

What's in the Milk? The Effects of Insulin-like Growth Factor-I on Mouse Development

Meghan George
Department of Biology,
Hartwick College
Oneonta, NY
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Thesis Advisor

Date

Department Chair

Date

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Meghan George
Dr. Laura Malloy, Advisor

ABSTRACT

Seeking to increase dairy milk production, some dairy farmers have employed a synthetic growth hormone known as bovine growth hormone (bGH) or bovine somatotropin (bST). Studies conducted by The U.S. Food and Drug Administration with the recombinant form of bST (rbST) have shown that the amount of bST in milk from injected cows is not statistically different from the amount of bST in milk from natural, non-injected cows. Thus the FDA has endorsed the use of this Monsanto product. However, the same studies have shown that the concentration of insulin-like growth factor-I (IGF-I) in milk from rbST cows is significantly increased compared to controls. IGF-I is a natural protein hormone that stimulates cell growth and tissue differentiation, while also binding to insulin receptors that regulate glucose and amino acid uptake into the cell. Therefore I tested the hypothesis that increased levels of IGF-I in the diet could induce insulin resistance in mice. A group of juvenile mice were randomly split into two treatment groups and a control group. The two treatment groups were orally fed either 0.1ng/ul x 26ul or 1ng/ul x 26ul for 19 days. Pre and post treatment glucose tolerance tests, body weight, and percent body fat were compared across groups. Data analysis using ANOVA demonstrated no changes in morphological characteristics – body weight, and percent body fat but did demonstrate that the mice in the high dose treatment group developed signs of insulin resistance. I conclude that increased levels of IGF-I in the diet may cause metabolic changes in the body which could potentially lead to serious diseases such as Type 2 Diabetes Mellitus.

INTRODUCTION

With the increasing need and desire for more dairy milk production, some dairy farmers have resorted to a newer technology of injecting their herds with a synthetic growth hormone. Bovine growth hormone (bGH), also known as somatotropin (ST), is naturally produced in the cow's pituitary gland and one of its effects is to regulate milk lactation. About sixty years ago it was discovered that natural bovine growth hormone, when injected into lactating rats and goats, increased milk yield. It was later discovered that bST could be genetically engineered. Just like natural bST, recombinant bST (rbST) can increase milk production in cows up to ten to fifteen percent when injected (Etherton and Bauman, 1998).

Along with an increase in lactation, there are many other effects of bST in the body so more nutrients can be used for lean tissue accretion or milk synthesis (Bauman, 1999). Somatotropin affects many target tissues and can cause either somatogenic effects or metabolic effects. Somatogenic effects occur when somatotropin stimulates cell proliferation, whereas metabolic effects include effects all nutrient classes like carbohydrates, lipids, proteins, and minerals (Etherton and Bauman, 1998). Biological effects from somatotropin are chronic and predominantly involve alterations in the ability to decrease lipogenesis and increase lipolysis by direct action on adipose tissue (Etherton and Bauman, 1998). A few of the most interesting and pertinent effects on dairy cattle after being injected with somatotropin include: an increase in protein accretion, a decrease in glucose uptake, and a

decrease in insulin stimulation of glucose metabolism and lipid synthesis in the adipose tissue –ie: That is, bST injection reduces the effectiveness of insulin. Treatment with bST can reduce whole-body glucose response to insulin tolerance tests by altering the responsiveness of modulate tissue to insulin causing a reduced response; this is referred to as “insulin resistance” (Etherton and Bauman 1998; Bauman, 1999). The changes in glucose response to insulin are almost exclusively related to effects on lipogenesis in adipose tissue (Dunshea et.al., 1992).

Currently, the U.S. Food and Drug Administration supports the safety and effectiveness of the recombinant bovine somatotropin and has approved the Monsanto Company’s manufacture and distribution of a patented recombinant bovine somatotropin (rbST) product, known as Posilac. Studies on milk from injected cows have demonstrated that the amount of bST in cow milk from injected cows is not statistically different from the amount of bST in cow milk from natural, non-injected cows (Etherton and Bauman, 1998). Further oral administration of bST to rats results in no overall body growth, likely because bST is not absorbed as an intact protein through the digestive tract (Seaman et. al, 1987). However, the concentration of insulin-growth factor-I (IGF-I) in milk from bST injected cows is significantly increased when compared to those who were not (Juskevich and Guyer, 1990). Also, Juskevich and Guyer note that IGF-I does not denature during the pasteurization process (1990). It has been shown that bovine IGF-I is identical in structure to human IGF-I (Honegger and Humbel, 1986). IGF-I is synthesized in the liver in response to somatotropin stimulation (Collier et al., 1991). IGF-I is a natural protein hormone produced in the liver and found in human saliva, that stimulates cell growth and differentiation in a variety of tissues (Di Cola et al., 1997). IGF-I also binds to insulin and IGF-I receptor sites, which then can regulate glucose and amino acid intake into the cell, gluconeogenesis, and promote lipogenesis (Di Cola et al., 1997). In newborn rodents, IGF-I can lower plasma glucose levels by binding to its own receptor in the absence of insulin receptors, thus mimicking insulin actions (Di Cola et al., 1997). IGF-I shares at least 49% structural homology with insulin and their receptors are quite similar (Ullrich et.al., 1986). Insulin and IGFs can interact with each other’s receptors in a concentration-dependent manner, although they have the highest affinity for their own receptor (Ullrich et.al.,1986).

Even though IGF-I concentrations in milk from rbST treated cows have been shown to be higher, and IGF-I has known metabolic effects no studies have researched the idea that increased IGF-I concentrations in the diet can cause direct metabolic effects. Further, in studies that focused on the effects of milk from rbST-treated cows, only anabolic effects were measured, dismissing that metabolism may also be influenced by bST induced peptides present in milk. Lack of interest in the effects of such proteins, including protein hormones, could be because these substances are naturally broken down in the digestive tract. For example, even though Seaman et.al. found a systemic antibody response to bST peptide fragments in the diet, the study suggested that the fragments must have been inactive because they noticed no effect on overall body weight and antibody responses are a “normal phenomenon” (1987). However, it is reasonable to suspect that peptides such as IGF-I may have an effect on surrounding tissue prior to breakdown in the gastrointestinal tract. Furthermore, studies of milk from bST treated cows and its effects have only been conducted in adult animals. This approach fails to recognize that juveniles are more vulnerable to their environment and dietary intake because they are in a crucial growing period in their lives. Therefore, it is reasonable to question if increased concentrations of IGF-I in the diet can

cause insulin resistance and a decrease in percent body fat in juvenile animals before its natural breakdown into fragments.

MATERIALS AND METHODS

Animals. Twenty-four female, recently weaned juvenile mice were obtained from Taconic Farms at three weeks of age. Mice were selected at random and put into cages in pairs for the two treatment groups. The four control mice were caged together. Food pellets and water were available ad libitum throughout the entire experiment except during fasting periods for glucose tolerance testing, when only water was available. Animal room temperature was kept at approximately 23°C with 56% humidity and the room was kept on a 12-h light cycle. Mouse cages were cleaned once a week or as needed. The mice were cared for in the accordance of the *Hartwick College Guide for the Care and Use of Laboratory Animals*. The two treatment groups were orally fed IGF-I in doses of 0.1ng/ul x 26ul or 1ng/ul x 26ul for 19 days. Pre and post treatment glucose tolerance tests and body weight were measured as was post treatment percent body fat.

IGF-I Dilutions and Administration. IGF-I was purchased through Gropep (Thebarton, SA Australia) in 10 microgram tubes. The dose of IGF-I was scaled to be comparable to that consumed by human children between the ages of 2 and 8. The amount of IGF-I typically found in a child's daily milk intake from rbST-treated cows and ten times that amount were calculated per kilogram for a human child (9.5kg) and then sized down to a 15g mouse. Dilutions were made by taking 1 milliliter of distilled water and adding it to the 10ug IGF-I tube to make 10,000ng/ml. 10 micro liters of the solution were removed and placed in a sterile micro tube to create a stock solution. The 2.6ng/ul concentration for treatment group number 1 was created by taking 10 micro liters of the stock solution and adding 990 micro liters of distilled water. The concentration for treatment group 2 was created by taking 100 microliters of the base solution and adding 900 microliters of distilled water. Mice were fed 26 micro liters of their specified concentrations depending upon their treatment group, once every day for 19 days.

Oral Glucose Tolerance Test (OGTT). OGTT tests were performed before the feeding protocol began and again after 19 days of the feeding regimen. Mice were fasted for 12 hours before the test. Each mouse was orally fed glucose at a dose of 1g/kg (Nobe et. al., 2004). Blood samples were taken just before glucose feeding, and then at 10, 20, 30 and 60 minutes after the fasting glucose reading. An Eckerd brand human glucometer was used and could accurately test mice blood sugar levels up to 3% variability (Messier and Kent, 1994).

Underwater Weighing and FFM. Mice were sacrificed by cervical dislocation after the second glucose tolerance test and two methods were used to determine body composition. In the first method used to determine body density mice were tied and weighted to suspend freely in a beaker of water, while attached to a scale. Mouse limbs and tails were secured so as not to touch the sides of the beaker. The water displaced by the mouse's suspension was captured and weighed. The strings and weights tied around the mouse were also taken into account for the mouse suspended weight. For the second method of determining body composition, the mouse was inserted into a 100mL graduated cylinder and their body volume was calculated from the volume of water displaced. Mouse density was calculated from each method; for the first method the water density was multiplied by the specific gravity of the mouse. For the second method the mouse dry weight was divided by the volume displaced in

the graduated cylinder, corrected for temperature. After calculating the densities, Siri's equation ($([4.95/\text{Density}] - 4.5) \times 100$) was used to determine the animal's percent body fat.

Ultimately, four mice were eliminated from the results figures and statistical analysis for one of two reasons: either there were known methodological errors during the glucose tolerance tests, or their pre-treatment glucose tolerance tests were abnormal suggesting a mouse was already diabetic before treatment. Consequently, one mouse's data from both the control group and the low dose group were disregarded and two mice from the high dose group were disregarded due to conflicted pre-treatment measurements.

Analysis. The areas under the glucose tolerance test curves for the pre-treatment and post-treatment were calculated for each mouse. The post-treatment area under the curve was divided by the pre-treatment area under the curve and multiplied by 100 to get a percent change for each mouse. One way ANOVA tests were used for statistical comparisons between the test groups and the assumptions were verified. The Student-Newman-Keuls, Duncan and Least Significant Difference (LSD) post hoc tests were used to determine which groups demonstrated statistically significant differences using a p-value of 0.05 (Dytham, 2006).

RESULTS

Analysis of Mouse Weights. The data in table 1 compares the average mouse's overall body weight throughout the duration of the experiment in each test group. The after treatment weight was divided by the before treatment weight to determine the percent weight gain after 19 days. The data shows that the mice were growing over time regardless of their assigned group and that there were no statistically significant nor dose-dependent differences among treatment groups in weight change over the course of the experiment (Fig. 1).

Table 1. Mouse average weights per test group before treatment and after treatment with percent change over duration of experiment. Parentheses indicate standard error range.

	Control	Low	High
Before	19.1g (+/- 0.7)	13.6g (+/- 0.8)	15.6g (+/- 1.2)
After	29.6g (+/- 0.6)	24.5g (+/- 0.9)	26.3g (+/- 0.8)
Percent (After/Before)	156% (+/- 7.2)	183% (+/- 8.4)	176% (+/- 14.9)

The data, presented in Table 1, were used to construct the graph shown in Figure 1.

Average Percent Weight Change

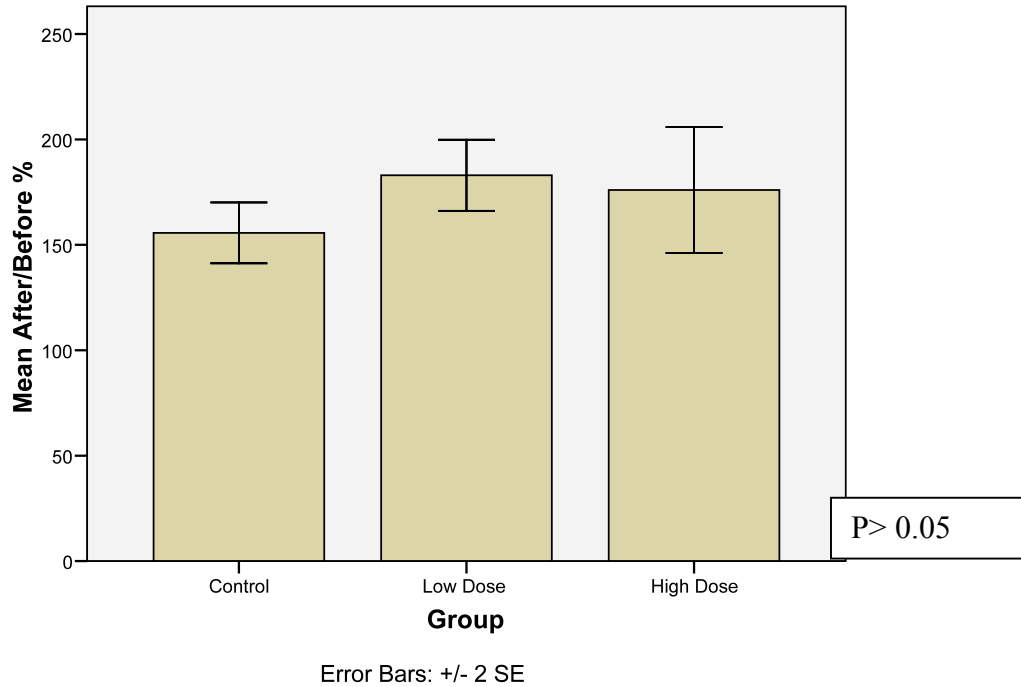


Figure 1. Comparisons of average percent change in overall body weight throughout the duration of the experiment. These data indicate that the overall percent weight gain in the two treatment groups was indistinguishable from the control group percent weight gain.

Glucose Tolerance Tests. Glucose tolerance test curves were generated by plotting average blood glucose following the glucose challenge in each treatment group both before and after the 19 day feeding protocol.

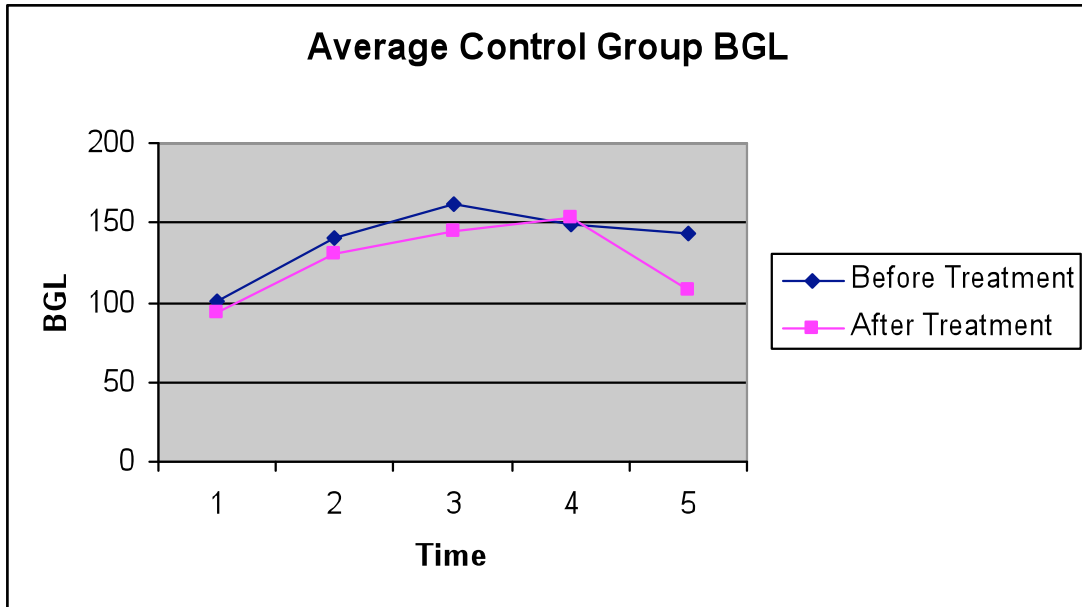


Fig. 2. The average blood glucose response to a glucose challenge before and after treatment for the three control mice. BGL stands for Blood Glucose Level and the 5 times were taken at 0, 10, 20, 30, and 60 minutes respectively.

Figure 2 demonstrates the average blood glucose response to a glucose challenge before and after treatment for the three control mice. There is an increase in blood glucose levels following an initial dose of glucose water that decreases over the course of an hour. The curve for mice after 19 days of treatment with regular diets is super-imposed upon the before treatment curve indicating there is no difference in glucose tolerance over the course of the 19 day feeding period.

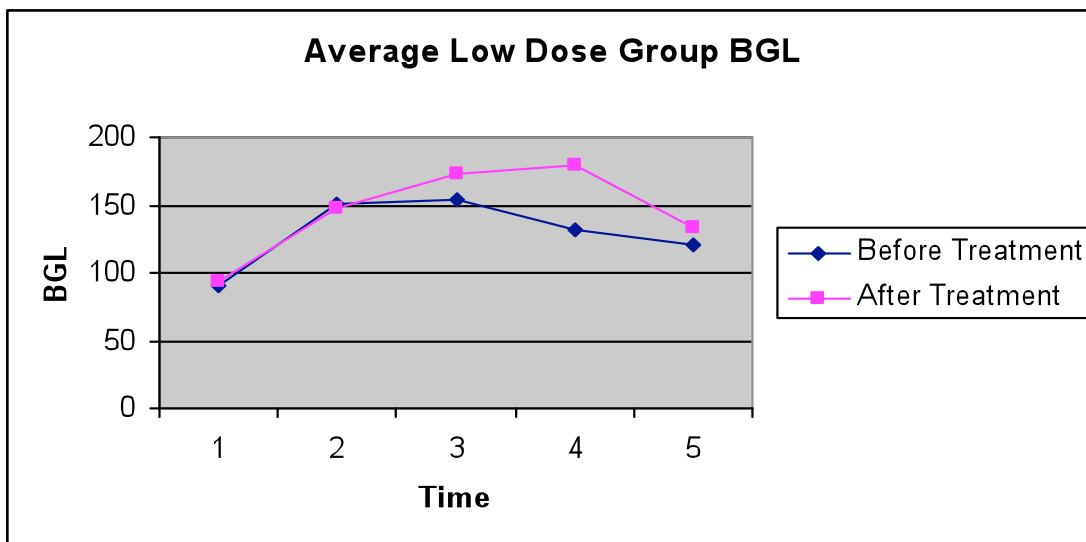


Fig. 3. The average blood glucose response to a glucose challenge before and after treatment for the nine low dose mice. BGL stands for Blood Glucose Level and the 5 times were taken at 0, 10, 20, 30, and 60 minutes respectively.

Figure 3. Demonstrates the average before and after treatment glucose challenge curves for nine low dose mice. The graph shows the after treatment curve starts as super-imposed on top of the before treatment graph, however the mice glucose levels are higher on average after the challenge and take longer to return back to normal.

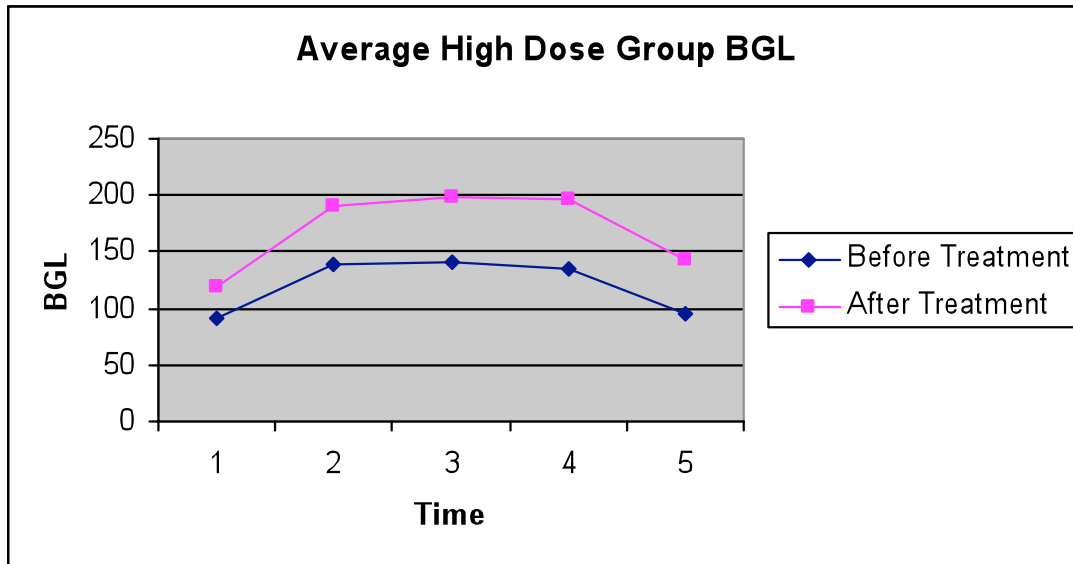


Fig. 4. Demonstrates the average blood glucose response to a glucose challenge before and after treatment for the eight high dose mice. BGL stands for Blood Glucose Level and the 5 times were taken at 0, 10, 20, 30, and 60 minutes respectively.

Figure 4. Shows the after treatment curve following a glucose challenge as well as the before treatment curve for eight high dose mice. The after treatment fasting glucose level starts higher than the before treatment fasting glucose level and the after treatment curve maintains its increased blood glucose state throughout the entire challenge.

GTT Response:BG over 60 minutes After/ BG over 60 minutes Before

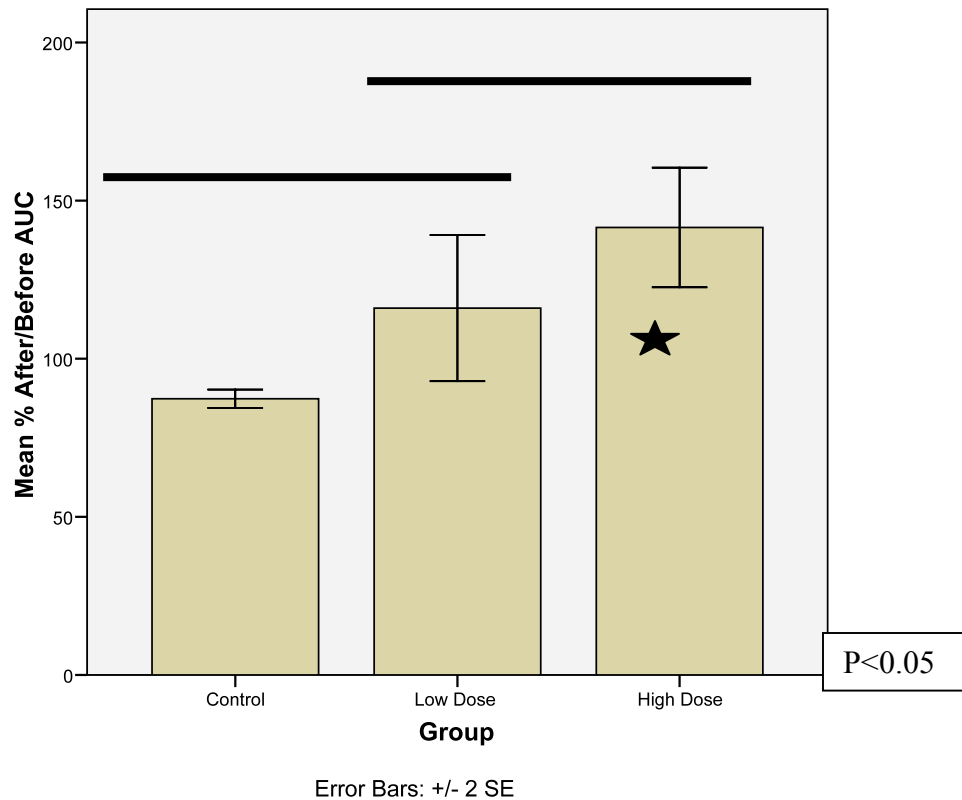


Fig. 5. Mean percent change of area under the curve measurements divided by 60 for the three test groups. Lines above the bars distinguish there was not a statistically significant difference between the test groups directly below. Star indicates the test group that is significantly different from the control.

Figure 5. Due to mouse variability and the peak variability between each glucose challenge graph, the area under the curve for each individual mouse over a 60 minute time period was averaged with the other mice in the same test group. There was no statistical significant difference between the individual time points for each mouse so the area under the curves proves to be a better method for comparisons across test groups. Figure 5 shows that the average area under the GTT curve for the control group was not significantly different from the low dose area under the curve. Likewise, the low dose group area under the curve is not significantly different from the high dose group. However, the high dose group is statistically significantly different from the control group and a dose-dependent response can be seen in the bar graph.

DISCUSSION

Our results found that orally administering IGF-I did not affect the overall body weight of the mice, however the results did show that increased doses of IGF-I caused a dose dependent decrease in glucose tolerance. The after treatment GTT curves are higher than the before treatment lines as the concentrations of IGF-I increase and the glucose levels in the blood take more time to return back to a normal glucose level (Figure 2,3,4). The mice fed the highest dose concentrations of IGF-I became insulin resistant, as their after treatment glucose tolerance test curves were much higher and took much longer to return back to a normal state (Figure 4). The high dose group showed that the area under the curves over a 60 minute period were significantly different from the control group with a p-value <0.05 (Figure 5). Therefore the results did support the hypothesis that increased IGF-I levels through oral intake would induce insulin resistance.

Our study is unique in that there are no current studies that look at the effects of IGF-I on metabolism, nor are there studies that focus on juveniles. Other studies have failed to recognize that milk from bST-treated cows has effects on metabolism (Seaman et.al.).

Previous reports also indicate that mouse overall body weight's are not affected after being fed a protein hormone (rbST) that typically stimulates cell proliferation (Seaman et. al, 1986). The results of this study agree with these findings, in that the increase in overall body weight for IGF-I fed mice is not significantly different from the control group (Figure 1). However, both previous studies and this study failed to consider that even though the overall body weight may increase, the percent body fat will decrease as lean tissue is developed. This study attempted but failed to address this issue since we were unable to make accurate measurements of body fat in each group. Although the results for this study did not support the hypothesis that percent body fat would decrease as an effect of oral doses of IGF-I, further research should be conducted in order to define a solid method for obtaining body composition densities in an affordable manner. Even though hydro-densitometry is convenient, it carries much room for error in measurements from specimen to specimen.

There is a continuing need for improvements in determining body composition as well as choosing one common formula for determining percent body fat (Kodama, 1971). Along with human error, there are factors such as expelling complete pockets of air out of the animal that make it very difficult to calculate. According to McArdle, Katch and Katch, air remaining in the lungs and other spaces, such as the gastrointestinal tract contributes to the buoyancy of the subject during hydro-densitometry experiments (2001). Air in cavities besides the lungs results in only a minor error, whereas residual lung volume should be calculated and figured in when calculating the density of the subject, which makes it difficult to compute.

Our results found that orally administering IGF-I did not affect the overall body weight of the mice, however the results did show that increased doses of IGF-I caused a dose dependent response. The after treatment curve lines are higher than the before treatment lines as the concentrations of IGF-I increase and the glucose levels in the blood take more time to return back to a normal glucose level (Figure 2,3,4). The mice fed the high dose concentrations of IGF-I became insulin resistant, as their after treatment glucose tolerance test curves were much higher and took much longer to return back to a normal state (Figure 4). The high dose group showed that the area under the curves over a 60 minute period were significantly different from the control group with a p-value <0.05 (Figure 5). Therefore the

results did support the hypothesis that increased IGF-I levels through oral intake would induce insulin resistance.

Future studies should refine this experiment by adding more test groups and therefore creating a greater dose-dependent response between the concentrations. Accurately identifying a method for calculating body composition density and using a percent body fat equation appropriate for the size of the animals is needed. It would be important to keep in mind that excess air pockets throughout the digestive tract or in the lungs causes increased buoyancy in the hydro-densitometry method. Future studies should also attempt to identify where exactly the protein hormone is broken down and if it is causing an effect on the body prior to its break down or if the break down products are creating the response. A blood plasma level testing for IGF-I fragments could help determine what is causing the response. Instead of determining the concentrations of IGF-I by comparing human and mouse body weights, it would be interesting to compare the concentrations by metabolism level because mice, having a higher metabolic rate, may be using the IGF-I faster than humans normally would and therefore the concentrations for the mice should actually be higher than in this study.

In conclusion, others have shown that IGF-I levels are increased in milk from cows treated with rbST (Juskevich and Guyer, 1990) and our study has shown that these increased levels can actually cause insulin resistance. Thus, milk from rbST treated cows has the potential to contribute to the development of Type II Diabetes Mellitus. This is a cause for concern among consumers drinking milk from rbST-treated cows over a period of time.

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