hormone (Harris 1946). Next, they removed the pancreases of dogs, which quickly caused the dogs to become diabetic (Harris 1946). Banting and Best ground up the pancreas and produced an extract, and when this extract was injected into the diabetic dog, the glucose levels of the dog quickly decreased (Harris 1946). Banting and Best knew that the mystery hormone was secreted by the islets of Langerhans (Harris 1946). The isolated hormone is now known as insulin and is known to be secreted by the β-cells in the islets of Langerhans. These discoveries provided the basis for current treatment of diabetes.

There are three main identifiable types of diabetes. Researchers believe that type I diabetes results from an autoimmune attack on the β-cells, resulting in their destruction (American Diabetes Association 1997). Without the β-cells, humans cannot produce insulin. Therefore, type I diabetes is also referred to as insulin dependent diabetes mellitus, IDDM. Individuals must take replacement injections of insulin on a daily basis. This disease was previously referred to as juvenile diabetes because its onset is much more common in children than adults.

The etiology of type II diabetes is much more varied than type I in some respects. Some newly diagnosed individuals with type II diabetes have insulin producing β-cells (American Diabetes Association 1997). However, in these cases individuals cannot produce enough insulin to maintain proper blood glucose levels. These individuals may
take oral medications to either increase the production of insulin in the body or to increase the sensitivity of the tissues to the insulin being produced (American Diabetes Association 1997). Therefore, type II diabetes is also known as non-insulin dependent diabetes mellitus, NIDDM.

Another recognized type of diabetes is gestational diabetes, which occurs in a mother during pregnancy (American Diabetes Association 1997). Affected mothers can control blood glucose levels by controlling their intake of food or by injections of insulin. After giving birth, the mothers' system of maintaining glucose homeostasis normalizes. Additionally, women with gestational diabetes have a greater risk of developing NIDDM later in life (American Diabetes Association 1997).

Diabetes research is well funded for several reasons. First, diabetes is the sixth leading cause of death by disease in the United States based on death certificate data (American Diabetes Association 1997). Complications that can result from diabetes include blindness, kidney failure, heart disease, stroke, and lower limb amputations (American Diabetes Association 1997). Second, there are approximately 15.7 million people in the United States with diabetes, 5.4 million of whom do not even know it (American Diabetes Association 1997). This number is rapidly increasing with approximately 2200 people diagnosed per day (American Diabetes Association). Because of this, many people strive to understand the cause of the disease, the way in which glucose homeostasis is maintained in the body, and the possible treatments for the disease. My research focused on how glucose is maintained in the body. In order to study this, we must consider all the hormones that are known to regulate plasma glucose levels in mammals.
In addition to insulin, there are other hormones that influence blood glucose levels. One of these hormones is glucagon. Glucagon stimulates gluconeogenesis and glycogenolysis. Gluconeogenesis is defined as the formation of glucose from non-carbohydrate sources, and glycogenolysis is the breakdown of glycogen, stored glucose, into usable glucose. Glucagon is secreted by the α-cells of the islets of Langerhans. This finding is significant for two reasons. First, the existence of glucagon answered many questions on how glucose levels are maintained during long periods without eating or during periods of extended exercise when hypoglycemia may occur. For example, when people sleep, they do not ingest any food. However, they are still using glucose. Glucagon provided the answer to how people obtain energy during these periods of fasting. Glucagon travels to the liver and initiates the breakdown of stored glucose and the metabolism of fats and amino acids for energy. Glucagon maintains proper glucose levels by acting in the opposite way of insulin by increasing blood glucose levels. The second significance of the discovery of α-cell secretion of glucagon focuses on the close proximity of these cells to the β-cells. This implied that the other cells in the islets of Langerhans might play some role in regulating glucose homeostasis. This belief was supported by the discovery of somatostatin hormone.

Researchers soon made an observation that determined what other hormones, besides insulin and glucagon, were present in the pancreas. Maurice Dubois (1975) used immunofluorescence to determine the hormones present on the human pancreas. He treated the tissues with anti-insulin, anti-somatostatin, and anti-glucagon sera and observed distinct portions of the pancreatic islets that corresponded to either the anti-
insulin, anti-somatostatin, or anti-glucagon sera (Dubois 1975). This indicated that all three hormones were secreted by different cells in the islet (Dubois 1975). Insulin was already known to be secreted by the β-cells, and glucagon was designated as being secreted by the α-cells. Therefore, the somatostatin secreting cells are now referred to as the δ-cells.

When researchers discovered a hormone produced in the pituitary that inhibited the secretion of growth hormone, somatostatin, they then tried to isolate the active portion of the hormone so that they could synthetically produce it and better understand its actions (Brazeau et al. 1973). Paul Brazeau et al. (1973) at the Salk Institute in La Jolla, California, attempted to isolate the factor that appeared to inhibit the release of growth hormone from the pituitary. They called the factor somatotropin release inhibiting factor (SRIF) and attempted to isolate the active portion of the hormone from the ovine hypothalamus (Brazeau et al. 1973). After determining that the isolated portion inhibited the secretion of immunoreactive rat or human growth hormones in vitro and in vivo in rats, they determined its structure using an Edman degradation (Brazeau et al. 1973). The sequence determined by the Edman degradation is as follows:

\[ \text{N-Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys-COOH} \]

By knowing the amino acid sequence, people could then produce a synthetic SRIF to be used in research. Because SRIF was released from the hypothalamus and inhibited the release of growth hormone from the anterior pituitary, there existed the possibility of the same situation for insulin. Insulin, in addition to mediating the uptake of glucose from
plasma into peripheral tissues, is also known as a growth hormone. Therefore, in vivo, SRIF, the synthetic active portion of somatostatin, might also inhibit the release of insulin from the β-cells.

Donna J. Koerker et al. (1974) made further progress in understanding the effects of SRIF on glucose homeostasis when they published a paper in which they determined the influence of SRIF on plasma insulin, glucagon, and glucose levels in male baboons. They began by inserting venous catheters and fasting the animals overnight, and they then restrained the baboons and intravenously administered SRIF to them (Koerker et al. 1974). During the administration of SRIF, researchers noted that the plasma glucose levels fell and quickly returned to control levels after terminating the infusion (Koerker et al. 1974). This led researchers to investigate the influence of SRIF on the glucagon and insulin levels in the baboons. Researchers used arginine instead of glucose to stimulate the β-cells; after initiating the SRIF infusion, both plasma insulin and glucagon levels decreased to less than 15% of pre-infusion levels (Koerker et al. 1974). Glucagon levels continued to be suppressed despite glucose levels falling below normal, and in addition, plasma concentrations of free fatty acids (FFAs) increased (Koerker et al. 1974). One explanation of this is that hypoglycemia signaled the release of FFAs which could then be metabolized into Acetyl CoA. The significance of this discovery was that, by administering SRIF and glucose, one could analyze the time course of glucose in the blood without insulin. By administering a glucose bolus, plasma glucose levels would not fall below normal, and the body would not release FFAs for energy. Furthermore, SRIF would inhibit the release of glucagon in response to the overnight fast, and this would also prevent the addition of glucose into the system outside of the bolus. Finally, SRIF would
also inhibit the β-cell release of insulin in response to the bolus. Therefore, SRIF was unique when compared to other inhibitors in the system of maintaining glucose homeostasis because it inhibited both insulin and glucagon secretion.

**Molecular Aspects:**

The question now becomes, how does SRIF inhibit secretion of insulin from the β-cells of the pancreas? One hypothesis was that somatostatin decreased the Ca$^{2+}$ entry through voltage-dependent Ca$^{2+}$ channels in the β-cell. This was hypothesized because by inhibiting the passage of Ca$^{2+}$ through these channels the overall permeability of the cell wall might be effected preventing the secretion of somatostatin from the cell. Another reason for this hypothesized is that often hormone actions are dependent on cascade systems that may be dependent on Ca$^{2+}$. Walter H. Hsu et al. (1991) investigated the effect of somatostatin on both insulin secretion from the β-cells and on the voltage dependent Ca$^{2+}$ channels. They did this by first cloning SV40 transformed hamster β-cell lines (Hsu et al. 1991). They then incubated the cells with glucose and monitored them for various changes including insulin secretion through radioimmunoassays and changes in [Ca$^{2+}$] (Hsu et al. 1991). They measured changes in both the overall [Ca$^{2+}$] and the membrane potential [Ca$^{2+}$] and concluded that the SRIF causes activation of K$^+$ channels and reduction in [Ca$^{2+}$] (Hsu et al. 1991). Hsu et al. (1991) also concluded that the level of SRIF interfered with the opening of the voltage dependent [Ca$^{2+}$] channels suggesting that the level of SRIF at the β-cell can regulate basal [Ca$^{2+}$] and insulin release.
Figure 1: Interaction of hormones to maintain glucose homeostasis with the intake of glucose or with the utilization of amino acids for energy

**Glucose as an Energy Source**

Mammals must ingest a carbon source and metabolize this source in order to obtain energy to survive. They can ingest a complex or simple carbohydrate as its carbon source. The digestive tract of mammals breaks down complex carbohydrates into simple sugars such as glucose and fructose. These nutrients are then absorbed through microvilli in the small intestine and into the blood. After entering the blood stream, glucose is dispersed throughout the body. Once it is transported into the peripheral tissues from the blood, glucose must be transported into the cells through glucose transporters on the cell surface. Then, glucose can be used to form ATP through cellular respiration. Without glucose, cells would resort to alternative sources of energy such as fatty acids. The breakdown of fatty acids will also produce harmful byproducts such as ketone bodies. Therefore, it is better to use carbohydrate sources of glucose to make ATP. The
phosphate bonds in ATP contain a great deal of energy, yielding 7.3 kcal/mol from hydrolysis (Stryer 1995). By breaking these bonds at the cellular level, mammals have the energy needed to replace dying cells and carry on many other necessary functions of life.

**Insulin Action**

In order for glucose to be broken down in cellular respiration and used for producing ATP, mammalian cells must transport glucose from the blood through the cell membrane and into the cell. There exists two main obstacles with the task of transporting glucose into the cell; first, a cell must be able to bring nutrients across cell membranes. Second, cells must prevent the nutrients from exiting back outside the cell if the concentration gradient is greater inside the cell than outside. Without the ability to perform these tasks, cells cannot maintain cell functions and will therefore die.

One important hormone for overcoming the obstacle of transporting glucose into the cell is insulin. Insulin influences blood glucose levels in several different ways. First, insulin binds to receptors on the cell surface that are specific to the hormone. Tyrosine kinase enzyme, the insulin receptor, binds with insulin causing the catalysis of the phosphorylation of tyrosine kinase enzyme is signaled. (Vander et al. 1994) This initiates a cascade of reactions that are not fully understood (Vander et al. 1994). The cascade is important in terms of amplifying the signaling effect of the insulin binding. In time, this cascade in time causes the glucose transporters to move to the cell surface and transport glucose into the cell from the blood. Thus, glucose moves from the plasma into the peripheral tissues across the cell membrane. Once glucose enters the cell, hexokinase catalyzes the phosphorylation of glucose. This prevents the glucose from exiting the cell immediately following its entry and overcomes the second task.
Another manner in which insulin functions in maintaining proper glucose levels is by inhibiting gluconeogenesis and glycogenolysis. Insulin inhibits gluconeogenesis by preventing the synthesis of several enzymes that are needed in gluconeogenesis (Vander et al. 1994). This prevents the production and release of glucose from the liver and kidneys.

Another influence of insulin action is dependent upon the β-cells. A greater β-cell mass in an individual will increase the rate of insulin production (Laury et al. 1989). This means that glucose levels may normalize more quickly after a glucose bolus if there is a greater β-cell mass.

Insulin action, $S_i$, therefore encompasses several different concepts. These concepts include the sensitivity of the tissues to insulin and the secretion of insulin in order to normalize plasma glucose levels. Insulin concentrations are dependent on the glucose concentration in the blood, on the length of time that the blood glucose has been at this concentration, and on the overall mass of the β-cells in the pancreas (Laury et al. 1989). Insulin's influence on blood glucose levels is dependent on insulin secretion and the sensitivity of the tissues to the insulin (Best et al. 1996). The sensitivity of the tissues to insulin is dependent on the number of insulin receptors on the cell surfaces that function because without this insulin cannot cause the cells to uptake glucose. The receptors can only bind to one insulin molecule at a time. Because of this, an insulin concentration exists which will cause all of the receptors to bind. In other words, this binding is saturable. Furthermore, the number of glucose transporters in the cell also influence the rate at which glucose leaves plasma and enters the cell because, as with insulin, glucose transporters can only transport one glucose molecule at a time.
Without proper function of insulin, insulin receptors, or glucose transporters, glucose that is consumed will not enter the cells and be used for energy, leading to several problems. First, glucose levels in the blood will increase. Furthermore, because the cells still lack the glucose they need, the mammalian body will respond metabolizing fatty acids and proteins. One byproduct of fatty acid metabolism is ketone bodies. These ketone bodies cause stress on many different systems of the body that will eventually lead to death if left untreated.

**Glucose Effectiveness**

Much research has determined that insulin action alone does not account for the maintenance of proper glucose levels (Best et al. 1996). The two main components of glucose homeostasis are insulin action, $S_i$, and glucose effectiveness, $S_o$ (Bergman et al. 1979). Glucose effectiveness is defined as the ability of glucose to suppress its own endogenous output and facilitate its own uptake in the peripheral tissues (Best et al. 1996). The concept of glucose effectiveness is based on the principle of mass action. The amount of glucose leaving the plasma and entering the cells through the glucose transporters will increase as the concentration of glucose in the blood increases independent of an insulin response (Best et al. 1996). This system operates on a positive feedback loop. Furthermore, the presence of a glucose level above normal will prevent the liver from performing gluconeogenesis and outputting glucose into the blood (Best et al. 1996).

**The Experiment**

Researchers in this field have debated the contributions of both $S_i$ and $S_o$ to glucose homeostasis. Recent studies indicate that $S_o$ contributes to approximately 83% of
glucose disposal in obese individuals and 99% of glucose disposal in NIDDM with severe insulin resistance and low levels of insulin secretion (Best et al. 1996). So has also been shown to have a significant influence on glucose levels in dogs (Best et al. 1996). Because rats are commonly used in studies relating to diabetes and glucose homeostasis, I investigated the role that So played in maintaining glucose levels. I hypothesized that So alone could not account for glucose homeostasis and that S1 played a role in maintaining proper plasma glucose levels.

Other factors that are known to effect plasma glucose levels are glucagon and non-esterified fatty acids (NEFAs). These fatty acids do not form an ester bond with glycerol and therefore can readily undergo fatty acid metabolism as an alternative energy source. Thus, we also monitored them because they were expected to play a significant role in regulation of plasma glucose levels.

**Methods:**

I performed the experiments on Sprague-Dawley male rats which were housed individually and exposed to a 12L:12D light cycle. The lights came on at 6:00 A.M. and went off at 6:00 P.M.. The rats ate Rat Chow® and had access to water *ad lib*.

**Catheterization:** One week after the rats were delivered to the lab, we inserted the catheters. We anesthetized the rats with sodium pentobarbital (Somnotol 40 mg/kg intraperitonially) and inserted polyethylene catheters (PE-50 with silastic tips) into the left femoral vein, right jugular vein, and left carotid artery. In order to prevent the catheter from being pushed out due to blood flow, we cut the silastic tubing to 3.3 cm long for the artery and 4 cm long for the veins. Next, we tunneled the tubing subcutaneously and exteriorized them at the nape of the neck leaving ~2-3 cm free. To prevent clotting, we
backfilled the lines with 10-50 U/ml of heparin, sealed them, and flushed them with heparinized saline (~40-80 U/ml heparin) approximately every 3 days. During surgery, we gave the rats atropine (0.04 mg/kg) to minimize fluid build up in the lungs and postoperatively gave them antibiotic (Baytril) and analgesic (Torbugesic).

Infusion: We fasted the rats at approximately 12 hours prior to the experiment until the end of the experiment. We also performed the experiment during the initial stage of the light cycle to insure the rats would be less active during the experiment. Next, we placed the rats in the holding containers and waited 30 minutes before we began the SRIF and tracer infusion at a rate of 0.15 ml/min. The concentration of the SRIF infused into the rats was 1.0-3.0 μg/kg/min. We began administering SRIF approximately 115 minutes prior to the injection of glucose. The 1 ml injection of glucose contained approximately 0.3g/kg of 10% dextrose. We administered this injection through the jugular vein and infused SRIF or saline through the femoral vein. We took blood samples at various intervals before and after the infusion from the carotid artery for approximately four hours.

Glucose Assays: I used the Sigma Trinder kit to analyze for glucose in the plasma samples. I performed all assays in duplicate. First, I prepared a standard curve of glucose ranging from 100-800mg/dl using the standards of 100, 300, and 800 provided by Sigma. I used a glucose reagent that had been prepared two to three months before the assays were done. To prevent the decomposition of the metabolites, I kept the plasma samples on ice during the assay and stored them at -20°C after the experiment. I added 5μl of plasma and 300μl of glucose reagent to each well in a 96-well microlitre plate and incubated the samples for approximately 20 minutes. Finally, I used a Cansen plate reader to read the absorbance at 505 nm.
NEFA: I performed the assays for NEFAs in a manner similar to the glucose assays, using a Waco NEFA C kit. I prepared a standard curve using the standards provided by Sigma and diluting them to the desired concentrations. These concentrations were 1.98, 1.00, 0.500, 0.390, 0.200, 0.100, and 0.000 mmol. Again, I kept the plasma samples on ice and added 4 µl of plasma to each well. Next, I added 80 µl of reagent A to the well and incubated the plate for 10 minutes at 37°C. Then, I added 160µl of reagent B and again incubated the plate for 10 minutes at 37°C. Finally, I performed a colorimetric analysis on the plate at 492 nm. Again, I performed all assays in duplicate.

Tracer Assay: First, I added 20µl of plasma, 100µl of distilled water, 40µl titrated barium hydroxide solution, and 40µl titrated zinc sulfate solution to an Eppendorf tube. Then, in order to separate the precipitate from the solution, I vortexed the tubes and then centrifuged them at 2000xg rpm for 20 minutes. I then extracted the supernatant, placed it into another set of tubes and centrifuged again to remove all the precipitate. Next, I pipetted 1 ml of the supernatant into glass scintillation vials and placed them under a vacuum oven and allowed them to dry overnight. The next morning, I added 1ml of distilled water and 10 ml of scintillation liquid and had the samples counted on a Beta counter. All assays were done in duplicates.

Insulin Assays: I performed the insulin assays on the plasma samples using the Linco radioimmunoassay (RIA) Insulin Assay kit. I added a specific amount of plasma to a borosilicate tube. Because I did not have the same amount of blood for all sample times, I had to vary the amount in each tube, but I kept the ratio of other solutions constant between each sample. Then, I added assay buffer until the total volume in the tube was 200 µl and then added 100µl of antibody to each tube. I then vortexed the tubes and
incubated at 4°C for 18-24 hours. Then, we added 100 µl of 125I-anti-insulin Ab to the tubes and again vortexed and incubated them another 18-24 hours. Next, we added gamma globulin, polyethylene glycol, and precipitation buffer, centrifuged the tubes, and removed the supernatant. We then counted the tubes for their 125I content and determined the concentration of insulin in terms of pmol/L. Again, we performed all assays in duplicates.

Validation of Methods: In several cases, we had to validate the methods prior to the assay. We expected the insulin concentrations in several of the samples to be low enough to require a new insulin sensitive kit from Linco. For this reason, we needed to test this new kit from Linco and a new method modified by Dr. Silia Chadan of Dr. Finegood's lab to determine whether a precipitate would form. First, we prepared a solution of 3 g γ-globulin/l and a solution of 250 g polyethylene glycol (PEG)/l. Before adding the precipitating reagent, we added 150 µl of γ-globulin. Then, after 15 minutes, we added 450 µl of PEG solution and 1.0 ml of precipitating reagent. We tested the method several times using the insulin standards and noted that a suitable amount of precipitate formed at the bottom of the tube. Therefore, we performed the insulin assays on the plasma samples.

Data Analysis:

Insulin: First, we determined the total radioactivity in the 125I solution by counting the disintegrations per minute of 100 µl of 125I. The 125I that was added would competitively bind with this other insulin in the solution. This binding was proportional to the amount of insulin that was bound to the antibody. Next, we determined the maximal binding without antibody, B0, using 300 µl of buffer. Then, we prepared a non-specific binding using 200
µl of buffer and 100 µl of antibody. We defined the non-specific binding as the amount of antibody binding that occurred without any insulin. We subtracted non-specific binding from the total to give us the amount of insulin binding to the antibody and subtracted the nsb from the B₀. We divided new B₀ by the new total and multiplied by 100% gave the percent of specific binding. A standard curve was determined using the standards provided by Linco and had an R² = 0.9811. To determine the percent binding, we performed the following calculation where nsb is non-specific binding:

\[
\frac{((cpm - nsb) \times 100\%)}{B₀}
\]

We used this to produce a standard curve of %B/B₀ versus concentration of insulin and determine the best fit equation for this curve which was:

\[
\frac{\%B}{B₀} = 9.3846 \left(\frac{ng}{ml}\right)^{-0.9463}
\]

We then determined the %B/ B₀ of our unknown samples in the same manner as our standards. Using the best fit equation, we determined the concentration of insulin in the plasma samples in terms of ng/ml based on the counts per minute, cpm, of the sample. From this, we multiplied the ng/ml by 150 to determine insulin concentration in terms of pmol/L.

**Tracer:** I calculated the amount of tracer in the plasma sample by taking the counts per minute (cpm) and dividing by the percent efficiency of the counter in order to determine the amount in disintegration per minute. I then multiplied this by 1000 to obtain (DPM)/ml and divided this by 2.22e6 to obtain µC/ml.
**Glucose:** I determined the concentration of glucose in the plasma samples by first graphing the concentration versus the optical density of the standards. Using the equation for the standard curve that had an \( R^2 = 0.960 \) to 1.000, I substituted the optical densities of the unknown plasma samples and then determined the concentration of the glucose. I then graphed the sample concentrations versus the time that I took the samples.

**NEFA:** I determined the concentration of NEFA in the standards in the same manner as the concentration of glucose. I did a new standard curve for this assay and the \( R^2 \) values ranged from 1.000 to 0.960.

**Results and Discussion:**

The glucose time course for both the control and the SRIF group appear to be similar with glucose levels dropping slightly less quickly in the SRIF group (Figure 1). Areas under the curve for both also indicate that the glucose levels of the SRIF group did not normalize as quickly as the control glucose levels with the area under the curve for the control and the SRIF group at 3740 and 4176 respectively (Figure 2). However, the standard deviations in these groups do overlap meaning that the area under the curve for the SRIF group could be less than or equal to that of the control also. This is most likely the result of there only being two animals in each group. Because of this, I cannot draw any conclusion in terms of statistical significance or in terms of the overall influence of glucose effectiveness in maintaining proper glucose levels in these rats. However, based on the data that I do have, I believe that the data supports my hypothesis that insulin action does play a role in glucose homeostasis. I also believe that it also shows that glucose effectiveness contributes greatly in normalizing glucose levels in the rats because the glucose levels still normalized fairly quickly after the glucose bolus.
Figure 2: We administered a glucose injection and a saline infusion to the control group, which is represented by the diamonds, and a glucose injection and SRIF infusion to the SRIF group, which is represented by the squares. The SRIF glucose levels normalize less quickly than the control.

Figure 3: The area under the curve is less for the control than for the SRIF group. The standard deviation of the groups also show that it is possible that area under the curve for the SRIF group could be less than or equal to the area under the curve for the control.

However, I find it more difficult to draw conclusions given the results of the NEFA assays and of the insulin assays. Because we injected a glucose bolus, we expected
that the amount of NEFAs in the plasma would remain constant or decrease. When NEFA is in the blood being used for gluconeogenesis and other methods of producing glucose, one would expect the glucose levels would not only represent the glucose bolus but also the NEFAs.

![Graph showing NEFAs over time](image)

Figure 4: Both the control group, represented by the diamonds, and the SRIF group, the represented by the squares, have an increase in NEFAs in their blood at the time of the glucose injection at 130 minutes.

One can observe that the time course of NEFAs in the plasma follow a similar time course to that of glucose (Figure 3). There is an increase in fatty acids at time equal to 130 minutes in both the control group, which was administered a saline infusion and glucose injection, the SRIF group, which was administered a SRIF infusion and glucose injection. This was the time that we administered the glucose. The peak is not quite as high in the control as it is in the SRIF group. We also had these results in the rats injected with the saline bolus. We assumed three possible reasons for this. First, we added heparin which contains fatty acids to each of the collection tubes to prevent the blood from
clotting. However, this could not account for the peak because one would expect a constant amount of NEFA throughout because we added the same amount to all the tubes. Furthermore, if we assume pipetting error might account for this, then we would have to assume that the pipetting error that was made for all the tubes was made at the same point in every set-up. Because we did not believe that this was probable, we assayed the 10% glucose solution for NEFAs. We could not detect any NEFAs in the solution unless the assay was allowed to incubate overnight. Because of this, we decided it would be best to purchase new standard glucose solutions for any future experiments. If we still observe a peak, one might also believe that the animal may be stressed. Rats often seek alternative sources of energy in stressed situations which means that the rats’ liver would release fatty acids that could be metabolized into Acetyl CoA and used to make ATP.

![Graph](image.png)

Figure 5: The control group is represented by the diamonds, and the SRIF group is represented by the squares. The control group has a large increase in insulin in the blood in response to the glucose injection. The SRIF group also has an increase in plasma insulin levels after the glucose injection, but the increase is not as large as the control.
Figure 6: We can also observe that the area under the curve for the SRIF group is approximately half as great as the area under the curve for the control. We can also observe that the insulin response was not completely suppressed in the SRIF group.

We also had difficulty drawing conclusions about the overall effects of $S_G$ on glucose homeostasis because of the results of the insulin assays (Figures 4 and 5). Again, in order to study $S_G$, we needed to suppress the insulin response to the glucose bolus. One can see that there is a peak in the insulin levels of the SRIF group following the bolus injection (Figure 4). By examining the area under the curve for insulin assays, we believed that the response was not completely suppressed but rather decreased by about 50% (Figure 5). As with the glucose assays, we noted that the standard deviation was also high for this group with the control and SRIF deviations being 1194 and 659 respectively. Koerker et al. (1974) found that the ability of the SRIF to inhibit insulin secretion was dependent on the ratio between the amount of SRIF infused and the amount of insulin injected. Therefore, in future trial, we should either decrease the amount of glucose injected or increase the amount of SRIF infused.
Looking at the time course of the tracer during the experiment, we observed many problems with the determining the tracer concentrations in the plasma samples (Figure 6). The efficiency listed in the Beta counter manual did not match the efficiency that we obtained with stock solutions. Furthermore, we observed that the resulting efficiency in the stock, assuming that the listed stock radioactivity was correct, was not consistent between different aliquots, making it impossible to calculate the efficiency of the Beta counter. Thus, we listed all values in terms of counts per minute in the plasma samples. There is also quite a bit of fluctuation in the tracer counts during the infusion. We expected that the amount of tracer in the blood should decrease initially as a result of uptake in the tissues. We did observe this (Figure 6). However, we also expected that the amount of tracer should continue to decrease throughout the experiment especially after the glucose bolus because the increased glucose levels should increase peripheral uptake
through glucose effectiveness and insulin action in those that were not given SRIF.

However, we did not observe this in either group (Figure 6). We expected that, based on previous research, the rate of uptake for both the SRIF group and the control should be the same. If the insulin response had been suppressed, this would have further supported the belief that insulin action had little influence on the rate of glucose uptake in rats.

**Conclusion:**

Based on an n = 2 for both my control and my SRIF group, I did not fully suppress the insulin response to the bolus injection. I decreased the response by approximately 50%. Because I did not completely suppress the response, I can only conclude that a decreased insulin response may result in glucose levels normalizing more slowly. I also believe that this may be influenced by an increase in NEFA levels after the time of the injection. This increase may be due to the animal being stressed during the injection.

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**Works Cited:**


