The Effect of Varying Levels of GH Treatment on the Body Composition of Obese and Diabetic Mice

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This thesis titled
The Effect of Varying Levels of GH Treatment on the Body Composition of Obese and
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by

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ABSTRACT

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The Effect of Varying Levels of GH Treatment on the Body Composition of Obese Mice
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This study examined the effects of various doses of Growth Hormone (GH) on obesity and diabetes in mice. Male C57Bl/6J mice were either placed on a low-fat (LF) or high-fat (HF) diet at 3 weeks of age and maintained on that diet for 16 weeks. The HF group was divided into five injection groups and received either daily saline injections or increasing doses of bGH, while the LF group was injected with saline. Injections were performed daily for 6 weeks. Obese mice injected with the highest dose of GH (5 µg/g BW) had the greatest increase in lean mass and the greatest decrease in fat mass. Remarkably, these animals did not differ significantly from the LF saline-injected controls after 6 weeks of GH treatment and had virtually no change in overall body weight while continuing on a HF diet. Furthermore, the highest dose of GH improved blood glucose levels to near LF control levels, despite the known diabetogenic effect of GH, but was unable to return plasma insulin to control levels suggesting some level of insulin resistance.

Approved: __________________________________________________________

Darlene E. Berryman

Associate Professor of Human and Consumer Sciences
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CHAPTER 1: INTRODUCTION

The rates of obese and overweight individuals globally have reached epidemic proportions. The World Health Organization (WHO) estimates that as of 2005 more than 1.6 billion adults are clinically overweight (BMI > 25 kg/m²), with 400 million considered clinically obese (BMI > 30 kg/m²) (WHO, 2006b). Continuing with this current trend, by the year 2015 approximately 2.3 billion adults will be overweight and 700 million of them will be obese (WHO, 2006b). Obesity-related complications are also occurring at alarming rates. Specifically, type 2 diabetes mellitus is estimated to affect nearly 6% of the world’s population (Adeghate, Schattner, & Dunn, 2006). In the United States, the Centers for Disease Control and Prevention (CDC) estimates 20.8 million people have diabetes (approximately 7.0% of the population) (CDC, 2005). These alarming disease rates have prompted researchers to search for new methods to treat obesity.

Type 2 diabetes mellitus comprises approximately 90% of all diabetes cases (WHO, 2006a). Obesity is considered a major risk factor, present in about 80% of patients (Adeghate et al., 2006). A schematic illustrating the prevalence of obesity and diabetes as of 2001 is shown in Appendix A. The progression of type 2 diabetes includes insulin resistance, hyperglycemia and eventual pancreatic β-cell dysfunction (Kahn, Hull, & Utzschneider, 2006). Body fat distribution is critical in the development of insulin resistance. Fat tissue located centrally has been associated with a greater risk of insulin resistance while individuals with fat tissue located more peripherally have a lower risk of developing insulin resistance (Kahn et al., 2006).
Growth hormone (GH) is secreted from the anterior pituitary in a pulsatile manner. GH is of interest in the treatment of obesity due to its lipolytic/antilipogenic effects as well as its anabolic effect on the growth of lean muscle. GH levels have also been shown to decrease in an obese state, which further supports the argument to use GH as a potential therapeutic agent in the treatment of obesity (Shadid & Jensen, 2003). However, GH has also been shown to have diabetogenic effects by antagonizing insulin’s actions (Kopchick & Andry, 2000). Mouse models that express bovine GH (bGH) and therefore have higher GH activity, have been shown to have a significantly shorter lifespan, decreased fat mass, increased lean muscle mass, develop insulin resistance and increased renal damage (Berryman et al., 2006; Chen et al., 1995). Similarly, studies involving acromegalic patients, who secrete excess GH, also experience similar complications including hypertension, diabetes, sleep apnea, cardiovascular disease, cancer and a mortality rate 2-3 times higher than that of the normal population (Molitch, 1992). These negative consequences of GH may make it a poor option for treatment of obesity.

Recent studies have been conducted using human GH (hGH) injections to treat obese human subjects. Currently, there are few studies that report significant and favorable body composition changes when daily standard doses of hGH are administered to humans (Attallah, Friedlander, & Hoffman, 2006). Due to the possible harmful effects of excess GH, especially related to the health risks associated with diabetes, it would be useful to use an animal model first to study the effects of daily GH injections not only on
body composition, but also in regard to other physiological parameters related to obesity and diabetes.

The C57Bl/6J mouse model has evolved as a useful model for obesity and diabetes-related studies. This model has been shown to progress from obesity to insulin resistance and eventual type 2 diabetes when fed a high-fat diet, and remain lean on a low-fat diet. Furthermore, they display the same distribution and deposition of fat as seen in humans with high-fat feeding (Rebuffe-Scrive, Surwit, Feinglos, Kuhn, & Rodin, 1993; Surwit, Kuhn, Cochrane, McCubbin, & Feinglos, 1988). The ability to induce obesity through diet makes this mouse model very useful for studies involving obesity and its subsequent progression to diabetes.

**Statement of the Problem**

The high rate of overweight and obese individuals and the associated health problems are of great concern. GH treatment has been used in an attempt to decrease adiposity and increase lean muscle mass. However, there are few studies that have demonstrated a significant effect of GH injections on body composition. Due to its diabetogenic effect, the addition of GH to an obese, possibly insulin resistant state may be more harmful causing exaggeration of the diabetic state. It is also unclear what dosage of GH would be effective in changing body composition in an obese state. Obese animal models are excellent for studying these effects because of the possible health risks involved with administering high doses of GH to obese humans. Also, research with animal models allows for the collection of many different parameters that would be difficult if not impossible to obtain using humans. Compared to human studies, animal
models also offer the researcher more ability to control factors pertaining to their external and internal environment such as diet, temperature and humidity, light/dark cycles, time of procedures and genetic similarity.

**Research Questions**

1. Does the body composition (lean mass and fat mass) of male, obese C57Bl/6J mice change with varying dosages of daily GH injections compared to male, obese or lean C57Bl/6J mice injected with a saline solution?

2. Are the plasma levels of glucose, insulin and IGF-1 altered in male, obese C57Bl/6J mice when given various dosages of daily GH injections when compared to lean and obese saline-injected controls?

3. Do organ weights change in male, obese C57Bl/6J mice when given various dosages of daily GH injections as compared to lean and obese saline-injected controls?

**Purpose of the Study**

This study aims to determine changes in an obese/diabetic state that are related to daily injections of GH. Most of the previous studies have used obese human subjects and injected varying concentrations of GH. There are few studies using mice which assess whole body composition changes with daily GH injections. In this study, not only will whole body composition be measured to assess the obese state, but also physiological parameters, such as glucose and insulin to assess the diabetic state. Tissue collection at the conclusion of the study will also allow for further investigations. This study will also help to determine if an effective and safe dosage of GH exists. GH injections may serve
as an aid to weight loss. However, because of GH’s diabetogenic effects, it is still unclear as to whether this type of treatment would be beneficial or harmful to current health status. This research will provide valuable insight for possible outcomes of this type of treatment on obese and diabetic human subjects.

**Limitations/Delimitations**

1. This study uses mice and may not be fully generalized to the human population.
2. Mice in this study are bred to be genetically equivalent; however, some variations may exist.
3. Multiple animals (2-3) will be housed in one cage possibly leading to hierarchical changes that affect food consumption and possible variations in adiposity.
4. Stress levels of the animals during the study may fluctuate due to procedures. This may cause variations in results unrelated to the GH injections.
5. The injection of the foreign protein, bGH, into the mice may result in an increased immunological response and possible production of antibodies.
6. The nuclear magnetic resonance (NMR) technology used to measure body composition is very sensitive; however, due to movement of the animals, variations may exist.

**Definition of Terms**

Adipose tissue: An organ of the body made up of various depots in which excess energy is stored as fat.
Growth hormone (GH): A 191-amino-acid protein, secreted by somatotroph cells located in the anterior pituitary gland, which promotes growth of tissues (Kopchick & Andry, 2000).

IGF-1: Insulin-like growth factor 1, belongs to a family of molecules including IGF-2 and insulin, that can bind to one of the IGFBP (IGF binding protein) as well as the IGF1R and insulin receptor. It is involved mainly in growth, development and differentiation (LeRoith & Yakar, 2007).

Insulin resistance: A physiological condition in which the tissues become insensitive to insulin’s actions and are no longer able to respond to insulin signals properly. Insulin secretion from the pancreas is increased in an attempt to maintain normal glucose levels (Kahn et al., 2006).

Lipolysis: Hydrolysis of triglycerides stored in adipocytes to free fatty acids (FFA) and glycerol (Wang & Fotsch, 2006).

Obesity: Accumulation of fat tissue in which body weight becomes above what is healthy for a given height or increases the likelihood of certain diseases. Body Mass Index (BMI) is a measurement used to compare weight and height, where a BMI > 30 is considered obese (CDC, 2006).

Type 2 diabetes mellitus: An endocrine disorder characterized by insulin resistance and eventual pancreatic β-cell loss leading to increased plasma glucose levels and several serious complications. Most commonly associated with obesity, older age and genetics (CDC, 2005).
CHAPTER 2: REVIEW OF LITERATURE

The high rates of overweight and obese individuals present many social and economic problems. With the proportion of obese adults approaching epidemic proportions, research has focused on ways to treat and prevent obesity. GH is a logical therapeutic target because of its ability to decrease fat mass and increase lean muscle mass. However, there are potentially harmful consequences that may accompany this type of treatment. This review will include an overview of obesity, the interaction of GH with adipose tissue as well as potential uses for and consequences of this type of treatment.

**Obesity**

The prevalence of obesity worldwide has increased dramatically over the past 20 years. The CDC reports that in the United States obesity among adults age 20-74 years has increased from 15% in 1976-1980 to 32.9% in 2003-2004 (CDC, 2006). The WHO estimates that of adults age 15 and older, approximately 1.6 billion are overweight and 400 million are obese globally (WHO, 2006b). Furthermore, the WHO estimates that by the year 2015, 2.3 billion adults will be overweight and 700 million will be obese (WHO, 2006b). Equally alarming are the growing rates of childhood obesity. In the United States, the CDC estimates that in the same 20-year time frame (1976-1980 through 2003-2004) the number of overweight children increased from 5.0% to 13.9% in ages 2-5, 6.5% to 18.8% in ages 6-11, and 5.0% to 17.4% in ages 12-19 (CDC, 2006). These alarming statistics indicate a growing public health concern of epidemic proportions.
Obesity is defined as having excess body fat. Clinically, this is assessed by measuring body mass index (BMI), which takes into account both weight and height. A BMI greater than 30 is considered obese (CDC, 2006). Many health problems have been identified as comorbidities with obesity. The incidence of type 2 diabetes, cardiovascular disease, hypertension, dyslipidemia, metabolic syndrome, gallbladder disease, osteoarthritis, sleep apnea and certain cancers has been shown to increase with obesity (Wyatt, Winters, & Dubbert, 2006). It is estimated that in the United States, the annual medical expenses for the treatment of overweight, obesity and attributable diseases is between $51.5-78.5 billion (Finkelstein, Fiebelkorn, & Wang, 2003). The annual estimates for obesity alone are between $26.8-47.5 billion (Finkelstein et al., 2003). Due to the prevalence of obesity, its related complications and the economic strain it contributes, a new urgency is driving the need for research of obesity prevention and treatment.

**Type 2 Diabetes**

The incidence of diabetes has increased drastically, affecting nearly 6% of the world’s population (Adeghate et al., 2006). The WHO estimates that more than 180 million people worldwide are affected, with the number expected to double by the year 2030 (WHO, 2006a). Type 2 diabetes is estimated to make up nearly 90% of all cases (WHO, 2006a). A link has been established between obesity and the development of type 2 diabetes. Of all reported cases, nearly 80% of type 2 diabetic patients are obese (Adeghate et al., 2006).
The progression of type 2 diabetes includes the development of insulin resistance, impaired glucose tolerance and eventual pancreatic β-cell dysfunction (Kahn et al., 2006). A 1963 study showed that when obese nondiabetic individuals were given an increased glucose load, their circulating insulin increased, while their circulating glucose remained constant (Karam, Grodsky, & Forsham, 1963). Another study involving the Pima Indian population showed that impairment of both insulin secretion and insulin action are present prior to the development of type 2 diabetes, indicating that dysfunction of pancreatic β-cells and insulin resistance may occur early in the disease progression, potentially before impaired glucose tolerance (Weyer, Bogardus, Mott, & Pratley, 1999). Studies with the ob/ob mouse (a mouse that lacks the gene for leptin and causes marked obesity) (Malik & Young, 1996) showed a marked increase in insulin resistance (Mayer, Andrus, & Silides, 1953). The development of glucose intolerance may develop later in the stages of type 2 diabetes. Studies have shown that in male rhesus monkeys, the development of glucose intolerance occurred several years after the development of insulin resistance (Hansen & Bodkin, 1986).

Potentially the distribution of adipose tissue is a more important indicator of risk for type 2 diabetes and insulin resistance than overall obesity. Studies involving both lean and obese individuals have shown that an increase in central obesity, or intra-abdominal adipose, is related to increased insulin resistance regardless of BMI. Android obesity, or obesity localized to the midsection, is considered to be related to diabetes in 93% of males and 60% of females (Vague, 1956). In lean individuals, increased insulin resistance is related to an increase in subcutaneous and intra-abdominal fat of 45% and
70%, respectively (Cnop et al., 2002). Regression analysis showed that intra-abdominal fat explained 54% of the variance in insulin sensitivity between lean groups (Cnop et al., 2002). High rates of both obesity and type 2 diabetes have led to increased research into the relative importance of adipose tissue from various regions in the development of insulin resistance.

**Growth Hormone**

Growth hormone is a 191-amino-acid polypeptide secreted from the somatotrophs of the anterior pituitary. GH, as its name implies, is a pivotal hormone in longitudinal bone growth. However, GH also has a significant impact on nutrient metabolism, including carbohydrate, fat, protein and mineral metabolism. For example, it promotes both lipolysis and lean muscle accretion. This important effect has made GH treatment a new and important area of obesity research. However, it also has potentially harmful effects which may or may not be exaggerated in obese individuals, such as increased insulin resistance. The danger of treating obese and potentially type 2 diabetic patients with a hormone known to increase the diabetic state is not yet understood in an animal model.

**GH Secretion**

The release of GH from the anterior pituitary is controlled mainly by two peptides, growth hormone-releasing hormone (GHRH) and somatostatin, both of which are secreted by the hypothalamus (Tannenbaum & Ling, 1984). GH is secreted in a pulsatile manner, with increased secretion occurring during sleep cycles (Takahashi,
Kipnis, & Daughaday, 1968). In humans, GH levels are high after birth and then decline until puberty. During puberty, GH levels as well as secretion rates are increased during both sleep and wake periods (Finkelstein, Roffwarg, Boyar, Kream, & Hellman, 1972). In adulthood, GH secretion decreases between the ages of 20 and 70, with eventual levels in the elderly reaching 12-20% of those seen in puberty (Ho & Hoffman, 1993).

Differences in secretion are also seen in obesity, with levels of GH decreased compared to normal-weight individuals. However, these concentrations have been shown to increase in obese, nondiabetic subjects when in a fasted, hypoglycemic state (Herrold & Tzagournis, 1970).

The release of GH is controlled by a negative feedback loop, with ultrashort-loop, short-loop and long-loop feedback mechanisms. A diagram of the feedback mechanism of GH is shown in Appendix B. GH has been suggested to act directly on the pituitary to inhibit GH release in the ultrashort-loop mechanism. As concentrations of GH in the blood rise, the release of somatostatin and GHRH from the hypothalamus are increased and decreased, respectively, to further decrease the release of GH from the anterior pituitary, resulting in a short-loop feedback. The long-loop feedback mechanism results from insulin-like growth factor 1 (IGF-1) in the serum released in response to GH, acting at the hypothalamus and anterior pituitary to inhibit GH secretion (Yamasaki, Prager, Gebremedhin, Moise, & Melmed, 1991). However, other hormones and compounds in the body have also been shown to impact GH secretion and inhibition. Leptin, a hormone which regulated metabolism of nutrients and food intake, decreased overall spontaneous GH secretion in both fed and fasted rats, with the fasted animals exhibiting an amplified
response (Carro, Senaris, Considine, Casanueva, & Dieguez, 1997). Various other hormones and neurotransmitters also affect the stimulation and inhibition of GH, either by themselves or through regulation of somatostatin and GHRH.

Alterations in GH secretion and concentrations are also seen with exogenous stresses. In humans, the greatest amplitude and frequency of GH secretion occurs during sleep (Obal, Payne, Kapas, Opp, & Krueger, 1991). Light/dark cycles have less of an effect on GH secretion when compared to slow-wave sleep phases (Obal et al., 1991). Psychological stress may also be related to GH secretion, though the relationship between stress and GH secretion in humans is not clear. For example, exercise and undernutrition increase GH secretion in humans, depending on the intensity and duration of exercise as well as muscle mass (Weltman, Wideman, Weltman, & Veldhuis, 2003). However, in individuals with decreased GH levels, such as the obese or the elderly, exercise fails to stimulate the release of excess GH (Kanaley, Weatherup-Dentes, Jaynes, & Hartman, 1999; Pyka, Wiswell, & Marcus, 1992). In humans, a 2-day fast results in a fivefold increase in the 24-hour secretion of GH, a twofold increase in the number of daily pulses and in the overall mass released during each pulse (Hartman et al., 1992).

Certain nutritional states also have an impact on GH secretion. Glucose, free fatty acids (FFA) and amino acids have all been shown to impact the release of GH in various models and disease states. In primates, a hyperglycemic state is related to decreased levels of GH, while hypoglycemia results in increased secretion of GH (Himsworth, Carmel, & Frantz, 1972). In humans, FFA have been related to decreased secretion of GH (Imaki et al., 1985). Certain amino acids, especially essential and branched chain
amino acids, stimulate GH release in various models (Giustina et al., 1992; Stewart, Koerker, & Goodner, 1984). Thus, GH secretion and nutrient status are intimately related: GH has a significant impact on nutrient status, and nutrient status is a significant regulator of GH secretion.

**GH Receptor Binding and Signal Transduction**

In order to promote its various effects, GH must bind to the GH receptor on the target tissue. In serum, GH is found either free or bound to the GH binding protein (GHBP), a soluble portion of the GHR. It has been shown that approximately 40-60% of GH is bound to GHBP in normal serum (Baumann, Amburn, & Shaw, 1988). The GHBP is a truncated portion of the GHR, that corresponds to the extracellular domain (Hadden & Prout, 1964). In some species, including humans, the GHBP is formed by proteolytic cleavage of the GHR (Dastot et al., 1996). In other species, including monkeys, mice and rats, the GHBP is formed by alternative splicing of the GHR mRNA (Baumbach, Horner, & Logan, 1989; Martini et al., 1997; Talamantes, 1994). Regardless of the mechanism, most species appear to have GHBP, suggesting an important evolutionarily conserved function for this binding protein. An important role of GHBP appears to be increasing the half-life of GH by decreasing both its clearance and degradation rate (Baumann et al., 1988).

The GHR is a membrane spanning protein consisting of intracellular, transmembrane and extracellular domains. The presence of GHR has been shown to be abundant in liver tissue. However, other tissues such as muscle, bone, adipose, kidney and embryonic stem cells have also been shown to express the receptor to some extent
(Kelly, Djiane, Postel-Vinay, & Edery, 1991; Ohlsson et al., 1993). There are two
important sites on the GH molecule that are necessary for correct binding to the receptor,
often referred to as site-1 and site-2. When binding occurs, site-1 is bound first by GH,
followed by site-2 binding. The GH molecule binds to a preformed receptor dimer,
resulting in signal transduction (Brown et al., 2005). Binding of the GHR leads to
tyrosine phosphorylation of the GHR and phosphorylation of the JAK2 (janus kinase 2)
molecule, followed by phosphorylation and signal transduction by a host of other
molecules (Zhu, Goh, Graichen, Ling, & Lobie, 2001), most notably STAT 5 (signal
transducer and activator of transcription). Ultimately, these second messenger systems
have the ability to alter the expression of various genes related to GH function (Kopchick
& Andry, 2000).

Disruption of GH binding to the receptor is demonstrated by the GH antagonist
(GHA) model. This molecule is able to bind at site-1, but because of specific mutations,
site-2 is unable to bind to the receptor properly. Glycine located at position-119 for bGH
and -120 for hGH, is critical for proper GH binding and action. When substituted with
any other amino acid other than alanine, site-2 binding is disrupted and no signal
transduction occurs (Chen, Chen, Yun, Wagner, & Kopchick, 1994; Chen, Wight, Mehta,
Wagner, & Kopchick, 1991; Ross et al., 2001). Further studies of this model have shown
that GHA binds the GHR but is not able to promote intracellular signaling (Clackson,
Ultsch, Wells, & de Vos, 1998; Harding et al., 1996).
**Biological Effects of GH**

The binding of GH to GHR results in altered expression of numerous proteins. The end result is that GH impacts various pathways. As mentioned previously, the main impact of GH is on longitudinal bone growth. This is illustrated by the impact of an excess of GH leading to gigantism or acromegaly. However, GH has other functions, impacting other tissues and processes of the body, like nutrient metabolism and proportion of lean versus fat mass (or body composition). In humans, pulses of GH are followed by an increase in circulating FFA, indicating an increase in lipolysis (Moller, Jorgensen, Alberti, Flyvbjerg, & Schmitz, 1990). The deposition and storage of lipids in adipose tissue, or lipogenesis, has also been shown to decrease with GH. GH has also been shown to impact carbohydrate metabolism. The diabetogenic effect of GH has been described in numerous studies. When GH was introduced to both normal and diabetic rats an increase in insulin resistance was reported (Milman & Russell, 1950). Although not clearly understood, the mechanism in which GH interferes with glucose metabolism is thought to be due in part to the cross talk between insulin and GH (Dominici et al., 2005). More recently, this cross talk has been found in adipose tissue to be related to p85α-subunit of PI-3 (phosphoinositide-3) kinase, a protein involved in mediating the insulin effects on adipose tissue (del Rincon et al., 2007). When p85α is disrupted in mice, an increase in insulin sensitivity and hypoglycemia is observed (Terauchi et al., 1999). In relation to GH status, animals with low GH levels have reduced levels of p85α and decreased insulin levels (del Rincon et al., 2007). Conversely, animals with excess GH have increased levels of p85α and are hyperinsulinemic (del Rincon et al., 2007).
Additionally, the ability of insulin to activate PI-3 kinase activity is decreased in animals with excess GH (del Rincon et al., 2007). The increase in the p85α subunit by GH negatively effects the insulin pathway in adipocytes by decreasing PI-3 kinase activity (del Rincon et al., 2007). This evidence supports the cross talk theory and provides evidence that the p85α subunit and PI-3 kinase are central to anti-insulin effects of GH.

Various animal models have been used to demonstrate the effects of chronic, high levels of GH. The bGH transgenic mouse model has been shown to have hyperinsulinemia (Quaife et al., 1989) and increased insulin resistance (Valera et al., 1993). In humans, acromegalic patients, who have high levels of GH, have also been shown to have insulin resistance, impaired glucose tolerance and increased risk of developing diabetes (Ezzat et al., 1994). The use of the GHA in acromegalic patients has shown an improvement of both fasting blood glucose and insulin resistance with no change in body weight (Rose & Clemmons, 2002). These results suggest a possible role of GHA in the treatment of diabetes.

GH also has a significant impact on body composition, in part due to its impact on nutrient metabolism. When GH-deficient adults were treated with GH, a significant increase in lean body mass and a significant decrease in fat mass were observed (Salomon, Cuneo, Hesp, & Sonksen, 1989). These studies support the importance of GH on the regulation of body composition.

**GH Treatment in Humans**

GH has been used to treat various diseases in humans. Because of its lipolytic/antilipogenic effects, GH has been considered to be a potential treatment for obesity.
However, due to the diabetogenic effect of GH, using this treatment in an obese group may lead to further complications. Despite these risks, multiple studies have addressed the effectiveness of GH treatment on the development of obesity. A summary of these studies is shown in Table 1. For example, when rhGH at 0.1 mg/kg IBW was given to obese subjects under dietary restrictions, the loss of fat mass was not found to differ from the control group, while the loss of lean body mass was attenuated (Clemmons, Snyder, Williams, & Underwood, 1987). This same study also found no difference in fasting blood glucose or serum insulin levels (Clemmons et al., 1987). In a similar study, obese, diet-restricted subjects were injected with GH at 0.1 mg/kg IBW for 11 weeks with no significant difference in the rate of fat loss or difference in glucose tolerance (Snyder, Clemmons, & Underwood, 1988). Additionally, these subjects had increased serum IGF-1 throughout the study, even after anabolic effects had ceased (Snyder et al., 1988).

Another study measuring the effect of low-dose (0.02 U/kg/day) GH continuous infusion in obese men found no change in insulin or IGF-1 levels (Oscarsson, Lindstedt, Lundberg, & Eden, 1996).

Additional studies have examined the lipolytic action of GH. When obese women were injected with GH at 0.03 mg/kg IBW for 5 weeks, an ~50% decrease in lipoprotein lipase (LPL, a hormone involved in the hydrolysis of lipids) was observed in both subcutaneous and intra-abdominal adipose tissues (Richelsen et al., 1994). Additionally, FFA concentrations increased after treatment, indicating an increase in lipolysis (Richelsen et al., 1994). The decrease in LPL activity was also examined and it was found that the decrease was in both the adipose tissue-specific and muscle-specific forms.
## Table 1

### Summary of Human GH Injection Studies on Treatment of Obesity

<table>
<thead>
<tr>
<th>Reference</th>
<th>Subjects</th>
<th>Length of Study</th>
<th>Age of Participants</th>
<th>GH dosage</th>
<th>Diet</th>
<th>Effect of GH Injections</th>
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<tr>
<td>Clemmons, Snyder, Williams, &amp; Underwood, 1987</td>
<td>8, obese (5 women, 3 men)</td>
<td>11 weeks</td>
<td>23-50 years (mean 34.4)</td>
<td>3 weeks, 0.1 mg/kg IBW every other day</td>
<td>Hypocaloric (24 Cal/kg, 1 g protein/kg IBW)</td>
<td>NS NS NR Increased NS NS</td>
</tr>
<tr>
<td>Snyder, Clemmons, &amp; Underwood, 1988</td>
<td>20, obese (16 women, 4 men)</td>
<td>13 weeks</td>
<td>20-54 years</td>
<td>0.1 mg/kg IBW every other day, weeks 2-12</td>
<td>Hypocaloric (18 Cal/kg IBW, 1.2 g protein/kg IBW)</td>
<td>NS NS NR Increased NS NS</td>
</tr>
<tr>
<td>Snyder, Clemmons, &amp; Underwood, 1989</td>
<td>11, obese women</td>
<td>10 weeks</td>
<td>21-49 years</td>
<td>0.1 mg/kg IBW every other day, 3 weeks</td>
<td>Hypocaloric (high carbohydrate vs high fat)</td>
<td>NS Decreased NR Increased NS</td>
</tr>
<tr>
<td>Kim et al., 1999</td>
<td>24, obese (22 women, 2 men)</td>
<td>12 weeks</td>
<td>22-46 years</td>
<td>0.18 U/kg IBW/week</td>
<td>Hypocaloric (3135 kcal/kg IBW)</td>
<td>NS Decreased (greater visceral fat loss) Increased NS NS</td>
</tr>
<tr>
<td>Norrelund et al., 2000</td>
<td>15, obese women</td>
<td>4 weeks</td>
<td>mean 36.6 years</td>
<td>0.03 IU/kg IBW-0.08 IU/kg IBW</td>
<td>Hypocaloric (3135 kJ/day)</td>
<td>NS NR NR Increased NR</td>
</tr>
<tr>
<td>Oscarsson, Lindstedt, Lundborg, &amp; Eden, 1996</td>
<td>8, overweight men</td>
<td>2 weeks</td>
<td>42-59 years</td>
<td>0.02 IU/kg/day</td>
<td>No restrictions</td>
<td>NR NR NR Increased (significance not reported) Increased (significance not reported) Increased (significance not reported)</td>
</tr>
<tr>
<td>Berneis, Vosmeer, &amp; Keller, 1996</td>
<td>30, normal weight men</td>
<td>1 week</td>
<td>mean 25.4 years</td>
<td>0.15 IU/kg twice daily + methylprednisolome</td>
<td>No restrictions</td>
<td>NR Decreased NR NR Increased</td>
</tr>
<tr>
<td>Snyder, Underwood, &amp; Clemmons, 1995</td>
<td>11, obese</td>
<td>38 days</td>
<td>25-49 (mean 39)</td>
<td>0.05 mg/kg IBW for 28 days</td>
<td>Hypocaloric (15 kcal/kg IBW)</td>
<td>Decreased (remained greater than controls) Decreased NR Increased Increased Increased</td>
</tr>
<tr>
<td>Kamel, Norgren, Elimam, Danielsson, &amp; Marcus, 2000</td>
<td>10, obese boys</td>
<td>12 months</td>
<td>10-12 years</td>
<td>0.035 mg/kg/day for 6 months</td>
<td>Median BMI decreased</td>
<td>Decreased NS NR NS NS</td>
</tr>
<tr>
<td>Thompson et al., 1998</td>
<td>33, obese women</td>
<td>12 weeks</td>
<td>mean 67.1 years</td>
<td>0.025 mg/kg BW, daily</td>
<td>Hypocaloric</td>
<td>Decreased Increased Increased Increased NR NR</td>
</tr>
<tr>
<td>Ahn et al., 2006</td>
<td>24, obese, T2DM (12 women, 12 men)</td>
<td>12 weeks</td>
<td>mean 53.7 years</td>
<td>1-1.5 U/day, 5 days/week</td>
<td>Hypocaloric</td>
<td>No change in BMI Decreased (significant visceral fat loss) Increased Increased Decreased NS</td>
</tr>
<tr>
<td>Reference</td>
<td>Subjects</td>
<td>Length of Study</td>
<td>Age of Participants</td>
<td>GH dosage</td>
<td>Diet</td>
<td>Effect of GH Injections</td>
</tr>
<tr>
<td>---------------------------</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Weight</td>
</tr>
<tr>
<td>Richelsen et al., 1994</td>
<td>9, obese women</td>
<td>5 weeks</td>
<td>24-46 years</td>
<td>0.03 mg/kg IBW daily</td>
<td>No restrictions</td>
<td>Increased</td>
</tr>
<tr>
<td>Johannsson et al., 1997</td>
<td>30, obese men</td>
<td>9 months</td>
<td>48-66 years</td>
<td>9.5 µg/kg, daily</td>
<td>No restrictions</td>
<td>No change in BMI</td>
</tr>
<tr>
<td>Snyder, Underwood, &amp;</td>
<td>8, obese women</td>
<td>15 weeks</td>
<td>18-37 years</td>
<td>0.1 mg/kg IBW, daily</td>
<td>Hypocaloric (12 kcal/kg IBW, 1 g/kg IBW)</td>
<td>NS</td>
</tr>
<tr>
<td>Richelsen et al., 2000</td>
<td>18, obese women</td>
<td>4 weeks</td>
<td>~35 years</td>
<td>0.03 IU/kg·0.08 IU/kg, daily</td>
<td>Hypocaloric (740 kcal/day)</td>
<td>NS</td>
</tr>
<tr>
<td>Sartorio et al., 2004</td>
<td>20, obese women</td>
<td>3 weeks</td>
<td>61-75 years</td>
<td>0.1 IU/kg/week, daily</td>
<td>Hypocaloric (1100-1500 kcal/day)</td>
<td>NS</td>
</tr>
<tr>
<td>Herrmann et al., 2004</td>
<td>25, obese men</td>
<td>18 months</td>
<td>mean 55 years</td>
<td>9.5 µg/kg/day + 850 mg Metformin twice daily</td>
<td>No restrictions</td>
<td>NS</td>
</tr>
<tr>
<td>Tomlinson et al., 2003</td>
<td>24, obese (11 men, 13 women)</td>
<td>8 months</td>
<td>mean 41 years</td>
<td>0.4 mg/day</td>
<td>No restrictions</td>
<td>No change in BMI</td>
</tr>
<tr>
<td>Franco et al., 2005</td>
<td>40, obese women</td>
<td>12 months</td>
<td>51-63 years</td>
<td>0.13-0.67 mg/day</td>
<td>No restrictions</td>
<td>NS</td>
</tr>
<tr>
<td>Lucidi, Parlanti, Piccioni, Santesso, &amp; De Feo, 2002</td>
<td>6, overweight, obese men</td>
<td>1 week</td>
<td>33-50 years</td>
<td>3.3 µg/kg, daily</td>
<td>No restrictions</td>
<td>NS</td>
</tr>
<tr>
<td>Skaggs &amp; Crist, 1991</td>
<td>12, obese women</td>
<td>4 weeks</td>
<td>29-50 years</td>
<td>0.08 mg/kg IBW</td>
<td>No restrictions</td>
<td>NS</td>
</tr>
<tr>
<td>Albert &amp; Moordanian, 2004</td>
<td>59, obese (44 women, 15 men)</td>
<td>6 months</td>
<td>20-45 years</td>
<td>200-600 µg/day</td>
<td>Hypocaloric</td>
<td>Decreased</td>
</tr>
<tr>
<td>Pasarica, Zachwieja, Dejonge, Redman, &amp; Smith, 2007</td>
<td>30, overweight (visceral obesity) men</td>
<td>50 weeks</td>
<td>40-70 years</td>
<td>0.95 mg, daily for 24 weeks</td>
<td>No restrictions</td>
<td>Increased</td>
</tr>
</tbody>
</table>

*Note.* Only statistically significant changes are reported. NS=No significant difference from placebo or control; NR= Not reported.
of LPL (Richelsen et al., 2000). Lipid kinetic studies have shown that treatment with GH is associated with an approximate 25% increase in the rate of lipolysis with changes in protein or carbohydrate metabolism (Lucidi, Parlanti, Piccioni, Santeusanio, & De Feo, 2002). Additional studies have also reported increases in FFA concentrations, serum glycerol levels, triglycerides and LPL activity following GH injections (Johannsson et al., 1997; Richelsen et al., 2000; Snyder, Underwood, & Clemmons, 1995).

The location of adipose tissue loss has also been examined. When daily doses of GH at 0.03 mg/kg IBW were injected into obese women, a significant amount of fat mass was lost and a significant decrease in the visceral fat (7%) was observed (Richelsen et al., 1994). A 12-week study using daily injections of GH at 0.03 IU/kg IBW and a hypocaloric diet reported a significant decrease in fat mass and a significant decrease in the visceral fat area (Kim et al., 1999). Injections of 0.33 mg GH/day and a calorie restricted diet caused significant fat mass loss and reduced visceral fat area (Ahn et al., 2006). A 6-month study using overweight males with adipose tissue localized in the abdominal region concluded that 0.95 mg GH/day for 24 weeks caused significant fat mass loss, with significant decreases in visceral adipose tissue (Pasarica, Zachwieja, Dejonge, Redman, & Smith, 2007).

Several studies have also examined the effect of diet restriction and various diet compositions. When the composition of a restricted diet was altered to contain either more lipid or more carbohydrate, it was found that GH was more responsive with the high-carbohydrate diet, leading to an increase in IGF-1 and urinary C-peptide excretion (Snyder, Clemmons, & Underwood, 1989). More recent studies have shown that GH
treatment at 0.18 U/kg IBW/wk in obese, diet restricted subjects was related to an increase in lean body mass with a decrease in visceral fat mass (Kim et al., 1999). When obese subjects were placed on a hypocaloric diet which was 500 kcal/day less than what was needed for normal weight maintenance, both weight and fat mass decreased with GH injections (Albert & Mooradian, 2004). The administration of GH over 4 weeks at 0.03 IU/kg IBW - 0.08 IU/kg IBW with dietary restriction helped to preserve protein stores when compared to controls (Norrelund et al., 2000). Additionally, an increase in lipid oxidation was observed, while the serum insulin levels remained unchanged in the GH treated group (Norrelund et al., 2000). Additional studies have shown the benefits of both a hypocaloric diet and exercise. When patients were placed on a diet of 500 kcal/day less than needed for weight maintenance and followed an exercise regimen, fat mass was significantly decreased (Thompson et al., 1998).

Additional studies have involved using GH in conjunction with other therapies. A study involving the injection of both GH and glucocorticoids found that when injected together, resting energy expenditure and serum insulin both increased when compared to the control group. Similarly, the estimated fat mass of the combination group decreased significantly during the study (Berneis, Vosmeer, & Keller, 1996). The concurrent use of GH and metformin, an insulin-sensitizing agent, decreased fat mass, but had no significant effect on glucose metabolism or lean mass (Herrmann et al., 2004).

These studies have shown varying results, sometimes with little effect on the treatment of obesity. In most cases, the change in body composition was not significant, and the report was based on the trend of data. Of the 24 studies examined, 13 reported
significant decreases in fat mass (Ahn et al., 2006; Albert & Mooradian, 2004; Berneis et al., 1996; Herrmann et al., 2004; Johannsson et al., 1997; Kamel, Norgren, Elimam, Danielsson, & Marcus, 2000; Kim et al., 1999; Pasarica et al., 2007; Richelsen et al., 1994; Skaggs & Crist, 1991; Snyder et al., 1989; Snyder et al., 1995; Thompson et al., 1998). Only six studies report either a significant increase or significant retention of lean mass (Ahn et al., 2006; Kim et al., 1999; Pasarica et al., 2007; Richelsen et al., 1994; Tagliaferri et al., 1998; Thompson et al., 1998). The amounts of GH injected (0.0033-0.95 mg/kg) as well as the length of studies (1 week to 18 months) also varied in these studies. Additionally, the level of GH used to treat patients in most cases was fairly low, suggesting that an effective dosage needs to be determined. GH, specifically hGH, is also able to bind to the prolactin (PRL) receptor (Somers, Ultsch, De Vos, & Kossiakoff, 1994). Recently, studies have shown that PRL inhibits lipolysis (Brandebourg, Bown, & Ben-Jonathan, 2007) and animals that lack PRL receptors have decreased adipose tissue (Flint, Binart, Boumard, Kopchick, & Kelly, 2006). The confounding effects of GH binding to the PRL receptor may explain the discrepancies between many of these studies.

**Mouse Models in GH, Obesity and Diabetes Research**

An important model for studying both diet-induced obesity and type 2 diabetes is the C57Bl/6J mouse. For obesity and diabetes, this model has been shown to progress through the same stages of obesity and type 2 diabetes as seen in humans. Previously, it has been reported that on a diet consisting of 20.5% protein, 35.8% fat and 36.8% carbohydrate, C57Bl/6J mice became obese and gained weight faster than a similar strain,
C57Bl/AJ (Surwit et al., 1988). When glucose and insulin were measured in these animals, the obese C57Bl/6J mice were found to be significantly more hyperglycemic and hyperinsulinemic than lean C57Bl/6J as well as lean and obese C57Bl/A/J mice (Surwit et al., 1988).

GH has been shown to affect body composition in animals with altered GH function. When mice expressing excess bGH (excess GH action), GHA (expression of the GH antagonist) and GHR -/- (GH function absent) mice were compared, bGH animals were found to have a leaner phenotype than the other two models (Berryman et al., 2004). When various adipose depots of these models were compared, the majority of the excess adipose tissue in the GHA and GHR -/- models was found in the subcutaneous depot (Berryman et al., 2004). Furthermore, when the bGH and GHR -/- models were placed on a high-fat diet, GHR -/- mice showed a greater susceptibility to gaining fat mass (Berryman et al., 2006). A figure of these animal models and their relevant phenotypes is shown in Appendix C. These effects can be explained by previous studies observing the effect of GH on both lean and adipose tissues. In mouse models with altered GH states, a HF (high-fat) diet resulted in less accumulation of adipose tissues in animals with excess GH as compared to animals with an absence of GH signaling (GHR -/-) and controls (Berryman et al., 2006). In this same group of animals, accumulation of subcutaneous adipose tissue was significantly greater in animals that lacked GH signaling, indicating a possible depot-specific effect.

Few studies have examined the effects of administration of GH to treat obesity in animal models. To measure the acute response, GH was administered to both control and
obese mice. Glucose tolerance was found to be impaired to a greater extent in the obese animals after 12 hours (Shull & Mayer, 1956).

Conclusions

Obesity and related comorbidities have become a major health concern in the United States and worldwide. The search for a viable treatment has led many to consider the use of GH. This is an attractive choice due to the lipolytic and antilipogenic effect on adipose tissue. Additionally, GH has an anabolic effect on lean muscle mass. However, GH is diabetogenic causing an insulin resistant state and decreased glucose tolerance. The treatment of obesity, a state that is high risk for developing diabetes, with a known diabetogenic hormone needs to be further evaluated.

Previous studies have shown a possible benefit to treatment with GH. However, the dosages administered have not all shown significant effects. A dosage gradient administered to an obese animal model would help to identify an optimal GH dose as well as possible complications that accompany this treatment.
CHAPTER 3: MATERIALS AND METHODS

Previous studies have shown the potential of GH as a therapeutic treatment of obesity. In this study, various dosages of GH were used to evaluate its effectiveness on improving body composition of obese mice. Measurements of physiological parameters such as glucose, insulin and IGF-1 levels as well as organ weights will help to determine possible harmful effects of this type of treatment.

Animals

Mice in the C57Bl/6J background were used for this study. Previous studies have characterized this model as useful for the study of diet-induced obesity and diabetes due to their human-like progression from obesity, insulin resistance and type 2 diabetes when fed a high-fat diet (Surwit et al., 1988). A total of 49 male C57/Bl6J mice at 21 days of age were received from Jackson Laboratories (Bar Harbor, ME) and divided into two different experimental groups. The first group, consisting of 42 mice, was placed on a high-fat (HF) diet produced by Research Diets Inc. (New Brunswick, NJ), while the second group, consisting of the remaining 8 mice, was placed on a standard laboratory rodent chow (ProLab RMH 3000). The HF diet supplied 20% of total kilocalories from carbohydrates, 20% kilocalories from protein and 60% kilocalories from fat. The standard low-fat (LF) diet supplied 60% of total kilocalories from carbohydrates, 26% of kilocalories from protein and 14% of kilocalories from fat.

Mice were housed 2-3 per cage and food and water were supplied ad libitum. The cages were kept in a temperature- and humidity-controlled room and exposed to a 12-hour light/dark cycle. All procedures were approved by the Ohio University Institutional
Animal Care and Use Committee and fully complied with federal, state and local policies.

**Growth Hormone Preparation**

Bovine growth hormone (recombinant bovine somatotropin, rBST) was received as a generous gift from the Monsanto Company (St. Louis, MO). The GH was first dissolved in 1% acetic acid solution to a concentration of 10 mg/mL. This solution was then added to filtered and sterilized phosphate buffered saline (PBS) to a final concentration of 500 μg/mL. Six mL were aliquoted into conical tubes and frozen at -80º C until use.

Prior to injections, 6 mL of GH were placed in a 37º C water bath. The remaining doses of GH were completed using serial dilutions of 1:10 of the 500 μg/mL solution. Sterilized 1X PBS was used to complete all dilutions and for use as a control. A total of four doses were freshly prepared each day to the following concentrations: 500 μg/mL, 50 μg/mL, 5.0 μg/mL and 0.5 μg/mL.

**GH Injections**

The first group of mice (n=42) was split into five different groups of 8-9 mice after 16 weeks of HF diet treatment. Weight measurements and glucose measurements prior to the assignment were used to ensure that the groups did not differ from one another. Each group was assigned to one of four GH injection concentrations or the PBS control group. One set of eight mice were placed in the 1X PBS saline control group. The remaining groups were arranged as follows to receive various dosages of bGH daily based on body weight: 8 mice received 0.005 μg/g; 9 mice, 0.05 μg/g; 8 mice, 0.5 μg/g;
and 9 mice, 5.0 µg/g. The second group, consisting of 8 mice on a LF diet, was also used as controls. All mice in this group were placed in the 1X PBS saline-injected group. A summary of the assignment of mice to treatment groups is shown in Table 2.

Table 2

*Assignment of Mice to GH Injection Groups*

<table>
<thead>
<tr>
<th>Diet</th>
<th>LF</th>
<th>HF</th>
<th>HF</th>
<th>HF</th>
<th>HF</th>
<th>HF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Mice</td>
<td>7</td>
<td>8</td>
<td>8</td>
<td>9</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>GH Dose</td>
<td>1X PBS (0.01 mL/g)</td>
<td>1X PBS (0.01 mL/g)</td>
<td>0.005 µg/g</td>
<td>0.05 µg/g</td>
<td>0.5 µg/g</td>
<td>5.0 µg/g</td>
</tr>
</tbody>
</table>

Subcutaneous GH injections were performed daily for six weeks using the assigned GH dose for each group. An amount of 0.01 mL/g body weight was injected and the most current body weights were used. Injection sites were rotated weekly between the neck and the lower back to minimize possible exaggerated local effects and skin irritations. The injections occurred at 3:00 PM ± 3 hours daily.

Weight and Body Composition Measurements

Total body mass was assessed weekly throughout the study using a standard scale to the tenth of a gram. After 16 weeks on either a HF or LF diet, weekly body composition measurements were performed using the Bruker Minispec (The Woodlands,
This machine uses NMR technology to assess the total fat mass, lean mass, and fluid mass in grams of the animals (Barac-Nieto & Gupta, 1996). The measurements were performed at the same time each week, reducing potential variations caused by the daily injections. Total lean mass and weight were used to calculate percent lean mass. Total fat mass and weight were also used to calculate the percent fat mass. The total accumulation of lean mass and fat mass was assessed beginning with the second week of measurements. A summary of all measurements and time points is represented in Figure 1.

Phase 1 (not shown): Induce obesity and type 2 diabetes
• Place 50 mice on a HF diet for 16 weeks
• Measure weights weekly

Phase 2: GH treatment
• Daily GH injections
• Measure weights, body composition and food consumption weekly
• Glucose (G), Insulin (I) and IGF-1 measured periodically

Figure 1. Timeline, in weeks, and summary of phase 1 and phase 2 of the GH injection study.
Food Intake Measurements

Total food intake/cage was assessed weekly throughout the study by subtracting the total grams of food remaining in the cage from the total grams of food added the previous week. These measurements are reported as the average number of kilocalories consumed by each animal in each experimental group. Food was measured on a standard scale.

Blood Glucose Measurements and Collection of Plasma

Blood glucose measurement and plasma collection began after 16 weeks of diet treatment and continued every other week to the end of the study. The measurements and collections were completed between 9:00 a.m. and 12:00 p.m. Prior to glucose measurements and collection, the mice were fasted for 12 hours. An infrared heating lamp was used to enhance blood collection. To collect blood, the tip of the tail was removed and blood glucose measurements were taken using a LifeScan OneTouch glucometer (Milpitas, CA) with the first drop of blood. Immediately after the blood glucose measurements, blood was collected using Chase Natelson heparanized capillary tubes (VWR International, Bridgeport NJ) and stored on ice. It was then spun at 4° C for 10 minutes at 7,000 x g, to prevent lysis of the red blood cells. Plasma was collected and stored at -80° C until further analysis.

Glucose measurements were also taken in triplicate after the third week (19 weeks of age) of injections and continued every other week to the end of the study. The mice were not placed under an infrared lamp in order to reduce stress levels. The tip of the tail
was cut and the first three drops of blood were used for plasma glucose measurements using a LifeScan OneTouch Glucometer.

**Plasma Measurements**

Several plasma metabolite concentrations associated with diabetes, GH and obesity were measured using ELISA (enzyme linked immunosorbent assay) kits. Free IGF-1 concentration was measured using the Free IGF-1: DSL-10-9400, Coated Well ELISA (Diagnostic Systems Laboratories, Webster TX). Insulin concentrations were determined using the ultra sensitive mouse Insulin ELISA kit (ALPCO Diagnostics, Windham, NH) as per the instructions of the manufacturer.

**Glucose Tolerance Test**

Glucose tolerance tests were performed during the final week (week 7) of the injections. Mice were fasted for 12 hours prior to the measurements, which began at 9:00 a.m. For all mice, an interperitoneal injection of a 25% glucose solution was administered at 0.01 mL/g body weight. Glucose measurements were performed in duplicate using a OneTouch LifeScan Glucometer beginning before the injection and continuing 60-, 120-, 180-, 240- and 360-minutes after the injection.

**Tissue Collection**

Animals were sacrificed by cervical dislocation after 22 total weeks of diet treatment and 6 weeks of daily GH injections. The following tissues were collected: skin, inguinal adipose tissue, epididymal adipose tissue, retroperitoneal adipose tissue, mesenteric adipose tissue, kidney, heart, liver, muscle, pancreas and bone. All tissues
collected were weighed using a standard laboratory scale and flash frozen in cryogenic vials using liquid nitrogen. They were then stored at -80° C until further use.

**Statistical Analysis**

Statistics were performed on all body composition measurements, glucose measurements, insulin and IGF-1 concentrations, and tissue weights using SPSS version 14.0 software (Chicago, IL). Groups are reported as LF-PBS (LF diet, PBS injected), HF-PBS (HF diet, PBS injected), HF-0.005 (HF diet, 0.005 µg GH/g BW injected), HF-0.05 (HF diet, 0.05 µg GH/g BW injected), HF-0.5 (HF diet, 0.5 µg GH/g BW injected) and HF-5 (HF diet, 5 µg GH/g BW injected). Tissue weights were analyzed using analysis of variance (ANOVA) and group means are reported. The remaining measurements were analyzed using repeated measures analysis to determine variances within groups. Group means were then be compared using ANOVA and planned contrasts were used to determine statistical significance between groups. A value of $p<0.05$ was regarded as statistically significant.
CHAPTER 4: RESULTS

Weight Gain

The weight of mice on a HF diet increased as compared to controls on a LF diet. When placed on a HF diet, C57Bl/6J mice had increased body weight that became exaggerated over time compared to mice fed a LF diet (see Figure 2). During phase 2 of the study, the weights of all mice on a HF diet, regardless of GH dosage, remained greater than the LF-PBS group (see Figure 2).

Weight Accumulation

The weight change over the course of phase 2 (GH injections) of the study varied within treatment groups. Weight accumulation data from all time points in phase 2 of the study (GH injections) is shown in Figure 3. After 1 week of injections, there was no significant difference in weight change between groups. After 2 weeks of injections, the HF-5 group had a significant decrease in weight as compared to the HF-PBS and the HF-0.05 groups ($F(5,43)=3.546, p>.05$). After 3 weeks of injections, the weight increase of the HF-0.5 group was significantly less than the HF-0.05 group and the weight decrease of the HF-5 group was significantly different than all groups ($F(5,43)=10.521, p>.05$). After 4 weeks of injections, the weight decrease of the HF-5 group was significantly different from the HF-PBS, HF-0.005 and HF-0.05 groups ($F(5,43)=11.477, p>.05$). Similarly, the weight increase in HF-PBS and HF-0.05 groups was not significantly different from the HF-0.005 group, but was significantly different from the remaining treatment groups ($F(5,43)=11.477, p>.05$). After 5 weeks of treatment, the weight gain
Figure 2. Weight (grams) measurements of mice on a HF or LF diet injected with PBS, 0.005 μg GH/g BW, 0.05 μg GH/g BW, 0.5 μg GH/g BW or 5 μg GH/g BW during phase 1 and phase 2 of the study. Data are expressed as mean ± SEM.
**Figure 3.** Weight accumulation (grams) of mice on a HF or LF diet injected with PBS, 0.005 µg GH/g BW, 0.05 µg GH/g BW, 0.5 µg GH/g BW or 5 µg GH/g BW during phase 2 of the study. Means within each week with a common letter do not differ, $p > .05$. Data are expressed as mean ± SEM.
of the LF-PBS, HF-0.5 and HF-5 groups were significantly less than the HF-PBS, HF-0.005 and HF-0.05 groups ($F(5,43)=12.068, p>.05$). At the conclusion of the study, after 6 weeks of injections, the overall weight gain was significantly less in the LF-PBS and HF-5 groups compared to HF-PBS, HF-0.005 and HF-0.05 groups ($F(5,43)=13.761, p>.05$). The HF-0.5 group had significantly less weight accumulation than both the HF-PBS and HF-0.05 groups ($F(5,43)=13.761, p>.05$). The total weight accumulation after 6 weeks of injections was 0.06 ± 0.34 g in the LF-PBS, 4.75 ± 0.53 g in the HF-PBS, 3.69 ± 0.50 g in the HF-0.005, 4.12 ± 0.67 g in the HF-0.05, 1.46 ± 0.57 g in the HF-0.5 and 0.43 ± 0.66 g in the HF-5 groups (Figure 3). Using the HF-PBS as the standard control group, the total weight accumulation after 6 weeks of injections for the LF-PBS, HF-0.005, HF-0.05, HF-0.5 and HF-5 groups were 1.2%, 77.8%, 86.8%, 30.7% and 9.0%, respectively.

**Fat Mass Accumulation**

The accumulation of fat mass over the course of phase 2 of the study was altered with GH injections. Fat accumulation data from all time points in phase 2 are shown in Figure 4. After one week of injections, the increase in fat mass of the LF-PBS, HF-PBS, HF-0.005 and HF-0.05 groups did not significantly differ from one another. The fat accumulation in the HF-0.5 group did not differ from the LF-PBS group, but was significantly less than the HF-PBS, HF-0.005 and HF-0.05 groups and significantly greater than the HF-5 group. The HF-5 group had a significant decrease in fat accumulation as compared to all other treatment groups ($F(5,43)=6.905, p>.05$). After 2 weeks of injections, the accumulated fat mass of the HF-0.5 group was significantly less
Figure 4. Fat mass accumulation (grams) of mice on a HF or LF diet injected with PBS, 0.005 μg GH/g BW, 0.05 μg GH/g BW, 0.5 μg GH/g BW or 5 μg GH/g BW during phase 2 of the study. Means within each week with a common letter do not differ, p > .05. Data are expressed as mean ± SEM.
than the HF-PBS and HF-0.005 groups, and again, the accumulated fat mass of the HF-5 group was significantly less than all other treatment groups ($F(5,43)=31.325, p>.05$).

After 3 weeks of injections, the HF-0.5 group had significantly less fat mass accumulation than the HF-PBS, HF-0.005 and HF-0.05 groups. The HF-5 group had significantly decreased fat mass accumulation compared to all other treatment groups ($F(5,43)=40.667, p>.05$). After 4 and 5 weeks of injection, fat accumulation of the HF-0.5 group was significantly less than the HF-PBS, HF-0.005 and HF-0.05 groups. The HF-PBS injected group had significantly greater fat mass accumulation than the LF-PBS, HF-0.5 and HF-5 groups. As before, the HF-5 group continued to have significantly less fat mass accumulation compared to all other treatment groups ($F(5,43)=42.770, p>.05$).

By 6 weeks of injections, the LF-PBS and HF-0.5 groups had significantly less accumulated fat mass than the HF-PBS, HF-0.005 and HF-0.05 groups. The HF-5 group had significantly decreased fat mass compared to all other treatment groups ($F(5,43)=32.516, p>.05$). The total fat mass accumulation after 6 weeks of injections was 0.47 ± 0.09 g in the LF-PBS, 3.65 ± 0.40 g in the HF-PBS, 3.1 ± 0.42 g in the HF-0.005, 2.87 ± 0.49 g in the HF-0.05, 0.20 ± 0.66 g in the HF-0.5 and -4.09 ± 0.81 g in the HF-5 groups (see Figure 4). Using the HF-PBS as the comparison group, the total fat accumulation after six weeks of injections for the LF-PBS, HF-0.005, HF-0.05, HF-0.5 and HF-5 groups were 12.9%, 84.9%, 78.7%, 5.5% and -112.0%, respectively.

**Lean Mass Accumulation**

The lean mass accumulation over the course of phase 2 of the study was altered with GH treatment. Lean mass accumulation data from phase 2 of the study is shown for
all time points in Figure 5. After 1 week of injection treatment the lean mass
cumulation of the HF-0.5 group was significantly greater than the LF-PBS, HF-PBS,
HF-0.005 and HF-0.05 groups. The accumulation of lean mass in the HF-5 group was
significantly greater than all other treatment groups ($F(5,43)=26.253, p>.05$) and this
significant increase was maintained throughout the study ($F(5,43)=41.076, p>.05$),
($F(5,43)=56.361, p>.05$), ($F(5,43)=58.552, p>.05$), ($F(5,43)=33.262, p>.05$),
($F(5,43)=46.620, p>.05$). After 2 weeks of injection treatment, the accumulation of lean
mass in the LF-PBS injected group was significantly less than the HF-0.05, HF-0.5 and
HF-5 groups. The lean mass accumulation was significantly greater in the HF-0.5 group
than the LF-PBS, HF-PBS and HF-0.005 groups ($F(5,43)=41.076, p>.05$). After 3 weeks
of injection treatment, the accumulation of lean mass was significantly less in the LF-
PBS injected group than the HF-0.05, HF-0.5 and HF-5 groups. The lean mass
accumulation in the HF-0.5 group was significantly greater than the LF-PBS, HF-PBS
and HF-0.005 groups ($F(5,43)=56.361, p>.05$). After 4 weeks of injections, the
accumulation of lean mass in the LF-PBS injected group was significantly less than the
HF-PBS, HF-0.05, HF-0.5 and HF-5 groups. The HF-0.5 group gained significantly
more lean mass than the LF-PBS and HF-0.005 groups ($F(5,43)=58.552, p>.05$). After 5
weeks of injections, the LF-PBS group gained significantly less weight than the HF-0.5
and HF-5 groups. The HF-PBS, HF-0.005 and HF-0.05 groups did not differ
significantly in lean mass accumulation from either the LF-PBS or the HF-0.5 groups
($F(5,43)=33.262, p>.05$). After 6 weeks of injections, the LF-PBS injection group lost
significantly more lean muscle mass than all other treatment groups. The HF-PBS,
Figure 5. Lean mass accumulation (grams) of mice on a HF or LF diet injected with PBS, 0.005 µg GH/g BW, 0.05 µg GH/g BW, 0.5 µg GH/g BW or 5 µg GH/g BW during phase 2 of the study. Means within each week with a common letter do not differ, \( p > .05 \). Data are expressed as mean ± SEM.
HF-0.005, HF-0.05 and HF-0.5 groups did not differ significantly from one another \((F(5,43)=46.620, p>.05)\). The total lean mass accumulation after 6 weeks of injections was \(-1.35 \pm 0.22 \text{ g}\) in the LF-PBS, \(0.03 \pm 0.29 \text{ g}\) in the HF-PBS, \(0.02 \pm 0.12 \text{ g}\) in the HF-0.005, \(0.73 \pm 0.28 \text{ g}\) in the HF-0.05, \(0.87 \pm 0.29 \text{ g}\) in the HF-0.5 and \(3.87 \pm 0.36 \text{ g}\) in the HF-5 groups (see Figure 5).

**Blood Glucose Levels**

Blood glucose levels during phase 2 of the study were altered significantly upon treatment with GH. After 3 weeks of GH injections (data not shown), the LF-PBS injected group had significantly decreased blood glucose levels than all other treatment groups. The blood glucose levels of the HF-PBS, HF-0.005, HF-0.05 and HF-0.5 groups did not differ significantly from one another. The HF-5 group had significantly decreased blood glucose levels from the HF-PBS, HF-0.005, HF-0.05 and HF-0.5 groups and significantly greater blood glucose than the LF-PBS treatment group \((F(5,43)=34.160, p>.05)\). After 4.5 weeks of injections (data not shown), the LF-PBS and HF-5 groups did not differ significantly from one another and had significantly lower glucose levels than the other treatment groups. The HF-PBS, HF-0.005 and HF-0.05 groups had significantly greater blood glucose levels than the LF-PBS, HF-0.5 and HF-5 groups. The HF-0.5 group had significantly lower blood glucose levels compared to the HF-PBS, HF-0.005 and HF-0.05 groups and significantly greater blood glucose levels than the LF-PBS and HF-5 groups \((F(5,43)=34.074, p>.05)\). After 6 weeks of injections (see Figure 6), both the LF-PBS and HF-5 groups had significantly lower blood glucose levels than the other treatment groups and did not differ significantly from one another.
**Figure 6.** Week 6 blood glucose measurements of mice (mg/dL) on a HF or LF diet injected with PBS, 0.005 µg GH/g BW, 0.05 µg GH/g BW, 0.5 µg GH/g BW or 5 µg GH/g BW during phase 2 of the study. Means shown with a common letter do not differ, \( p < .05 \). Data are expressed as mean ± SEM.
The HF-0.5 group had significantly lower blood glucose than the HF-PBS and HF-0.05 groups and significantly higher blood glucose levels than the LF-PBS and HF-5 groups. The HF-PBS, HF-0.005 and HF-0.05 groups did not differ significantly from one another (F(5,43)=24.719, p>.05). After 6 weeks of injections, the blood glucose levels were 121 ± 7.9 mg/dL in the LF-PBS, 194 ± 5.0 mg/dL in the HF-PBS, 181 ± 6.8 mg/dL in the HF-0.005, 195 ± 5.6 mg/dL in the HF-0.05, 166 ± 4.3 mg/dL in the HF-0.5 and 137 ± 8.2 mg/dL in the HF-5 groups (see Figure 6).

**Serum Insulin Levels**

Serum insulin levels during phase 2 of the study remained unchanged upon treatment with GH. Prior to beginning GH injections, the insulin levels of the LF-PBS treated group were significantly lower than all other treatment groups (data not shown). The serum insulin levels of the HF-PBS, HF-0.005, HF-0.05, HF-0.5 and HF-5 groups were not significantly different from one another (F(5,43)=5.595, p>.05). After 2 weeks of injection treatment, the LF-PBS injected group had significantly lower serum insulin levels than the HF-0.005 and HF-0.05 treatment groups (data not shown) (F(5,43)=3.538, p>.05). After 4 weeks of injections, the LF-PBS injected group had significantly lower serum insulin levels than the HF-PBS, HF-0.005, HF-0.05 and HF-0.5 treatment groups (F(5,43)=3.835, p>.05) (data not shown). After 6 weeks of injections, the LF-PBS injected group had significantly lower plasma insulin levels than the HF-PBS, HF-0.005, HF-0.05 and HF-5 groups (see Figure 7). The HF-PBS, HF-0.005, HF-0.05, HF-0.5 and HF-5 treatment groups did not differ significantly from one another (F(5,43)=4.891, p>.05). After 6 weeks of injections the serum insulin levels were
Figure 7. Week 6 serum insulin measurements (ng/dL) of mice on a HF or LF diet injected with PBS, 0.005 µg GH/g BW, 0.05 µg GH/g BW, 0.5 µg GH/g BW or 5 µg GH/g BW during phase 2 of the study. Means shown with a common letter do not differ, $p>.05$. Data are expressed as mean ± SEM.
0.65 ± 0.04 ng/mL for the LF-PBS, 3.42 ± 0.40 ng/mL for the HF-PBS, 2.87 ± 0.37 ng/mL for the HF-0.005, 3.47 ± 0.53 ng/mL for the HF-0.05, 2.56 ± 0.57 ng/mL for the HF-0.5 and 2.69 ± 0.57 ng/mL for the HF-5 groups (see Figure 7).

Serum IGF-1 Levels

Serum IGF-1 levels during phase 2 of the study were altered significantly upon treatment with GH. The serum IGF-1 levels prior to beginning GH injections were significantly less in the LF-PBS (217.9 ± 12.5 ng/mL) injected group compared to HF-PBS (294.6 ± 7.2 ng/mL), HF-0.005 (374.2 ± 14.1 ng/mL), HF-0.05 (353.6 ± 18.5 ng/mL) and HF-5 (307.3 ± 19.5 ng/mL) groups. The serum IGF-1 levels of the HF-0.005 group were significantly greater than the LF-PBS, HF-PBS and HF-0.5 groups. The HF-0.05 group had significantly greater serum IGF-1 levels than the LF-PBS and HF-0.5 groups (F(5,43)=10.950, p>.05). After 2 weeks of GH treatment, the LF-PBS injected group had significantly lower serum IGF-1 levels than the HF-PBS, HF-0.005, HF-0.05 and HF-5 groups (data not shown). The HF-0.5 group had significantly lower serum IGF-1 levels than the HF-0.005 and HF-0.05 groups. The HF-5 group had significantly greater serum IGF-1 levels than all other treatment groups (F(5,43)=26.635, p>.05) and this was maintained throughout the study (F(5,43)=22.260, p>.05), (F(5,43)=27.373, p>.05). The LF-PBS, HF-PBS, HF-0.005, HF-0.05 and HF-0.5 groups did not differ significantly from one another (data not shown) (F(5,43)=22.260, p>.05). After 6 weeks of injections (see Figure 8), the serum IGF-1 levels of the LF-PBS injected group were significantly lower than the HF-PBS, HF-0.005, HF-0.05 and HF-5 groups (F(5,43)=27.373, p>.05). After 6 weeks of injections the serum IGF-1 levels were
Figure 8. Week 6 serum IGF-1 measurements (ng/dL) of mice on a HF or LF diet injected with PBS, 0.005 µg GH/g BW, 0.05 µg GH/g BW, 0.5 µg GH/g BW or 5 µg GH/g BW during phase 2 of the study. Means shown with a common letter do not differ, $p > .05$. Data are expressed as mean ± SEM.
184.5 ± 14.4 ng/mL for the LF-PBS, 269.5 ± 17.5 ng/mL for the HF-PBS, 270.7 ± 10.1 ng/mL for the HF-0.005, 257.1 ± 15.0 ng/mL for the HF-0.05, 253.0 ± 16.0 ng/mL for the HF-0.5 and 435.3 ± 24.2 for the HF-5 groups (see Figure 8).

**Organ Weights**

The weight of four adipose depots was altered significantly upon treatment with GH. Mean organ weights are shown in Table 3. The weights of heart ($F(5,43)=1.644, p>.05$), liver ($F(5,43)=1.560, p>.05$) and spleen ($F(5,43)=1.721, p>.05$) were not altered among any of the treatment groups. The kidney weight of the HF-0.005 group was significantly less than the HF-5 group; however, these groups were not significantly different from the remaining treatment groups ($F(5,43)=2.914, p>.05$). The weight of the inguinal (subcutaneous) adipose depot (see Figure 9) was decreased significantly in the LF-PBS and HF-5 groups as compared to the HF-PBS, HF-0.005, HF-0.05 and HF-0.5 groups ($F(5,43)=17.490, p>.05$). Using the HF-PBS group as the standard control group, the percent change in weight of the inguinal adipose tissue of the HF-0.005, HF-0.05, HF-0.5 and HF-5 groups were 6.25%, 7.35%, -14.25% and -59.36%, respectively. The weight of the epididymal adipose depot (see Figure 9) was significantly decreased in the LF-PBS injected group compared to all other treatment groups. The HF-5 group had a significantly smaller epididymal adipose depot compared to the HF-PBS, HF-0.005 and HF-0.05 groups ($F(5,43)=26.933, p>.05$). Using the HF-PBS group as the standard control group, the percent change in weight of the epididymal adipose tissue of the HF-0.005, HF-0.05, HF-0.5 and HF-5 groups were -9.12%, -7.17%, -12.27% and -35.81%, respectively.
Table 3

Organ Weight Measurements (grams) of Mice on a HF or LF Diet Injected with PBS, 0.005 µg GH/g BW, 0.05 µg GH/g BW, 0.5 µg GH/g BW or 5 µg GH/g BW

<table>
<thead>
<tr>
<th>Diet</th>
<th>Injection</th>
<th>Heart</th>
<th>Liver</th>
<th>Kidney</th>
<th>Spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td>LF</td>
<td>PBS</td>
<td>0.14 ± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.49 ± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.34 ± 0.02&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>0.19 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>HF</td>
<td>PBS</td>
<td>0.15 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.64 ± 0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.36 ± 0.01&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>0.19 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>HF</td>
<td>0.005 µg/g BW</td>
<td>0.17 ± 0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.55 ± 0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.31 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.19 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>HF</td>
<td>0.05 µg/g BW</td>
<td>0.14 ± 0.005&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.53 ± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.33 ± 0.01&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>0.19 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>HF</td>
<td>0.5 µg/g BW</td>
<td>0.15 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.24 ± 0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.33 ± 0.02&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>0.24 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>HF</td>
<td>5.0 µg/g BW</td>
<td>0.18 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.44 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.38 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.26 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Note. Means within each tissue group shown with a common letter do not differ, p > .05.

Data are expressed as mean ± SEM.
Figure 9. Mean weights (gram) of four adipose depots of mice on a LF or HF diet injected with PBS, 0.005 µg GH/g BW, 0.05 µg GH/g BW, 0.5 µg GH/g BW or 5.0 µg GH/g BW during phase 2 of the study. Means within each tissue group shown with a common letter do not differ, \( p > .05 \). Data are expressed as mean ± SEM.
respectively. The weight of the retroperitoneal adipose depot (see Figure 9) in the LF-PBS injection group was significantly smaller than the HF-PBS, HF-0.005, HF-0.05, and HF-0.5 groups. The HF-5 group had a significantly smaller retroperitoneal adipose depot than the HF-PBS, HF-0.005 and HF-0.05 groups ($F(5,43)=16.663, p>.05$). Using the HF-PBS group as the standard control group, the percent change in weight of the retroperitoneal adipose tissue of the HF-0.005, HF-0.05, HF-0.5 and HF-5 groups were -15.73%, -8.65%, -24.82% and -47.29%, respectively. The weight of the mesenteric adipose depot (see Figure 9) in the LF-PBS injection group was significantly smaller than the HF-PBS, HF-0.005, HF-0.05 and HF-0.5 groups. The mesenteric adipose depot in the HF-5 group was significantly smaller than the HF-PBS, HF-0.005 and HF-0.05 groups ($F(5,43)=12.959, p>.05$). Using the HF-PBS group as the standard control group, the percent change in weight of the mesenteric adipose tissue of the HF-0.005, HF-0.05, HF-0.5 and HF-5 groups were -7.64%, -0.44%, -28.40% and -51.02%, respectively.
CHAPTER 5: DISCUSSION

The increasing incidence of obesity and related complications has prompted intensive research into prevention and treatment of this condition, now considered to be a major epidemic. GH has become a logical target for therapy because of its effects on body composition, namely increasing lean mass and decreasing adipose tissue. However, the possible accompanying diabetogenic effects of GH may further enhance the complications associated with obesity. In this study, mice were placed on a HF or LF diet and then injected with saline or increasing levels of bGH. Body composition, weight and several metabolic parameters were measured periodically to assess the effectiveness of GH on the treatment of obesity. In terms of body composition, obese mice injected with the highest dose of GH (5 µg/g BW) had the greatest increase in lean mass and the greatest decrease in fat mass. Remarkably, these animals did not differ significantly in weight change from the LF-PBS controls after 6 weeks of GH treatment and had virtually no change in overall body weight while continuing on a HF diet. Additionally, the greatest impact on decreasing fat mass and increasing lean mass was observed with the highest dose of GH. Furthermore, the highest dose of GH improved blood glucose levels to near LF control levels, despite the known diabetogenic effect of GH, but was unable to return serum insulin to control levels suggesting some level of insulin resistance.

Recently, GH has been used as a potential therapeutic agent to treat obesity. Human studies have reported mixed results as to its effectiveness in reducing fat mass (Richelsen et al., 1994; Richelsen et al., 2000; Sartorio et al., 2004; Skaggs & Crist, 1991; Snyder, Underwood, & Clemmons, 1990; Tagliaferri et al., 1998). In calorically-
restricted patients receiving GH, there appears to be no effect on the loss of fat mass; however, the loss of lean mass is attenuated (Clemmons et al., 1987). Additional studies have shown small reductions in fat mass and gains or retention of lean mass in obese patients (Berneis et al., 1996; Kim et al., 1999; Norrelund et al., 2000). Few animal studies have been conducted assessing the effectiveness of GH on obesity, increasing concern over the safety of using this treatment in humans. GH is a known diabetogenic agent, having well documented anti-insulin effects. The use of this type of compound in obese, possibly insulin resistant or diabetic patients may cause additional diabetic complications.

Diet is an important variable when conducting studies on obesity. In most human studies involving GH injections, neither the caloric intake nor the caloric macronutrient content of patients is well controlled (Franco et al., 2005; Herrmann et al., 2004; Tomlinson et al., 2003). Additionally, in human studies the patient’s adherence to a specific diet is more difficult to control. In the present study, animals were placed on either a HF or a LF diet where diet composition and macronutrient content was controlled. The injection of GH, especially in the highest dose, caused a loss in fat mass and increase in lean mass even as these animals were maintained on a HF diet. The lower doses of GH had little to no effect as compared to the highest dose administered. As HF diets were used for all animals treated with GH, additional studies may provide insight as to the effectiveness of these low doses of GH in combination with a LF or reduced calorie diet.
GH treatment also caused changes in body composition. In the HF-PBS group, there was an accumulation of fat mass with no apparent change in lean mass leading to an overall increase in body weight. As the amount of GH injected increased, the loss of fat mass also increased as well as the accumulation of lean mass. The lowest doses of GH (HF-0.005 and HF-0.05) did not appear to effect body composition as these groups followed the same trend as the HF-PBS group. In the HF-0.5 group, there was no apparent gain or loss of fat mass with an overall increase in the amount of lean mass. The highest dose of GH (HF-5) showed a trend approaching no change in overall body weight. This dose of GH had the greatest effect on overall body composition. In this group, the loss of fat mass was accompanied by a dramatic increase in lean mass to almost the same degree, a trend that would be favorable for the treatment of obesity. This positive impact on lean and fat mass with GH treatment has been observed previously (Berneis et al., 1996; Kim et al., 1999; Norrelund et al., 2000). There was no change in overall body weight due to this complete reversal in body composition. Therefore, measuring body weight as opposed to body composition may not provide a clear picture of the effectiveness of GH on reducing fat mass.

The effectiveness of GH on both adipose tissue and lean tissue also seemed to vary with time. During the first 3 weeks of injections, the HF-5 group lost an average of 4.4 grams of fat mass. However, during the last 3 weeks of injections, there was no additional loss in fat mass. The lean mass accumulation appeared to be more consistent over the 6 weeks of injections. The HF-5 group continued to gain lean mass consistently over phase 2 of the study. After 3 weeks of injections, this group had gained 2.6 grams
of lean mass and after 6 weeks had gained 3.9 grams of lean mass. These results indicate that the injection of GH appears to have a more immediate impact on adipose tissue and lipolysis that diminished over time. The effect on lean mass does not appear to be as dramatic; however, the effect was consistent throughout the injections and may have continued to increase if the injections continued for longer than 6 weeks. Additional studies may provide additional information as to how long the anabolic effect on lean mass will continue and whether the catabolic effect on adipose tissue would return.

High GH levels usually result in high IGF-1 levels. The levels of GH in this study were related to the levels of IGF-1, with higher doses of GH causing increases in IGF-1. The HF-5 group had significantly higher IGF-1 levels than all other treatment groups, beginning at week 2 of phase 2 and continuing throughout the study. Interestingly, the animals on the HF diet had significantly higher IGF-1 levels than the LF-control group at the start of phase 2, suggesting that obesity or the intake of a high fat diet may contribute to increased IGF-1 levels. With the exception of week 4 during phase 2, the IGF-1 levels of the HF animals remained higher than those of the LF control group. It has been reported previously that increases in IGF-1 occur in an obese state, more specifically in the agouti mouse model (Martin et al., 2006). GH levels in these mice also tend to be increased, but the levels were not significant. The hyperinsulinemic state of the animals in this study may also contribute to the increased IGF-1 levels in the HF animals. Studies have shown that increased insulin levels are associated with increased IGF-1 gene transcription (Kaytor, Zhu, Pao, & Phillips, 2001; Phillips, Harp, Goldstein, Klein, & Pao, 1990). Additionally, studies in mice have shown an increase in
IGF-1 levels in animals fed a high fat, high carbohydrate diet (Venkateswaran et al., 2007). However, in humans both IGF-1 and GH levels have been reported to decrease with obesity (De Marinis et al., 2004; Eden Engstrom et al., 2006; Rasmussen et al., 1995; Rasmussen, Juul, Kjems, & Hilsted, 2006). The discrepancies between these reports and the connection of IGF-1 levels with high fat intake and obesity warrant further investigation.

The diabetogenic and antiinsulin effects make GH a less than ideal therapeutic agent for the treatment of obesity. To assess the progression of diabetes, glucose and insulin measurements were assessed every other week during phase 2 of the study. Additionally, triplicate glucose measurements were taken at various time points and a glucose tolerance test was performed at the end of the study. Glucose levels overall decreased as the amount of GH injected increased. Additionally, the HF-5 group had decreased blood glucose such that after 4.5 weeks of GH treatment their blood glucose levels did not significantly differ from those of the LF-PBS group. This was maintained throughout the conclusion of phase 2 of the study. The HF-0.5 group also showed a significant decrease in blood glucose compared to the HF-PBS group after 4.5 weeks; however, the levels were still significantly higher than the LF-PBS group. These decreases in blood glucose levels suggest that the loss of overall fat mass is able to overcome the diabetogenic effects of GH. Serum insulin measurements were also assessed to measure the diabetic status of the animals. Insulin levels were found to be higher in all HF groups when compared to the LF-PBS group. Specifically, in the HF-0.5 and HF-5 groups, which both had decreased blood glucose, the insulin levels were not
found to be different from the HF-PBS group. This indicates that while the blood glucose levels approached normal levels, the animals remained hyperinsulinemic and insulin resistant. While the diabetogenic effect of GH may have been overcome by the decrease in fat mass, the anti-insulin effects of GH may cause the hyperinsulinemic state to persist, suggesting that these properties of GH are distinct and can be separated from one another.

GH has been shown previously to influence organ size and weight. In addition, GH appears to have depot-specific effect on adipose tissue, with previous studies showing a decrease in the size of the subcutaneous, retroperitoneal and epididymal adipose depots in mice expressing excess GH (Berryman et al., 2004; Berryman et al., 2006). Models with reduced GH signaling (GH antagonist) and lack of GH signaling (GH receptor knockout) have increased adipose tissue, with the majority located in the subcutaneous region, suggesting that this depot may be more responsive to the absence of GH (Berryman et al., 2004; Berryman et al., 2006). Taking into account the differences in size between these animals, it appears that the depots affected by GH, in order from most to least responsive are subcutaneous (inguinal), retroperitoneal and epididymal, with no data provided on the size of the mesenteric depot (Berryman et al., 2004; Berryman et al., 2006). Significant differences in weights of adipose depots were found in this study after the injection of GH. The HF-5 group had the greatest decrease in the adipose depots measured, with varying decreases seen in the remaining groups. The inguinal (subcutaneous) depot appeared to be the most responsive to GH injections with a 59.4% decrease in size. However, the remaining adipose depots were also highly affected by the GH treatment, with all depots in the HF-5 group decreasing significantly in size. In this
study it appears that the depots are affected differently, with (in order of most to least change) the subcutaneous (-59.36%), mesenteric (-51.02%), retroperitoneal (-47.29%) and epididymal (-35.81%) depots decreasing in size by different amounts.

No significant differences were found in the weights of the other organs collected. However, trends were observed with some tissues suggesting that additional treatment with GH or higher doses may cause significant differences. Specifically, livers showed a decreasing trend with increasing concentrations of GH injections. The group with the highest doses of GH had livers that, while not significant, tended to be smaller than the LF-PBS group as well as all the HF groups. The spleen size of the HF-5 group tended to be larger than any of the other groups, a finding that has been reported previously for transgenic animals expressing excess GH (Debeljuk, Steger, Wright, Mattison, & Bartke, 1999). This indicates a possible inflammatory response to the bGH used for injections. Serum measurements of inflammatory markers as well as measurements of bGH antibody production would provide further insight on the inflammatory status of these animals. Spleen size has also been reported to increase in animals expressing excess GH (Debeljuk et al., 1999). Due to the possible immune response to the exogenous GH, it cannot be concluded that the increase in spleen size is due to the effects of GH signaling.

**Future Studies**

Additional studies are needed to resolve several findings in the present study. One issue that remains troubling is the increased insulin levels in the GH treated animals even when the fat mass was decreased and glucose levels normalized. The combined therapy of GH with oral hypoglycemic drugs used to treat diabetes may be helpful to
resolve the hyperinsulinemic state that is still present with the treatment of the highest GH dose. For example, combined therapy of GH with IGF-1, which is known to have insulin like action, may produce a more normal insulin and glucose state while maintaining the positive body composition effects. Previous studies have shown the positive effects of IGF-1 treatment on glucose metabolism in patients with type 1 and type 2 diabetes (Yuen & Dunger, 2007). Weight and, specifically, inguinal fat pad mass were also decreased upon the treatment of GH and IGF-1 in obese rats (Clark et al., 1996; Dubuis, Deal, Tsagaroulis, Clark, & Van Vliet, 1996). These studies indicate that a positive effect on diabetes and obesity may be achieved when using the hormones in tandem. Additionally, the true diabetogenic effects of the GH treatment can not be fully understood because of the lack of a GH injected LF control. Further studies injecting LF control animals with various levels of GH would show the overall insulin resistant and diabetogenic effect of GH, independent of obese status.

In the highest dose of GH, the accumulation of lean mass with the loss of fat mass may allow these animals to maintain their decreased fat mass state longer if GH injections are stopped. In effect, they may become more resistant to weight regain. Further studies may provide information on the attenuation of fat mass once injections are stopped and how long this effect persists. Other studies that manipulate diet, such as macronutrient content and caloric intake would also be helpful. The loss of lean muscle as well as fat mass when on a LF diet has been reported previously (Forbes, 1970). Related to this study, making the animals obese on the HF diet and then reversing their diet to LF either in the presence of injections of GH or PBS would be useful to determine
the extent of this lean mass loss with diet versus GH action. Due to the possible dose dependent trend of lean mass accumulation and fat mass loss, a longer study may also show a beneficial effect of the lower doses of GH and/or other, more dramatic consequences of all GH doses. Additionally, the most effective GH doses appeared to be 0.5 µg/g BW and 5 µg/g BW in this study. Future studies may use doses in this range to see if the positive body composition outcomes are maintained and if the negative anti-insulin effects are diminished. The timing of doses may also play a role in the effectiveness of GH. By altering the timing of injections to multiple injections per day or reducing injections to once every 2 days, body composition, glucose and insulin measurements would indicate positive or negative effects of the timing.

**Conclusions**

1. GH injections caused gain of lean mass and loss of fat mass in mice maintained on a HF diet. The gain of lean mass and loss of fat mass occurred to a greater extent with higher doses of GH.

2. No change in overall body weight was observed with the highest dose of GH. The gain in lean mass roughly equaled the loss in fat mass, accounting for the stable weights observed in these animals.

3. The catabolic effect of GH on fat mass declined over time, while the anabolic effect on lean mass was maintained.

4. IGF-1 levels were higher with the highest dose of GH; however, IGF-1 levels were significantly elevated in all HF groups.
5. Blood glucose levels decreased with the two highest doses of GH. Glucose levels with the highest dose of GH were not different from the LF controls after 4.5 weeks of treatment.

6. Plasma insulin levels were elevated in all HF treatment groups. High doses of GH lowered glucose levels, however; these animals remained hyperinsulinemic.

7. The weight of all four adipose depots measured was decreased with GH treatment. The subcutaneous depot was most responsive with a decrease of 59.4% in the HF-5 group.

8. No significant weight differences were observed in the heart, liver, kidney or spleen. Trends of decreasing liver size and increasing spleen size were observed in the HF-5 group.

9. Future studies may help elucidate and minimize the insulin resistant effect of GH as well as the long term effect of GH treatment on body composition.
REFERENCES


Rebuffe-Scrive, M., Surwit, R., Feinglos, M., Kuhn, C., & Rodin, J. (1993). Regional fat distribution and metabolism in a new mouse model (C57BL/6J) of non-insulin-
dependent diabetes mellitus. *Metabolism: Clinical and Experimental, 42*(11), 1405-1409.


APPENDIX A: STATE BY STATE ANALYSIS OF OBESITY AND DIABETES RATES IN THE UNITED STATES

(MOKDAD ET AL., 2003)

Note. Adapted from the CDC at http://www.cdc.gov/diabetes/statistics/maps/index.htm
APPENDIX B: GROWTH HORMONE RELEASE, CONTROL AND TISSUE EFFECTS (KOPCHICK & ANDRY, 2000)
APPENDIX C: PHENOTYPIC AND METABOLIC PARAMETERS OF TRANSGENIC MOUSE MODELS WITH ALTERED GH SIGNALING

<table>
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<th>GHA</th>
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<td>Very Low</td>
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