Diet-Induced Obesity in Growth Hormone Receptor Antagonist Mice

A thesis presented to

the faculty of

the College of Health Sciences and Professions of Ohio University

In partial fulfillment

of the requirements for the degree

Master of Science

Tianxu Yang

November 2010

This thesis titled

Diet-Induced Obesity in Growth Hormone Receptor Antagonist Mice

by

TIANXU YANG

has been approved for

the School of Applied Health Sciences and Wellness

and the College of Health Sciences and Professions by

Darlene E. Berryman

Associate Professor of Applied Health Sciences and Wellness

Randy Leite

Interim Dean, College of Health Sciences and Professions

ABSTRACT

YANG, TIANXU, M.S., November 2010, Human and Consumer Sciences, Food and Nutrition

Diet-Induced Obesity in Growth Hormone Receptor Antagonist Mice

Director of Thesis: Darlene E. Berryman

Growth hormone receptor antagonist (GHA) mice, with partial repression of GH signaling, have not been challenged with high fat (HF) feeding; therefore, both genders of GHA mice and wild type (WT) controls were fed a HF and a low fat (LF) diet to investigate the susceptibility to diet-induced obesity as well as examine gender differences. Body composition, body weight, and energy intake were measured weekly from 10-21 weeks of age. HF feeding increased body weight, fat mass and percent fat mass for all mice as compared to LF feeding. The body weight gain was due solely to increases in adiposity. Both female GHA and female WT mice exhibited a striking ability to resist fat mass gain as compared to males. Euglycemia was observed in GHA mice when exposed to a HF diet. GHA mice stored more fat mass especially in the subcutaneous pad even though most other organ weights decreased in GHA mice. Overall, GHA male mice were hyperphagic and more sensitive to diet-induced obesity than WT littermates.

Approved: _____

Darlene E. Berryman

Associate Professor of Applied Health Sciences and Wellness

ACKNOWLEDGEMENT

I would like to thank my favorite advisor, Dr. Darlene E. Berryman, who encouraged me to finish this meaningful research and my master's education at Ohio University. I appreciate her patience, time, encouragement, and countless advice about my study. I would also like to thank my committee members, Dr. Fang Meng and Dr. Shigeru Okada. I thank them for their support and advice on my thesis.

In addition, I want to give heartfelt thanks to Dr. John Kopchick and all colleagues in his lab at the Edison Biotechnology Institute. Finally, millions of thanks to my families for their endless love, understanding, support, and encouragement in my life.

TABLE OF CONTENTS

Page

ABSTRACT 3
ACKNOWLEDGEMENT 4
LIST OF TABLES 9
LIST OF FIGURES 10
LIST OF ABBREVIATIONS 13
CHAPTER 1: INTRODUCTION 15
Statement of Problem 20
Research Questions 21
Purpose of the Study 21
Limitations/Delimitations 22
Definitions of Terms 23
CHAPTER 2: REVIEW OF LITERATURE 27
Growth Hormone 27
History of Growth Hormone 27
Growth Hormone Gene and Protein 28
Structure of Growth Hormone 29
Regulation and Secretion of Growth Hormone 29
Growth Hormone Receptor 31
Growth Hormone Binding Protein 31
Growth Hormone-Mediated Signal Transduction 32
Biological Effects of Growth Hormone 33
Insulin-Like Growth Factor-1 35
Growth Hormone/Insulin-Like Growth Factor-1 Axis 36
Growth Hormone/Insulin-Like Growth Factor-1 Axis and Adipose Tissue 37
Mouse Models with Altered Levels of Growth Hormone Action 43
Adipose Tissue 49
Physiology of Adipose Tissue 49
Pathophysiology of Adipose Tissue 59

Growth Hormone Receptor Antagonist 61
Review of Growth Hormone Receptor Antagonist 61
Growth Hormone Receptor Antagonist Mouse Model 66
Summary 74
CHAPTER 3: MATERIALS AND METHODS 75
Animals 75
Dietary Manipulation 75
Measurements 76
Food Consumption 76
Weight Gain and Body Composition Measurement 76
Glucose Tolerance Test 79
Area Under the Curve 80
Tissue Weights and Distribution of Fat 80
Note 80
Statistical Analysis 81
CHAPTER 4: RESULTS 82
Body Weight 82
Body Composition 88
Absolute Fat Mass 88
Normalized Fat Mass 92
Lean Mass 96
Normalized Lean Mass 99
Fluid Mass 102
Normalized Fluid Mass 105
Food Consumption 108
Energy Consumption 108
Blood Glucose and Glucose Tolerance Test 111
Absolute Tissue/Organ Weights 118
Normalized Tissue/Organ Weights 126
CHAPTER 5: DISCUSSION 134
Growth Hormone Receptor Antagonist Mice Comparison on the Low Fat Diet 134

Diet-Induced Obesity in Growth Hormone Receptor Antagonist Mice 136
Fat Depots and Organ Weight Differences 139
Glucose Homeostasis 141
Gender Differences 143
Conclusion 147
Future Studies 148
REFERENCES 151
APPENDIX A: GHA AND WT MICE IN 11 WEEKS OF FEEDING STUDY 209
APPENDIX B: THREE-WAY REPEATED ANOVA RESULTS OF BODY WEIGHT IN GHA AND WT MICE 210
APPENDIX D: GENOTYPE COMPARISON: PERCENT VALUES IN BODY WEIGHT AND BODY COMPOSITION OF GHA MICE AS COMPARED TO WT CONTROLS OF THE SAME DIET AND GENDER AT THE BEGINNING AND END OF THE FEEDING STUDY 212
APPENDIX E: GENDER COMPARISON: PERCENT VALUES IN BODY WEIGHT AND BODY COMPOSITION OF FEMALE VERSUS MALE MICE OF THE SAME DIET AND GENOTYPE AT THE BEGINNING AND END OF THE FEEDING STUDY. 213
APPENDIX F: THREE-WAY REPEATED ANOVA RESULTS OF FAT MASS IN GHA AND WT MICE 214
APPENDIX G: PAIRED T-TEST RESULTS OF BODY WEIGHT AND BODY COMPOSITION IN GHA AND WT MICE AT WEEK 10 AND 21 OF FEEDING STUDY 215
APPENDIX H: THREE-WAY ANOVA RESULTS OF FAT MASS IN GHA AND WT MICE 216
APPENDIX I: THREE-WAY REPEATED ANOVA RESULTS OF PERCENT FAT MASS IN GHA AND WT MICE 217
APPENDIX J: THREE-WAY ANOVA RESULTS OF PERCENT FAT MASS IN GHA AND WT MICE 218
APPENDIX K: THREE-WAY REPEATED ANOVA RESULTS OF LEAN MASS IN GHA AND WT MICE 219
APPENDIX L: THREE-WAY ANOVA RESULTS OF LEAN MASS IN GHA AND WT MICE 220
APPENDIX M: THREE-WAY REPEATED ANOVA RESULTS OF PERCENT LEAN MASS IN GHA AND WT MICE 221

APPENDIX N: THREE-WAY ANOVA RESULTS OF PERCENT LEAN MASS IN GHA AND WT MICE 222

APPENDIX O: THREE-WAY REPEATED ANOVA RESULTS OF FLUID MASS IN GHA AND WT MICE 223

APPENDIX P: THREE-WAY ANOVA RESULTS OF FLUID MASS IN GHA AND WT MICE 224

APPENDIX Q: THREE-WAY REPEATED ANOVA RESULTS OF PERCENT FLUID MASS IN GHA AND WT MICE 225

APPENDIX S: THREE-WAY ANOVA RESULTS OF PERCENT FLUID MASS IN GHA AND WT MICE 226

APPENDIX T: THREE-WAY ANOVA RESULTS OF ENERGY EXPENDITURE IN GHA AND WT MICE 227

APPENDIX U: THREE-WAY ANOVA RESULTS OF BLOOD GLUCOSE IN GHA AND WT MICE 228

APPENDIX V: THREE-WAY ANOVA RESULTS OF AREA UNDER THE CURVE IN GHA AND WT MICE 229

APPENDIX W: PERMISSION TO REPRODUCE FIGURE 230

LIST OF TABLES

Page

Table 1: Summary Diet Manipulation of bGH and $GHR^{-/-}$ Mouse Data 47

Table 2: Summary GHA Mouse Data From Studies Using Standard Chow Diets 72

Table 3: Genotype Comparison: Percent Values in Body Weight and Body Composition of GHA Mice as Compared to WT Controls of the Same Diet and Gender at the Beginning and End of the Feeding Study. 86

Table 4: Gender Comparison: Percent Values in Body Weight and Body Composition of Female Versus Male Mice of the Same Diet and Genotype at the Beginning and End of the Feeding Study.....87

Table 5: Correlation of Glucose/AUC and Absolute Fat/Percent Fat Mass 117

Table 6: Factorial ANOVA Results of Absolute Tissue/Organ Weight in GHA and WT Mice 120

Table 7: Factorial ANOVA Results of Percent Tissue/Organ Weight in GHA and WT Mice 128

LIST OF FIGURES

Page

Figure 1: GH secretion and action 30

Figure 2: GH-mediated signal transduction 33

Figure 3: Comparison of percent body weight among male WT, bGH, and GHR$^{-/-}$ mice in a HF diet feeding 46

Figure 4: Male mice feeding manipulation during the 11-week diet study 78

Figure 5: Female mice feeding manipulation during the 11-week diet study 79

Figure 6: Body weights of male and female mice over the 10- to 21-week feeding study 84

Figure 7: Body weights of GHA and WT mice over the 10- to 21-week feeding study 85

Figure 8: Absolute fat mass of male and female mice over the 10- to 21-week feeding study 90

Figure 9: Absolute fat mass of GHA and WT mice over the 10- to 21-week feeding study 91

Figure 10: Percent fat mass of male and female mice over the 10- to 21-week feeding study 94

Figure 11: Percent fat mass of GHA and WT mice over the 10- to 21-week feeding study 95

Figure 12: Absolute lean mass of male and female mice over the 10- to 21-week feeding study 97

Figure 13: Absolute lean mass of GHA and WT mice over the 10- to 21-week feeding study 98

Figure 14: Percent lean mass of male and female mice over the 10- to 21-week feeding study 100

Figure 15: Percent lean mass of GHA and WT mice over the 10- to 21-week feeding study 101

Figure 16: Absolute fluid mass of male and female mice over the 10- to 21-week feeding study 103

Figure 17: Absolute fluid mass of GHA and WT mice over the 10- to 21-week feeding study 104

Figure 18: Percent fluid mass of male and female mice over the 10- to 21-week feeding study 106

Figure 19: Percent fluid mass of GHA and WT mice over the 10- to 21-week feeding study 107

Figure 20: Energy consumption of male and female mice over the 10- to 21-week feeding study 109

Figure 21: Energy consumption of GHA and WT mice over the 10- to 21-week feeding study 110

Figure 22: Fasting glucose of male and female mice at week 20 of feeding study 113

Figure 23: Fasting glucose of GHA and WT mice at week 20 of feeding study 114

Figure 24: Area under the curve of male and female mice over the 10- to 21-week feeding study 115

Figure 25: Area under the curve of GHA and WT mice over the 10- to 21-week feeding study 116

Figure 26: Absolute fat depot weights of male and female mice over the 10- to 21-week feeding study 122

Figure 27: Absolute fat depot weights of GHA and WT mice over the 10- to 21-week feeding study 123

Figure 28: Absolute organ weights of male and female mice over the 10- to 21-week feeding study 124

Figure 29: Absolute organ weights of GHA and WT mice over the 10- to 21-week feeding study 125

Figure 30: Normalized fat depot weights of male and female mice over the 10- to 21-week feeding study 130

Figure 31: Normalized fat depot weights of GHA and WT mice over the 10- to 21-week feeding study 131

Figure 32: Normalized organ weights of male and female mice over the 10- to 21-week feeding study 132

Figure 33: Normalized organ weights of GHA and WT mice over the 10- to 21-week feeding study 133

LIST OF ABBREVIATIONS

Arg	arginine
AUC	area under the curve
BAT	brown adipose tissue
bGH	bovine growth hormone transgenic
FFA	free fatty acid
GH	growth hormone
GHA	growth hormone receptor antagonist
GHBP	growth hormone binding protein
GHD	growth hormone deficiency
GH-N	growth hormone-normal gene
GHR	growth hormone receptor
$GHR^{-/-}$	growth hormone receptor/binding protein gene-disrupted or knockout
GHRH	growth hormone-releasing hormone
GTT	glucose tolerance test
hGH	human growth hormone
HF	high fat
iBAT	interscapular brown adipose tissue
IGF-1	insulin-like growth factor-1
IGF-1R	insulin like growth factor-1 receptor
IGFBP	insulin like growth factor binding protein
IL-6	interleukin 6

JAK-2	Janus kinase-2
LDL	low-density lipoprotein
LF	low fat
NMR	nuclear magnetic resonance
PAI-1	plasminogen activator inhibitor-1
PEG	polyethylene glycol
PI3K	phosphoinositide 3-kinase
PL	placental lactogen
PRL	prolactin
STAT	signal transducers and activators of transcription
TAG	triglyceride
TNF-α	tumor necrosis factor
UCP1	uncoupling protein 1
WAT	white adipose tissue
WT	wild type

CHAPTER 1: INTRODUCTION

Growth hormone (GH), like the name implies, is a vital hormone in the human body that regulates longitudinal bone growth (Greenspan, Li, Simpson, & Evans, 1949). Other important functions of GH include:

1. Modulating the metabolism of lipid (Raben & Hollenberg, 1959), carbohydrate (Goodman, 1965a), and nitrogen (Goodman, 1978);

2. Promoting the secretion of insulin-like growth factor-1 (IGF-1) from target organs such as liver, bone, muscle, and adipose tissue (Entingh-Pearsall & Kahn, 2004);

3. Regulating bone mineral and vitamin D metabolism (Gomez, 2006);

4. Maintaining the normal functioning of the immune system (Chen, Schuler, & Schultz, 1998), neurologic system (Higham & Trainer, 2008; Yoshizato, Fujikawa, Soya, Tanaka, & Nakashima, 1998), and cardiac development (Isgaard, Nilsson, Vikman, & Isaksson, 1989; Mathews, Enberg, & Norstedt, 1989).

The GH/IGF-1 axis exerts a profound impact on metabolism and body composition. Overall, GH profoundly increases lean mass (Florini, Ewton, & Coolican, 1996) and decreases fat mass (Felig, Marliss, & Cahill, 1971; Henneman & Henneman, 1960). It should also be noted that GH exerts its impact to some degree by blocking the effects of insulin. Indeed, a previous report revealed that elevated levels of GH can lead to hyperinsulinemia and insulin resistance; consequently, patients with high GH have a greater susceptibility of developing type 2 diabetes (Sonksen, Salomon, & Cuneo, 1991).

Acromegaly is a disease caused by excessive GH secretion, typically due to an adenoma of the anterior pituitary gland (Crushing, 1909). It is diagnosed later than its

onset by several years (Rajasoorya, Holdaway, Wrightson, Scott, & Ibbertson, 1994). The accompanying problems of the disease include rheumatologic (Waine, 1945), cardiovascular (Wahlander, Isgaard, Jennische, & Friberg, 1992), respiratory (Grunstein, Ho, & Sullivan, 1991), and metabolic consequences; there is also an increase in mortality (Bates, Van't Hoff, Jones, & Clayton, 1993). Although surgery, radiology, and somatostatin analogues drugs can be used to treat the condition, a growth hormone receptor antagonist (GHA), called pegvisomant and marketed under the brand name Somavert, has gained widespread acceptance as a highly selective and efficacious treatment for acromegaly.

Growth hormone deficiency (GHD) is an acquired pathophysiologic condition of pituitary gland, such as trauma, infection, pituitary tumors, and radiation treatment (Agha et al., 2004; Carpinteri, Patelli, Casanueva, & Giustina, 2009; Ronchi et al., 2009) and an idiopathic problem in childhood (Hindmarsh, Smith, Brook, & Matthews, 1987) with markedly decreased serum GH levels. This disease is also termed as hypopituitarism, which exhibits a series of features. There are abnormalities in body composition in GHD including reduced lean body mass (Salomon, Cuneo, Hesp, & Sonksen, 1989), increased fat body mass with a strikingly abdominal (increased waist circumference) and subcutaneous adiposity (Binnerts et al., 1992), reduced body water (Rosen, Bosaeus, Tolli, Lindstedt, & Bengtsson, 1993), reduced bone mineral density (Beshyah et al., 1995), increased fasting glucose and insulin levels (Johansson, Fowelin, Landin, Lager, & Bengtsson, 1995), and shortened lifespan (McGauley, 1989). In addition, other clinical symptoms include impaired cardiovascular and endothelial functioning (Rosen &

Bengtsson, 1990), reduced muscle strength (Cuneo, Salomon, Wiles, & Sonksen, 1990), reduced skin thickness (Black, Shuster, & Bottoms, 1972), and psychological complaints (Rosen, Wiren, Wilhelmsen, Wiklund, & Bengtsson, 1994). Thus, in many ways, decreased serum GH levels with GHD appears to be opposite to the status of acromegaly.

The GHA was first discovered by Chen, Wight, Wagner, and Kopchick in 1990. These researchers designed a mutant bovine GH gene that changed three critical amino acids in GH and that, when produced by transgenic mice, resulted in a dwarf animal (Chen, Wight, Wagner, & Kopchick, 1990). Subsequently, further studies revealed that only changing the glycine at position 119 of bGH and 120 of hGH was needed for the same antagonistic effect (Chen, Wight, Mehta, Wagner, & Kopchick, 1991). Failing to stimulate any intracellular signaling pathway, the GHA competes with normal GH for binding to the GH receptor, effectively antagonizing or blocking a large portion of GH function. Due to the effective suppression of GH function, a slightly modified version of the GHA, which has a longer half-life, has been approved by the FDA to treat acromegaly. The name of the FDA approved drug is pegvisomant.

GHA transgenic mice produce GHA continually and have a phenotype characteristic of GH deficiency. Since GH promotes fat breakdown and muscle gains, a decrease in GH action should result in fat accumulation and less muscle and bone. As expected, GHA transgenic mice exhibit an obese and dwarf phenotype (Berryman et al., 2004). The GHA mice also show a preferential accumulation of fat mass in the subcutaneous region (Berryman et al., 2004). However, GHA mice appear to have fairly normal insulin sensitivity and lifespan despite their obesity (Coschigano et al., 2003).

Further, serum leptin is significantly increased in GHA mice, while these mice exhibit increased food consumption when normalized to body weight, indicating that GHA mice may have some level of leptin resistance (Berryman et al., 2004). In addition, higher levels of serum free fatty acids (FFA) and triglycerides (TAGs) have been found in GHA mice (Yakar et al., 2004). Finally, in the GHA transgenic mice, although the concentration of GH in the blood circulation is high, the chronic and continued expression of GHA results in lower IGF-1 levels than normal (Chen et al, 1990). Overall, the GHA mouse model is similar to a clinic state of GHD.

Surwit and colleagues (1995) reported that the C57Bl/6J background mouse strain is susceptible to diet-induced obesity and diabetes when fed a high fat (HF) diet, which is similar to what occurs with humans. In addition, C57Bl/6J become hyperinsulinemic and hyperglycemic on HF diets, while mice on low fat (LF) diets remain euglycemic and lean (Surwit, Kuhn, Cochrane, McCubbin, & Feinglos, 1988). Thus, transgenic or gene disrupted mice maintained under this background strain provide a means to assess the impact of a particular gene on diet-induced obesity.

Previous studies have illustrated that other strains of mice with altered GH (GH receptor knockout or $GHR^{-/-}$ and bovine GH transgenic or bGH mice) have altered susceptibility to diet-induced obesity. Bovine GH or bGH mice, a transgenic mouse model expressing bovine GH, have high GH and IGF-1 levels with a giant stature (Palmiter et al., 1982), increased lean mass, reduced fat mass (Bengtsson, Brummer, Eden, Bosaeus, & Lindstedt, 1989), insulin resistance (Foss et al., 1991; Oscarsson, Wiklund, Jakobsson, Petruson, & Bengtsson, 1994), and disturbed lipoprotein

metabolism (Nikkila & Pelkonen, 1975; Oscarsson et al., 1994). Importantly, these mice have a striking ability to resist diet-induced obesity, despite hyperphagia, dyslipidemia, and diabetes when fed a HF diet (Olsson et al., 2005). Similar results were also reported by another group that further showed that energy expenditure was not responsible for the resistance to diet-induced obesity (Berryman et al., 2006; Dominici, Arostegui Diaz, Bartke, Kopchick, & Turyn, 2000; Hauck, Hunter, Danilovich, Kopchick, & Bartke, 2001; Trainer et al., 2000). In contrast to bGH mice, the GH receptor gene disrupted model or $GHR^{-/-}$ mice have high GH but low IGF-1 levels with a short stature and slightly reduced glucose levels and severely reduced insulin levels (Coschigano, Clemmons, Bellush, & Kopchick, 2000; Dominici, Arostegui Diaz, Bartke, Kopchick, & Turyn, 2000; Hauck, Hunter, Danilovich, Kopchick, & Bartke, 2001; Zhou et al., 1997). These mice exhibit a dramatic increase in susceptibility to diet-induced obesity and have increased insulin levels and islet cell mass when challenged by a HF diet (Berryman et al., 2006; Robertson, Kopchick, & Liu, 2006).

GHA transgenic mice exhibit an increased fat mass starting as early as 6-weeks of age and a decreased lean mass at later phases when fed a standard diet. Again, this mouse line is capable of maintaining a normal glucose level and higher levels of leptin (Magon, 2009). However, GHA transgenic mice have never been challenged with a non-standard diet, so their susceptibility to diet-induced obesity has not been tested. Thus, the purpose of this study is to determine how GHA mice (previously characterized as obese with normal insulin sensitivity) in the C57Bl/6J strain (known to be susceptible to diet-induced obesity and diabetes) respond to a HF and a LF diet, which may facilitate an

understanding how humans treated with GHA respond to the drug. Further, because GHA models also can be considered similar to GHD obese state, these results may provide insights into diet-induced obesity in GHD.

Statement of Problem

The study of GHA transgenic mice is clinically relevant to humans for two reasons. First, transgenic mice for GHA can provide information about the chronic use of this drug in humans. GHA has been used to treat patients with acromegaly because this drug can drastically decrease IGF-1 level as well as glucose and insulin concentrations; thereby, some clinical symptoms are improved (Holly et al., 1991; Trainer et al., 2000). Despite its widespread use, little is understood about its long term impact. Further, as GHA mice are essentially in a state of GH deficiency throughout life, these mice also provide insight into the long-term outcomes of a GH deficient state. Current studies about GHA mice show that these transgenic mice develop an increased fat mass and a lower lean mass. Also, this trend is evident in early life, and male mice have more fat mass than females suggesting gender specific effects. GHA mice maintain a lower or normal glucose and insulin level (Chen et al., 1990). However, all previous data collected are based on a standard diet; there is no information showing the susceptibility of these mice to diet-induced obesity. In humans, obesigenic diets are more common than the LF diets fed to these mice, making it important to determine how these mice will respond to a high calorie diet. In studies with humans, there are many uncertain factors that are difficult to control; for example, complex genotypes and multiple unpredicted exogenous factors including uncontrolled diet, activity, and medical treatment. Unlike

humans, the GHA mice have a uniform genotype and environmental factors, such as diet, are more easily controlled.

Research Questions

In this study, male and female GHA transgenic mice and littermate controls in the C57Bl/6J background strain were exposed to a HF or a LF feeding regimen for 11 weeks. For all studies, GHA male and female mice were compared with age- and gender-matched littermate control mice. The research questions addressed were:

1. How will the body composition (lean mass, fat mass, and body fluid) change in GHA mice (male and female) in comparison to littermate controls when exposed to a HF versus a LF diet?
2. How will calorie intake change in GHA mice (male and female) in comparison to control mice when fed on a HF versus a LF diet?
3. How will the levels of fasting glucose and glucose tolerance change for GHA mice (male and female) and littermate controls when fed on a HF diet versus a LF diet?
4. How will the tissue and fat depot weights change in GHA mice (male and female) in comparison to controls when challenged with a HF diet versus a LF diet?

Purpose of the Study

The C57Bl/6J mouse strain is susceptible to diet-induced obesity and diabetes when fed a HF diet, which is similar to how humans develop obesity and diabetes. Male transgenic GHA mice in the C57Bl/6J genetic background exhibit a dwarf and obese phenotype at least by 6 months of age on a LF diet. However, GHA transgenic mice maintain a normal glucose and insulin level with lower level of IGF-1. The purpose of

this study is to challenge this strain with a higher fat, higher calorie diet to test the susceptibility of GHA mice to diet-induced obesity and diabetes. While previous studies focused on susceptibility to diet-induced obesity in other mouse lines ($GHR^{-/-}$ and bGH transgenic mice), no dietary manipulation study has yet been done in the GHA mouse line. Studies with humans are difficult for ethical reasons. Since the transgenic GHA mice produce GHA continually, it is a good model to explore how the different diets affect progression of obesity and diabetes without any harm to clinical populations. GHA mice in the C57Bl/6J background strain are also similar to the clinical state of GHD in humans; therefore, the results from this study may also provide insight into the treatment of this condition.

Limitations/Delimitations

1. Inherent errors of measurements made with specific equipment, such as nuclear magnetic resonance (NMR) exist; these factors cannot be avoided completely.
2. Some stress to the animals is inevitable during particular manipulations, such as measuring body composition and collecting blood. This may change the data to some extent. Wherever possible, attempts were made to minimize stress to the mice during the dietary manipulation phase of the study.
3. Since two or three mice were located in one cage and mice can obtain food freely, it was hard to calculate the food consumed accurately by each mouse in the same cage.
4. The data from mice cannot be fully extrapolated to the human's condition due to species differences.

Definitions of Terms

Acromegaly. Acromegaly is an acquired progressive condition due to excessive GH in the body, which is caused by a benign pituitary somatotroph adenoma. The typical features are broadened and thickened bones of extremities and face. Many physiologic systems, endocrine, rheumatic, neurotic, cardiac systems, are also disrupted, which lead to increase mortality (Chanson & Salenave, 2008; Crushing, 1909).

Adipocyte. Adipocyte is the lipid laden cell of adipose tissue, and it secrets a variety of cytokine-like factors (Unger & Orci, 2002).

Adipokines. A group of cytokine-like factors that are secreted and that take part in various metabolic activities such as regulation of energy homeostasis, steroid conversion and sexual maturation, insulin-lipid-glucose metabolism, dietary behavior, insulin sensitivity, vascular remodeling, coagulation, and other relevant vascular behaviors (Berti, Kellerer, Capp, & Haring, 1997; Kubota et al., 2002; Ravussin et al., 1997; Steppan et al., 2001).

Bovine GH mice. Bovine GH or bGH mice are a transgenic mouse line. These mice were made by using a metallothionein transcriptional regulatory element linked to the first exon and intron of the bGH cDNA. These mice have excessive levels of GH. Features of these mice are similar to the acromegalic state including characteristics such as a giant stature, high level of IGF-1 and insulin, normal glucose, short lifespan, and insulin resistance (Berryman et al., 2004).

Growth hormone receptor antagonist (GHA) mice. GHA mouse is a transgenic engineered mouse line that expresses GHA. The antagonist mice have a mutated glycine

119 of bGH constitutively expressed under a metallothionein promoter. These GHA mice exhibit a dwarf stature and obese phenotype, low levels of IGF-1, yet normal levels of glucose, insulin, and lifespan (Chen, White, Wagner, & Kopchick, 1991; Coschigano et al., 2003). Most excess fat mass is accumulated in the subcutaneous region (Berryman et al., 2004; Chen, White et al., 1991).

Growth hormone deficiency (GHD). A clinical state characterized by altered body composition, bone mineralization, serum lipids, glucose tolerance, and insulin-induced hyperglycemia. GHD individuals are short and obese (Coschigano et al., 2003; Murray, Adams, & Shalet, 2004).

Growth hormone receptor knockout ($GHR^{-/-}$) mice. These are mice with a deletion of most of the fourth exon and part of the fourth intron of the GH receptor. These mice have essentially no functional GH signaling through the receptor despite having the ability to make GH. These mice exhibit severe postnatal growth retardation, absence of GHR and GH binding protein, low level of IGF-1, glucose, and insulin, high levels of GH, extreme insulin-sensitivity, and long lifespan (Coschigano et al., 2003; Zhou et al., 1997).

Hyperglycemia. Hyperglycemia is a pathological high level of glucose in the blood, which occurs with diabetes, results in multiple microvascular and microvascular complications, and increases morbidity and mortality (Capes, Hunt, Malmberg, Pathak, & Gerstein, 2001).

Hyperinsulinemia. This is the clinical condition of having excess levels of circulating insulin in the blood. It is a common feature of people with diabetes mellitus type 2, insulin resistance, and obesity (Ginsberg, Kimmerling, Olefsky, & Reaven, 1975).

Insulin-like growth factor-1 (IGF-1). It is a member of the insulin-like growth family and is a major protein upregulated by GH. Thus, many of the effects of GH are due to IGF-1, which plays a crucial role in postnatal normal growth (Jones & Clemmons, 1995).

Insulin resistance. Insulin resistance is associated with type 2 diabetes and excessive adipose tissue. This pathophysiological condition suggests that insulin fails to effectively promote glucose uptake and storage in tissues especially in muscle and fat cells. In order to maintain the normal serum glucose levels, the pancreas secretes much more insulin than normal (Campfield, Smith, Guisez, Devos, & Burn, 1995; Goodyear et al., 1995; Kahn & Flier, 2000).

Leptin. Leptin is a cytokine secreted mainly from adipocytes. Leptin alters food intake, energy expenditure, body weight, lipid and glucose metabolism, insulin sensitivity and resistance, hypertension, and immunity (Campfield, Smith, Guisez, Devos, & Burn, 1995; Rondinone, 2006).

Somatostatin analogue. Somatostatin analogues are pharmaceutical agents that can be used to treat acromegaly. They are growth hormone releasing hormone antagonists. They act at the central nervous system to suppress GH hypersecretion in acromegaly by binding to somatostatin receptors. Analogs are used because the analogs

are more stable and effective than somatostatin to treat acromegaly (Newman et al., 1995; Vance & Harris, 1991).

CHAPTER 2: REVIEW OF LITERATURE

GH influences a number of important physiologic functions. Both overproduction and deficiency of GH lead to metabolic abnormalities. A relatively new pharmaceutical agent, the GHA, inhibits the action of GH. Mice transgenic for GHA show a dwarf stature and relatively normal insulin and glucose levels. However, the GHA mice are also obese. It is well known that diet is an important factor that affects body composition, yet no research has explored the impact of diet on GHA mice. This literature review will provide an overview of GH, adipose tissue, and the GHA. In addition, this review will explain the interaction between GH and adipose tissue, and compare several mice models with altered GH levels.

Growth Hormone

History of Growth Hormone

GH has a long research history. In 1886, Pierre Marie discovered that acromegalic patients had enlarged pituitary glands (Kopchick & Andry, 2000). Since then, in 1910, some animal experiments further revealed that growth factors existed in the pituitary gland (Crowe, Cushing, & Homans, 1910). According to Evans and Long's research (1921) on the anterior lobe of the pituitary, an extract from the anterior lobe of the pituitary stimulated rats to exhibit a greater rate of growth than was first assumed (Evans & Long, 1921). The year of 1944 marked a milestone in GH research in which bovine GH was first isolated from the ox anterior lobes in a lab at the University of California, Berkeley (Li & Evans, 1944). Subsequently, advances in genetic technology allowed for the cloning and expression of GH in *Escherichia coli* bacteria (Martial,

Hallewell, Baxter, & Goodman, 1979). With the successful discovery of the GHR in rabbits and humans, the high affinity between GHR, GH binding protein, and GH were recognized as important factors for growth in 1987; not only was GHR on liver membranes and GH binding protein recently purified, but also the rabbit GH receptor clone was subsequently isolated (Leung et al., 1987). During the same period, the three-dimensional structure of porcine GH, which includes four antiparallel α-helices arranged in a left twisted helical bundle, was revealed; this structure has facilitated additional research on the characteristics of GH (Abdel-Meguid et al., 1987).

Growth Hormone Gene and Protein

GH is a member of the large hormone family that includes prolactins (PRLs), placental lactogens (PLs), and more recently discovered members such as PRL-like protein-B, PLP-C (Roby et al., 1993), mouse proliferin, somatolactin, and other molecules. Having evolved approximately 350 million years, this ancient GH gene consists of 5 exons and 4 introns and is located on the long arm of chromosome 17 in humans (Owerbach, Rutter, Martial, Baxter, & Shows, 1980). Human GH (hGH) spans 3000 nucleotides and consists of five closely related genes including GH-N (growth hormone-normal gene), CS-L (chorionic somatomammotropin-like gene), CS-A (chorionic somatomammotropin-A), GH-V (growth hormone-variant), and CS-B (the chorionic somatomammotropin-B) genes (Hirt et al., 1987), which have a high identity in coding and flanking regions (Seeburg, 1982). GH is synthesized as a precursor protein; subsequently, the signal peptide attached to the amino terminal is cleaved when GH is secreted. GH-N encodes both a 22-kDa GH and a 20kDa GH isoform. The former

is the most abundant in plasma while the latter only comprises about 5-10% in plasma (Lewis et al., 1980). The GH amino acid sequences have approximately 75-77% similarity among humans, rats, and bovine species (Nicoll, Mayer, & Russell, 1986). Therefore, research in animal models provides new insights and avoids ethical problems that may emerge by conducting experiments with humans.

Structure of Growth Hormone

GH is composed of 191 amino acids and the molecular mass of GH is about 22,000 Daltons. The three-dimensional structure of porcine and human GHs was determined in 1987 and 1992, respectively (Abdel-Meguid et al., 1987; de Vos, Ultsch, & Kossiakoff, 1992). Both species of GH have similar structures, consisting of four α-helices with 21-30 amino acids in length each (Abdel-Meguid et al., 1987; de Vos, Ultsch, & Kossiakoff, 1992). The three-dimensional structure shows some similarity in an unusual up-up-down-down topology with a left-handed bundle orientation. There are highly conserved Cys residues evident in GH, PRLs, and PLs (Nicoll et al., 1986), suggesting some basic structural similarity between these family members as well as their importance in maintaining the three-dimensional structure of the molecule.

Regulation and Secretion of Growth Hormone

GH secretion is regulated by multiple mechanisms. Two antagonistic acting hormones, growth hormone-releasing hormone (GHRH) and somatostatin (somatotropin release-inhibiting factor), control the majority of GH secretion by somatotrophs cells in the anterior pituitary gland (Brazeau et al., 1973; Tannenbaum, 1991; Tannenbaum & Ling, 1984). As the name suggests, GHRH stimulates somatotroph cells to synthesize

GH while somatostatin inhibits GH secretion (see Figure 1). Other factors, such as ghrelin, physical activities, stress, fasting, or nutrition intake, also influence GH secretion.

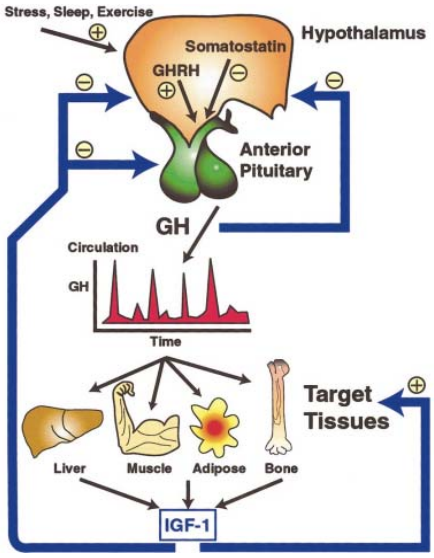


Figure 1. GH secretion and action. From "Growth hormone (GH), GH receptor, and signal transduction," by J. J. Kopchick and J. M. Andry, 2000, *Molecular Genetics and Metabolism, 71*, p. 295. Copyright 2000 by Academic Press. Adapted with permission of the author.

Numerous studies show that GH is released in a pulsatile manner and most GH secretion happens at night, particularly after the onset of deep sleep (Quabbe, Schilling, & Helge, 1966). The peak of GH secretion is due to the action of maximal concentration of GHRH and the minimal level of somatostatin (Tannenbaum & Ling, 1984). Once secreted, the clearance rate of serum GH is very rapid, only lasting less than 20 minutes.

The rapid clearance is due to most GH being degraded and cleared by the kidney and liver (Thompson, Rodriguez, Kowarski, & Blizzard, 1972).

Growth Hormone Receptor

The binding of GH to its receptor is the first step of multiple actions of GH. GHR is located on the cell surface in which the level of GHRs varies depending on the tissue. At least in rats, liver and adipose tissue express a very high amount of GHR, while moderate levels are reported for skeletal muscle, intestine, kidney, and other organs. In contrast, there is no GHR existing in the testis, thymus, and spleen (Tiong & Herington, 1991). In humans, GHRs are expressed in several tissues but mainly located in liver and adipose tissue (Esposito, Paterlini, Kelly, Postel-Vinay, & Finidori, 1994; Sobrier, Duquesnoy, Duriez, Amselem, & Goossens, 1993; Werther, Haynes, & Waters, 1993). The GHR gene is located on chromosome 5 (5p13-p14) (Arden, Boutin, Djiane, Kelly, & Cavenee, 1990) and GHR protein is a transmembrane glycoprotein of 620 amino acids (Leung et al., 1987).

Growth Hormone Binding Protein

GH binding protein (GHBP) is a soluble extracellular domain of the GHR that is found in the serum of vertebrates (Hadden & Prout, 1964). The molecular mass of GHBP is approximately 55 kDa. The means by which GHBP is generated depends on species; that is, in mice and rats, GHBP is due to alternative splicing of the mRNA while in humans, GHBP is generated through proteolytic digestion.

The biological function of GHBP remains controversial although it is clear that GHBP binds to GH in serum. GHBP binding of GH has been suggested to maintain

appropriate levels of functional GH in serum (Baumann, Amburn, & Shaw, 1988) or function to antagonize of GH from binding to the GHR.

Growth Hormone-Mediated Signal Transduction

GH binds to a GHR dimer located on the cell membrane to elicit a cascade of intracellular signals that is responsible for the biological impact of GH. Two GHRs bind asymmetrically to sites on the GH. With the hGH·$(GHR)_2$ complex formed, the second GHR will move relative to the first receptor; specifically, the second GHR undergoes a rotation and a vertical movement (Brown et al., 2005). The conformational change of GHR leads to a rotation of the transmembrane domain, which further activates Janus kinases (JAK2) by transphosphorylation in the cytoplasmic domain (Brown et al., 2005). Thus, the GH-dependent signal cascade is initiated. Activated JAK2 induces signal transducers and activators of transcription (STATs) phosporylation that causes STATs to translocate to the nucleus, where they bind to DNA and promote or block the transcription of GH responsive genes including IGF-1 (Rosenfeld & Hwa, 2009). Thus, GH binding to GHR induces a cascade of signal transduction, which results in IGF-1 gene expression, synthesis, and output (see Figure 2) (Madsen, Friberg, Roos, Eden, & Isaksson, 1983; Murphy & Friesen, 1988). GH also activates other signaling pathways, such as the phosphoinositide (PI) 3-kinase signaling pathway, to induce insulin resistance in adipocytes (Takano et al., 2001). The mechanism likely involves a subunit of p85α. That is, overexpression of p85α, an isoform of the PI 3-kinase subunit, inhibits PI 3-kinase signaling and insulin action (Ueki, Algenstaedt, Mauvais-Jarvis, & Kahn, 2000). For example, excess GH increases p85α expression in white adipose tissue (WAT)

inducing insulin resistance in bGH mice; however, reduced p85α expression in GH deficient lit/lit mice results in hypoinsulinemia (del Rincon et al., 2007).

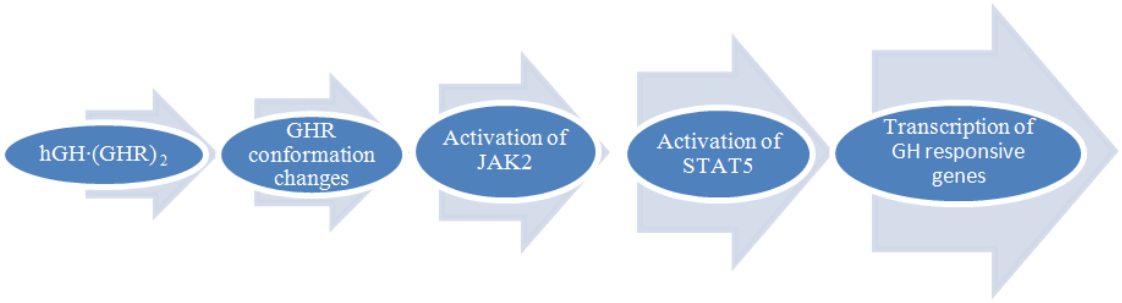


Figure 2. GH-mediated signal transduction.

Biological Effects of Growth Hormone

GH acts on a variety of tissues in the body and plays a significant role in metabolism. One main action of GH is to promote postnatal longitudinal growth. Longitudinal skeletal growth is mediated by GH in a dose dependent manner that causes the proliferation of chondrocytes in the epiphyseal growth plate in which GH stimulates DNA synthesis and differentiation as well as clonal proliferation (Casanueva, 1992; Madsen, Friberg, Roos, Eden, & Isaksson, 1983). Based on the theory of GH's dual effects (Green, Morikawa, & Nixon, 1985), not only does GH lead to differentiation of precursor cells, but it induces these cells to synthesize IGF-1 locally, which also influences differentiation through autocrine and paracrine mechanisms (Casanueva,

1992). Unbalanced GH secretion leads to abnormal body growth; that is, over-expression results in gigantism or acromegaly while GH deficiency results in dwarfism.

GH plays a crucial role in reducing fat mass. In mature adipose cells, GH exhibits a lipolytic effect approximately 1-2 hours after administration, thus increasing the hydrolysis of TAGs and release of FFA and glycerol while decreasing FFA re-esterification (Van Vliet et al., 1987). This lipolytic process is independent of IGF-1 (Casanueva, 1992). Clinical studies further support a lipolytic function of GH on adipose tissue (Dietz & Schwartz, 1991). For example, patients with GH deficiency present a mildly obese phenotype (Bonnet, Vanderschueren-Lodeweyckx, Eeckels, & Malvaux, 1974) while patients with acromegaly are very lean (Katznelson, 2009). Animal models with altered GH levels consistently show similar trends in adipose tissue. For example, in bGH transgenic mice, a mouse model with excess GH, total body fat mass is significantly decreased compared to control littermates, while models with deficiencies are relatively obese (Berryman et al., 2004)

Skeletal muscle is also an important GH-target tissue, where GH enhances amino acid uptake and increases protein synthesis. Accordingly, athletes abusing GH and patients with acromegaly have elevated amounts of lean tissue (Katznelson, 2009; Khaleeli et al., 1984; Rennie, 2003). Using mice, it has been shown that GH increases muscle-specific IGF-1 that stimulates myotube hypertrophy (Clark, Schuenke, Keeton, Staron, & Kopchick, 2006; Sotiropoulos et al., 2006), leading to an increased myonuclear number and facilitating fusion of myoblasts (Sotiropoulos et al., 2006), thus increasing

muscle mass. Consistent with GH's effect on the muscle, bGH mice also exhibit larger muscle fibers (Dudley & Portanova, 1987).

GH also influences carbohydrate metabolism, but its actions are dependent on timing. During the first few hours of GH administration, GH exerts an insulin-like effect, enhancing glucose utilization, lipogenesis, and amino acid metabolism. However, after three hours, an anti-insulin effect occurs, thus inducing opposite effects, including lipolysis, hyperglycemia, and hyperinsulinemia. Overall, the overriding effect is to antagonizes insulin's actions (Campbell & Rastogi, 1969; Davidson, 1987; Rizza, Mandarino, & Gerich, 1982).

GH has other functions. For example, GH increases total body extracellular fluids. Other GH actions include regulating immune systems (Murphy, Durum, Anver, Frazier, & Longo, 1992), modulating psychological reactions (Yoshizato et al., 1998), and prompting cardiac development and hypertrophy (Cittadini et al., 1996). Overall, as an important endocrine mediated factor in the body, GH regulates many aspects of growth, metabolism, and nutrient partitioning.

Insulin-Like Growth Factor-1

As mentioned above, GH is derived from the anterior pituitary and acts on distant target sites such as bone, muscle, reproductive organs, immune and nervous systems (Martini et al., 1995; Ohlsson et al., 1993). However, many of the actions of GH are mediated through IGF-1. Similar to GH, IGF-1 plays a crucial role in postnatal somatic growth (Stewart & Rotwein, 1996), especially during pubertal growth spurts (Yakar, Liu, & Le Roith, 2000; Yakar et al., 1999).

IGF-1 is a member of a family of insulin-related peptides such as IGF-1, IGF-2, and insulin (Rinderknecht & Humbel, 1978). Structurally, IGF-1 is a small peptide that contains 70 amino acids and that has a molecular weight of 7649 Da. IGF-1 and insulin share a common ancestor of the proinsulin gene (Rinderknecht & Humbel, 1978), and IGF-1 has the ability to bind to the insulin receptor (Ullrich et al., 1986) albeit with weaker affinity than insulin.

IGF-1 binds to the IGF-1 receptor (IGF-1R) on cell membranes and assorted IGF binding proteins (IGFBPs) in the serum. The latter behaves as protection for IGF-1 from degradation in the serum (Kato, Faria, Stannard, Roberts, & LeRoith, 1993; Zapf, Hauri, Waldvogel, & Froesch, 1986). IGF-1 binds to IGF-1R on cell surfaces to activate intercellular signaling pathway, leading to cellular proliferation and differentiation among other functions (Kato et al., 1993). The IGF-1R is a member of the tyrosine kinase growth factor receptor family (Kato et al., 1993) and is present in almost all tissues, whereas IGF-1 expression is the highest in the liver. In addition, IGF-1 has functions in an autocrine or paracrine manner by most tissues (Yakar et al., 1999).

Growth Hormone/Insulin-Like Growth Factor-1 Axis

Animal and clinical evidence support that GH enhances IGF-1 expression in the liver and nonhepatic tissues (Lowe, Lasky, LeRoith, & Roberts, 1988). On the other hand, IGF-1 forms a negative feedback on GH secretion through acting on the hypothalamus and the anterior pituitary. Therefore, GH and IGF-1 work collectively to balance the expression of the other.

Growth Hormone/Insulin-Like Growth Factor-1 Axis and Adipose Tissue

Acute and chronic effects of growth hormone on adipose tissue. As stated previously, GH exerts an overall lipolytic effect although timing is important. However, GH was proposed to possess an opposing role in regulating lipid metabolism (Honeyman & Goodman, 1980). That is, GH plays an insulin-like role favoring antilipolytic and lipogenic processes in the early stage (Adamafio & Ng, 1984). For instance, in rat adipocytes, glucose uptake and inhibition of lipolysis are increased with added GH at the first hour (Carter-Su, Rozsa, Wang, & Stubbart, 1988; Goodman, 1965b). The acute effects of GH are attributed to increased uptake of carbohydrate by the adipocytes (Henderson, Morgan, & Park, 1961). However, 3-4 hours after injection of GH, the glucose utilization in adipose tissue is apparently reduced in vitro. Again, the chronic effect of GH is lipolytic through inhibiting lipoprotein lipase and enhancing hormone-sensitive lipase activity (Dietz & Schwartz, 1991), which stimulates TAG breakdown. For example, GH treatment reduces abdominal obesity (Kamel, Norgren, Elimam, Danielsson, & Marcus, 2000).

Effects of growth hormone on gender difference and adiposity. GH levels are different between genders, which influences growth and body composition. Female rats and mice have lower GH levels than male animals and exhibit an irregular interval. Male rats and mice have a higher GH peak, which causes or at least contributes to a muscular phenotype (Eden, 1979; MacLeod, Pampori, & Shapiro, 1991). In acromegalic men, total mass, lean mass, and bone mineral content are greater than female patients while fat mass is lower in men and negatively related to GH and IGF-1 levels, suggesting males are

more affected than females (Sucunza et al., 2008). It has been well documented that abdominal fat mass is negatively related to testosterone (Seidell, Bjorntorp, Sjostrom, Kvist, & Sannerstedt, 1990) and GH levels (Hansen, Vahl, Jorgensen, Christiansen, & Hagen, 1995; Vahl, Jorgensen, Jurik, & Christiansen, 1996). Therefore, the sexual dimorphism of GH interacts with gonadal hormone in regulating adiposity.

Depot-specific effect of growth hormone. Interestingly, GH has a fat-depot-specific effect (Bengtsson et al., 1993; Rosenbaum, Gertner, Gidfar, Hirsch, & Leibel, 1992). In humans, fat mass weight and adipocyte size in the abdominal region is strikingly reduced when patients with GHD were treated by GH (Bengtsson et al., 1993; Rosenbaum et al., 1992). In mice, reduced GH causes a two- to three-fold fat mass increase in epididymal and subcutaneous sites (Pomp, Oberbauer, & Murray, 1996). Several mouse models with reduced GH action also exhibit an increased, but non-uniform distribution of fat mass (Berryman et al., 2004). Both $GHR^{-/-}$ and GHA mouse exhibit a disproportionate amount of fat mass in the subcutaneous depot (Berryman et al., 2004; Berryman et al., 2010; Magon, 2009).

Growth hormone receptor in adipose tissue. It is well known that GHR plays an important role in mediating effects of GH in adipose metabolism. As mentioned before, GHR is widely expressed in many tissues, being highest in liver and adipose tissue in rats (Tiong & Herington, 1991). GHR expression varies according to different fat depots and species. The number of GH binding sites in rats is greater in epididymal than subcutaneous and retroperitoneal adipocytes (LaFranchi, Hanna, Torresani, Schoenle, & Illig, 1985). As compared to perirenal and subcutaneous depots, GHR mRNA is highly

expressed in omental fat in pigs (Brameld et al., 1996). In women, GHR mRNA is expressed in subcutaneous and intraabdominal fat mass (Fisker et al., 2004). In addition, diet and GH interaction influences GHR expression. That is, GH treated pigs fed with low protein have increased GHR expression in adipose tissue (Brameld et al., 1996). GH treatment in GHD patients increases truncated GHR mRNA expression in the abdominal subcutaneous adipose tissue (Fisker et al., 2001). Additionally, another study reported that GHR mRNA expression was up-regulated during the process of preadipocyte-adipocyte differentiation in 3T3-L1 mouse cells (Fleenor, Arumugam, & Freemark, 2006; Zou, Menon, & Sperling, 1997).

Growth hormone on adipocyte differentiation and proliferation. GH has an effect on adipogenesis including preadipocytes differentiation and proliferation depending on the cells used in studies (Doglio, Dani, Grimaldi, & Ailhaud, 1986; Morikawa, Nixon, & Green, 1982; Vassaux, Negrel, Ailhaud, & Gaillard, 1994; Yarwood, Kilgour, & Anderson, 1998). With regard to 3T3-F442A preadipocytes, GH stimulates the differentiation process (Morikawa et al., 1982) while inhibiting proliferation (Tang, Jeoung, & Sonenberg, 1995). When using primary preadipocytes (Vassaux et al., 1994) and 3T3-L1 cells (Tominaga, Morikawa, & Osumi, 2002), GH inhibits preadipocyte differentiation; however, it promotes proliferation in primary cultures (Wabitsch et al., 1996). In later stage, GH inhibits the terminal differentiation in primary rat preadipocytes (Hansen, Madsen, Teisner, Nielsen, & Billestrup, 1998). In children with GH deficiency, GH treatment increases the subcutaneous adipocytes number while decreasing size dramatically (Bonnet et al., 1974). Overall, GH promotes preadipocytes

differentiation into mature adipocytes, while reducing the volume of mature adipocytes and body fat (Wabitsch, Hauner, Heinze, & Teller, 1995).

Effects of insulin-like growth factor-1 on adipose tissue. IGF-1 plays a lipogenic role in vivo. Since IGF-1 is a member of the insulin family and can bind to insulin receptors, it impacts adipose tissue in a manner similar to insulin. That is, high levels of IGF-1 promote lipogenesis and inhibit lipolysis. IGF-1 stimulates expression of sterol response element-binding protein-1 (Smith, Cong, Gilliland, Clawson, & Thiboutot, 2006) through activating mitogen-activated protein kinase, phosphoinositide 3-kinase (PI3K), and Akt pathway, thus promoting lipogenesis (Smith, Gilliland, Clawson, & Thiboutot, 2008). IGF-1 decreases non-esterified fatty acid concentration (Schmitz, Hartmann, Stumpel, & Creutzfeldt, 1991) and stimulates lipogenesis in epididymal fat pats (Guler, Zapf, & Froesch, 1987). Generally, GH and IGF-1 have opposite effects on body fat metabolism.

Region-specific expression of insulin-like growth factor-1. As reported previously, IGF-1 mRNA is highly expressed in WAT and liver. GH treatment will restore the IGF-1 levels in WAT for hypophysectomized rats (Peter, Winterhalter, Boni-Schnetzler, Froesch, & Zapf, 1993). Like fat-specific effects of GH, IGF-1 mRNA is expressed in a site-specific manner. IGF-1 mRNA is high in retroperitoneal and epididymal fat pads but lower in mesenteric and subcutaneous depots (Villafuerte et al., 2000). IGF-1 is regulated by GH, e.g., porcine GH increases the amount of IGF-1 mRNA expressed in the subcutaneous fat depot in growing swine (Wolverton, Azain,

Duffy, White, & Ramsay, 1992). Region-specific distribution of IGF-1 mRNA is related to adipogenesis.

Insulin-like growth factor-1 and adipocyte differentiation. IGF-1 promotes preadipocyte differentiation and proliferation (Christoffersen et al., 1998; Sekimoto & Boney, 2003). IGF-1 plays a role in clonal expansion in adipose tissue because preadipocytes are sensitive to IGF-1 (Zezulak & Green, 1986). A previous study revealed that IGF-1 mRNA is localized in both adipocytes and stromal-vascular cells which also includes preadipocytes, thus sufficient concentration of IGF-1 can induce adipocytes differentiation in vitro and in vivo (Peter et al., 1993). A subsequent study finds that IGF-1 alone regulates the early preadipocytes differentiation in mesenteric and subcutaneous depots and synergistically with insulin improves maturation of adipocytes in vivo (Sato et al., 2008). Therefore, GH/IGF-1 axis cooperates to regulate adipogenesis.

Growth Hormone/Insulin-Like Growth Factor-1 Axis and Glucose Homeostasis

The GH/IGF-1 axis plays a profound role in regulating glucose metabolism. GH and IGF-1 have opposite functions on glucose metabolism. GH antagonizes action of insulin while IGF-1 has a similar function to insulin. The mechanism how GH and IGF-1 affect glucose homeostasis is still unclear.

GH is viewed as a diabetogenic factor. In the 1930s, researchers found that hypophysectomy-reduced hyperglycemia in diabetic dogs (Houssay & Biasotti, 1930). GH stimulated hepatic gluconeogenesis and glycogenolysis while peripheral glucose utilization declined (Bak, Moller, & Schmitz, 1991; Fowelin, Attvall, von Schenck, Smith, & Lager, 1991; Press, Tamborlane, & Sherwin, 1984). In adipocytes studies,

PI3K pathway regulates GH induced insulin resistance. Excess GH causes increased p85α (the subunit of PI 3-kinase) expression in adipose tissue, thus leading to insulin resistance (del Rincon et al., 2007). With regard to the diabetogenic properties of GH, it was found that nonesterified fatty acids (NEFA) are produced by GH prompting hepatic glucose output and decreasing peripheral glucose oxidation in glucose-fatty acid cycle (Ferrannini, Barrett, Bevilacqua, & DeFronzo, 1983; Sjogren et al., 2001). In summary, GH induces a high level of serum glucose, and the glucose cannot be normally utilized by the tissue.

It still remains unclear how IGF-1 influences glucose homeostasis. When IGF-1 gene is disrupted, serum IGF-1 levels dramatically decline to 15-25%. However, GH increases approximately six-fold. In spite of IGF-1 deficiency, there is no apparent difference in glucose concentration, whereas the liver IGF-1-deficient animal exhibits hyperinsulinemia and insulin resistance (Sjogren et al., 2001). In humans, both the intravenous (Zenobi, Graf, Ursprung, & Froesch, 1992) and subcutaneous infusion of IGF-1 cause hypoglycemia by increasing peripheral glucose uptake, oxidation, and suppressed hepatic glucose production (Boulware, Tamborlane, Rennert, Gesundheit, & Sherwin, 1994). Hence, IGF-1 decreases glucose concentration, like insulin. Overall, IGF-1 is considered an insulin sensitizer while GH antagonizes insulin function (Goodman, 2009).

Transgenic animals with GH over expression develop insulin resistance, significant hyperinsulinemia, and hyperglycemia (Valera et al., 1993). In humans, patients with acromegaly also develop the above symptoms, and up to 40% of patients

become diabetic (Colao et al., 2000; Ezzat et al., 1994). Interestingly, adults with GHD appear to have hyperglycemia, severe insulin resistance and hyperinsulinemia due to increased central fat mass. The molecular mechanisms involved are not clear (Alford, Hew, Christopher, & Rantzau, 1999).

Mouse Models with Altered Levels of Growth Hormone Action

Mouse models with altered GH action have proven a valuable means to study the physiological impact of GH in the whole animal. Many features of these mouse models share striking features with human clinical conditions of altered GH function and have begun to provide insight into the molecular mechanisms responsible for the some of the reported clinical phenotypes associated with altered GH action. The specific mouse model that will be the focus of this thesis, GHA transgenic mice, will not be discussed here but will be more thoroughly addressed in subsequent sections.

bovine Growth Hormone Transgenic Mice

The bGH transgenic mice overproduce circulating GH. These mice exhibit features similar to acromegaly: gigantism, organomegaly, increased lean mass, and reduced fat mass. Interestingly, bGH mice have more fat mass than wild type (WT) mice before 6 weeks of age. The body composition changes during aging are gender-specific. For example, the changes of lean and fat mass in females occur later than in males (Palmer et al., 2009). In terms of specific fat depots, percentage of retroperitoneal, epididymal (Berryman et al., 2004), subcutaneous, and mesenteric (Palmer et al., 2009) fat masses are lower in bGH versus WT mice. This reduced mass is unique as the absolute weights of almost all organs are greater in bGH than siblings especially kidney,

liver, and heart (Berryman et al., 2004; Palmer et al., 2009). Furthermore, bGH mice have the ability to resist diet-induced obesity, although these mice exhibit hyperphagia, suggesting altered nutrient partitioning (see Table 1). Indeed, a HF diet has been shown to induce weight gain but half of that weight gain is attributed to gains in lean mass (Berryman et al., 2006) (see Figure 3). When feeding bGH mice a HF diet, all fat pads increase but are still lower than controls, whereas kidney and liver weights are still higher than control groups (Berryman et al., 2006; Olsson et al., 2005). In addition, concentrations of glucose, insulin, and lipids also show significant alterations. On a LF diet, the bGH mice are insulin resistant with slightly impaired (Berryman et al., 2006; Kopchick et al., 1999) or normal glucose tolerance (Balbis, Dellacha, Calandra, Bartke, & Turyn, 1992; McGrane et al., 1990) (see Table 1). Also on a LF diet, the levels of very-low-density lipoprotein (VLDL), free fat acids (FFA) and hepatic TAG dramatically decreased, whereas high-density lipoprotein, low-density lipoprotein (LDL), and total cholesterol levels increased in bGH mice compared to controls (Frick et al., 2001; Olsson et al., 2005). As a result, bGH mice have a better lipoprotein profile and maintain relevant good lipoproteins in circulation. However, bGH mice have markedly decreased lifespan in comparison to their siblings (Wolf et al., 1993).

Growth Hormone Receptor/Binding Protein Knockout Mice

The GHR/binding protein (GHR/BP) knockout ($GHR^{-/-}$) mouse is generated by disrupting the GHR/BP gene via homologous recombination (Zhou et al., 1997). Absent GHR produces a dwarf stature as well as strikingly low IGF-1 and high GH levels in circulation. IGF-1 and IGFBP-3 (main form of IGFBP) levels in $GHR^{-/-}$ mice sharply

decrease to approximately 20% and less than 10% of the controls, respectively (Coschigano et al., 2003). Meanwhile, both insulin and fasting glucose levels are reduced to 26-10% and 65-86% compared to controls, respectively, whereas insulin sensitivity increase remarkably (Coschigano et al., 2003). Again, this mouse line has a higher percentage of fat mass as well as excess fat mass accumulated in the subcutaneous region (see Table 1). In particular, $GHR^{-/-}$ mice exhibit higher retroperitoneal and subcutaneous fat mass when normalized to body weight; whereas epididymal mass is not significantly different (Berryman et al., 2004) or decreases compared to littermates (Coschigano et al., 2003). Furthermore, percentage of lean mass dramatically decreases with aging (Bonkowski et al., 2006; Egecioglu et al., 2005). Consistent with their smaller body size, all organs weights are less than controls (Berryman et al., 2004). Interestingly, leptin levels are drastically increased 4.8 times, as might be expected with their excess fat mass (Egecioglu et al., 2005). Additionally, $GHR^{-/-}$ mice tend to be more sensitive to diet-induced obesity as compared to littermate controls, again suggesting unique nutrient partitioning. In one HF feeding study, $GHR^{-/-}$ mice was the largest in percent weight change among $GHR^{-/-}$, bGH, and WT littermates (see Figure 3). Specifically, when challenged with a HF diet, $GHR^{-/-}$ mice appear to increase all fat mass especially in epididymal, retroperitoneal, subcutaneous, and scapula compartments (Berryman et al., 2006; Robertson, Kopchick, & Liu, 2006) (see Table 1). $GHR^{-/-}$ mice with the C57Bl/6J background live nearly 26% longer than control littermates (Coschigano et al., 2003). The $GHR^{-/-}$ mouse model is comparable to a clinical disease in humans called Laron syndrome (growth hormone insensitivity syndrome), in which individuals exhibit a

severe postnatal growth retardation and deranged metabolism including a very short stature, facial dysmorphism, truncal obesity, delayed puberty, and recurrent hypoglycemia (Li et al., 1990; Zhou et al., 1997).

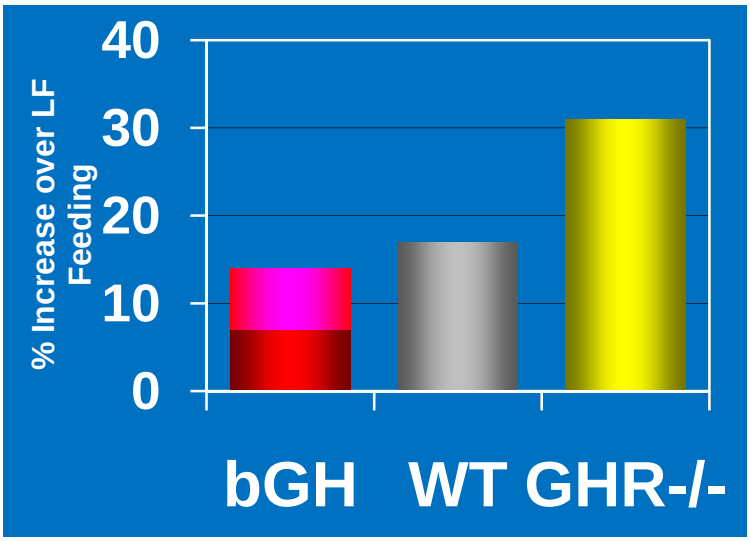


*Figure 3.*Comparison of percent body weight among male WT, bGH, and GHR$^{-/-}$ mice in a HF diet feeding. From "Effect of growth hormone on susceptibility to diet-induced obesity," by D. E. Berryman, E. O. List, D. T. Kohn, K. T. Coschigano, K. T. Seeley, and J. J. Kopchick, 2006 [Unpublished PowerPoint presentation]. Reprinted with permission of the author.

Table 1

Summary Diet Manipulation of bGH and GHR$^{-/-}$ Mouse Data

Reference	Gene	Gender	Diet	Age (Duration)	Food Intake, Energy Intake, and EE	Body composition	Glucose	Insulin
Olsson et al., 2005	bGH	♂	HF[1] LF[2]	6 m (8wk feeding)	• On HF: food intake was not changed, but energy intake and EE ↑ • On LF: NS in intake but EE ↑	• bGH mice are lean and resistant to diet-induced weight gain • Retro & BAT weights were not changed; Epi ↓	↑in HF induced diabetic condition	HF & LF led to insulin resistance and hyperinsulinemia
Berryman et al., 2006	bGH	♂	HF[3] LF[4]	10 wk (12 wk feeding)	bGH mice consumed more food & were hyperphagic when feeding HF	• bGH mice are lean • HF: all absolute weights of fat pads ↑, but relatively less than controls, suggesting that protecting from excess fat storage	HF and LF increased glucose	Either diet stimulated higher insulin levels
Berryman et al., 2006	GHR$^{-/-}$	♂	HF[3] LF[4]	10 wk (12 wk feeding)	When fed HF, GHR$^{-/-}$ mice consumed more energy with no increase in food intake	• GHR$^{-/-}$ mice were relatively obese whether on HF and LF • On HF, BW gain was attributed to gains in fat mass • Fad pads significantly increased 2- to 2.7-fold in all depots • On LF, GHR$^{-/-}$ had greater proportional Sc	Glucose < WT in either diet	Insulin levels were lower and exhibiting insulin sensitivity in HF & LF
Robertson et al., 2006	GHR$^{-/-}$	♂	HF[5]	3.5m (17 wk feeding)	NR	• GHR$^{-/-}$ mice are obese on HF • Fat pads increased in visceral, leg, and scapula with HF • No change in perirenal with HF feeding	Dramatically↓	↓ on HF, but no insulin resistance or prompt insulin sensitivity

Note. EE: energy expenditure; Epi: epididymal fat mass; Intra: intraabdominal fat mass; Retro: retroperitoneal fat mass; Sc: subcutaneous fat mass; BW: body weight; WT: wild type mice.
NR: not reported. NS: no significantly statistical difference.
1. HF diet (R-638, AnalyCen Nordic AB, Lidkoping, Sweden) (in energy percent): 39.9% fat, 17% protein, 0.7% fiber, and 42.3% nonfat energy.
2. LF diet (R-34, Lactamin, Vadstena, Sweden) (in energy percent): 9.4% fat, 20.2% protein, 0.8% fiber, and 69.6% nonfat energy.
3. HF diet: Dyets (Bethlehem, PA); 20g of fat (19g butter oil and 1g soybean oil) per100g of diet, provided 4.54kcal/g of diet.
4. LF diet: Dyets (Bethlehem, PA); 3g butter oil and 1g soybean oil per100g of diet, provided 3.81kcal/g.
5. HF diet (Research Diets, New Brunswick, NJ), no details are provided.

Adipose Tissue

In the past, adipose tissue was regarded as a simple energy warehouse where excess energy was stored. Nearly two centuries ago, researchers began to hypothesize that adipose tissue was also an endocrine gland (Lindberg, 1970). When leptin was discovered in 1994, adipose tissue became more widely recognized as an endocrine organ (Zhang et al., 1994). Adipose tissue has broad physiological functions. In addition to energy storage, insulation, and thermoregulation, it also has a role in reproduction, inflammation, angiogenesis, hypertension, immunity, regulation of proliferation, and others (Fruhbeck, 2008). Many of these various actions are conducted by secreting a variety of enzymes, hormones, growth factors, adipokines, and other factors. Many receptors of these factors are also found in adipocytes, suggesting adipose tissue regulates local and systemic metabolism by cross talking (Fruhbeck, 2008).

Physiology of Adipose Tissue

Types of Adipose Tissue

In mammals, based on the morphological structure, distribution, and function, adipose tissue consists of two main types: brown adipose tissue (BAT) and white adipose tissue (WAT). Both types of adipose tissues have the capacity to store energy to varying degrees; however, BAT also has a role in regulating body temperature (Louis Casteilla, 2008; Matthias et al., 2000). BAT secretes a less amount of adipokines as compared to WAT, suggesting that WAT has more endocrine functions (Farmer, 2008; Vazquez-Vela, Torres, & Tovar, 2008).

Adipose tissue, both WAT and BAT, is composed of a specialized loose connective tissue with lipid-laden adipocytes. Besides adipocytes, other cells such as macrophages, fibroblasts, blood cells, endothelial cells and pericytes are found in this tissue and account for around 50% of total cell population (Fruhbeck, 2008). Therefore, adipose tissue is a complex and heterogeneous tissue.

Brown Adipose Tissue

BAT is a reddish brown adipose tissue that contains numerous multilocular cells. The brown adipocyte size varies from 15μm to 50μm, which is smaller than white adipocytes. Structurally, the nucleus is located in the center of brown adipocytes surrounded by multiple small lipid droplets. BAT is filled with a large amount of blood vessels and abundant mitochondria and lysosomes. BAT appears brown in color due to a high level of cytochromes in the mitochondria and hemoglobin in the vascular system (Malina, 2004). Abundant mitochondria in BAT cells conduct an oxidative phosphorylation which releases heat (Fruhbeck, 2008). Therefore, the major function of BAT is to facilitate newborn babies and small animals to adjust to cold weather by converting fat mass to heat through thermogenesis (Mouroux, Bertin, & Portet, 1990; Rafael & Heldt, 1976) and to maintain the constant body temperature when hibernating (Nedergaard, Connolly, & Cannon, 1986). In an acute cold challenge, the sympathetic nervous system stimulates thermogenesis through the oxidation of BAT lipids and by increasing the number of mitochondria and blood flow (Lindberg, 1970). Also, the proliferation and differentiation of the precursors into BAT adipocytes help with adapting body's metabolic need when exposed to an acute cold acclimation (Himms-Hagen,

1990). Unlike WAT, BAT is distributed only in several regions in humans, including depots around the kidneys, in the back of the neck, and in the interscapular region of the back (iBAT) in newborn infants. Unexpectedly, a recent study find that BAT still exists in adult humans rather than reducing after infancy (Truong et al., 2004). The hypermetabolic BAT is detected in the cervical, supraclavicular, paravertebral, mediastinal, para-aortic, and suprarenal regions (but not interscapular) by fluorodeoxyglucose positron emission tomography (Nedergaard, Bengtsson, & Cannon, 2007).

White Adipose Tissue

Structure. WAT is a white-yellowish mass made up of countless unilocular signet-ring cells (Napolitano, 1963). White adipocytes are apparently larger than brown adipocytes, with the average diameter of white adipocytes ranging from 25μm to 150μm. WAT adipocytes are composed of a single, large TAG lipid vacuole and a compressed nucleus and cell organelles, which are squeezed in the narrow area between the lipid droplet and the cell membrane (Malina, 2004). It is reported that the lipid content accounts for 89-90 percentage of the cell volume (Hull, 1966). Apart from adipocytes, precursors cells, preadipocytes (Hauner, Wabitsch, & Pfeiffer, 1988; Weisberg et al., 2003), macrophages, fibroblasts, and endothelial cells are found in WAT filtrating the aperture of adipocytes (Weisberg et al., 2003). It is well known that there is a vast network of capillaries that exist among the interconnected adipose tissue cells, making adipose tissue a well-innervated and highly vascularized structure. In contrast to BAT,

WAT is evident throughout the body. The majority is located at subcutaneous areas, and the rest partly gathers around the viscera, kidneys, liver, and other organs (Malina, 2004).

Physiology and function. Not surprisingly, the amount of WAT is far more than BAT at birth. Approximately 16% of total body weight is WAT compared to BAT (2-5%, as stated above) (Fruhbeck, 2008). With aging, WAT dramatically increases in both size (from 30-40μm at birth to 80-100μm in adulthood) and in cell number (from 5 billion at birth to 30-50 billion in adulthood). The basic function of WAT is to be the source and reservoir of energy as lipids for the organism (Himms-Hagen, 1990). When excess food is taken in, TAGs are formed and stored in WAT. On the other hand, when the body is in a state of hunger, TAGs in fat depots are mobilized and broken down into free fatty acids that are released into the circulation in order to provide enough metabolic fuel (Cahill, 1976). In addition, the subcutaneous WAT provides thermal protection from cold temperature (Mohamed-Ali, Pinkney, & Coppack, 1998; Wang, Mariman, Renes, & Keijer, 2008).

Since leptin was discovered, WAT has been regarded as a multifunctional organ through autocrine, paracrine, and endocrine pathways rather than only a repository site for energy (Fruhbeck, Aguado, & Martinez, 1997; Mohamed-Ali et al., 1998). Adipocytes secrete various hormones, growth factors, enzymes, cytokines, complement factors, and matrix proteins, termed adipokines, which are involved in fat metabolism, vascular system, inflammation and the immune system (Fruhbeck, 2008; Fruhbeck et al., 1997; Hotamisligil, Shargill, & Spiegelman, 1993; Kintscher et al., 2008; Rondinone, 2006; Tordjman, Guerre-Millo, & Clement, 2008). Many target organs or systems,

including the brain, bone (Ducy et al., 2000), pancreas (Morton, Emilsson, de Groot, Pallett, & Cawthorne, 1999), liver (Cohen, Novick, & Rubinstein, 1996), skeletal muscles (Kellerer et al., 1997), immune system (Lord et al., 1998), and blood vessels (Sierra-Honigmann et al., 1998), are regulated by adipokines (Rajala & Scherer, 2003).

Adipokines play a role in inflammation, obesity, and insulin resistance. For example, tumor necrosis factor-α (TNF-α), interleukin 6 (IL-6), IL-8, plasminogen activator inhibitor-1 (PAI-1), angiotensinogen, and others are directly or indirectly involved in blood pressure, inflammation, atherogenesis, fibrinolysis, angiogenesis, apoptosis and immunity. Epidemiological studies allude that there is an association between inflammation and insulin resistance (Festa et al., 2000). In addition, some adipokines also take part in immunity and energy balance regulation (Fruhbeck, 2008). For instance, adiponectin reduces body weight (Matsuzawa, 2005; Yamauchi et al., 2001) and decreases insulin resistance (Yamauchi et al., 2001). However, TNF-α, resistin, and IL-6 play opposite roles in obesity-associated insulin resistance and the pathogenesis of type 2 diabetes (Hotamisligil, Murray, Choy, & Spiegelman, 1994; Senn et al., 2003; Steppan et al., 2001). That is, TNF-α regulates inflammatory response and impairs insulin signaling via serine phosphorylation triggered by obesity (Peraldi, Hotamisligil, Buurman, White, & Spiegelman, 1996). In the current review, leptin will be discussed further due to its relevance to this thesis.

Leptin

Structure and its receptor. Leptin is a 16-kDa polypeptide consisting of 167 amino acid residues and is predominantly produced by adipocytes. This protein is

encoded by the ob gene that was first discovered by a naturally occurring mutation in leptin-deficient obese mice (Campfield et al., 1995; Fei et al., 1997; Lee et al., 1996; Mercer et al., 1996; Stephens et al., 1995; Tartaglia et al., 1995; Zhang et al., 1994). The central nervous system plays an important role in reducing weight, and leptin receptors are found in the hypothalamus (Campfield et al., 1995; Stephens et al., 1995). The leptin receptor, ObRa, is produced in the choroid plexus and in microvessels (Mercer et al., 1996; Tartaglia et al., 1995). Leptin receptors are also expressed in a number of peripheral tissues, such as the lungs, kidney, lymph nodes, liver, adipose tissue and skeletal muscle (Fei et al., 1997; Lee et al., 1996; Tartaglia et al., 1995). Thus, leptin has the ability to impact a variety of tissues.

Mechanism of function. In the brain, leptin acts on two pathways: one is the anorexigenic pathway in which leptin stimulates on pro-opiomelanocortin neurons, cocaine, and amphetamine-related transcript neurons; the other is to inhibit the orexigenic pathway which is controlled by neuropeptide Y and agouti-related protein (Beales, Farooqi, & O'Rahilly, 2009). Through these pathways in the brain, elevated leptin decreases appetite and increases energy expenditure. In contrast, decreased leptin stimulates food intake and decreases energy consumption (Friedman, 2002; Rondinone, 2006).

Leptin has additional functions in the body. In terms of glucose metabolism, leptin down-regulates glucose transport into adipocytes and stimulates muscle cells to utilize glucose indirectly (Fruhbeck et al., 1997; Haque et al., 1999; Kahn, Alquier, Carling, & Hardie, 2005; Kamohara, Burcelin, Halaas, Friedman, & Charron, 1997).

Regarding insulin sensitivity, leptin promotes translocation of fatty acids into mitochondria and accelerates beta-oxidation in the mitochondria, thus improving insulin sensitivity (Kahn, Alquier, Carling, & Hardie, 2005) and lypolysis (Fruhbeck et al., 1997). Overall, leptin decreases food intake, increases energy expenditure, and causes weight loss (Beales et al., 2009; Clement et al., 1998; Farooqi et al., 2002; Pelleymounter et al., 1995). Other less well-characterized functions of leptin in other tissues will not be discussed here.

Leptin deficiency. A homozygous mutation on the ob gene and leptin receptor gene (lepr) leads to a severe obese phenotype in both humans and mice (Montague et al., 1997; Zhang et al., 1994). Symptoms include severe hyperphagia, intensive food-seeking behavior, aggressive behavior without food, and hyperinsulinemia (Farooqi et al., 2002). Leptin deficiency can be improved by injecting recombinant human leptin (Bluher, Shah, & Mantzoros, 2009).

Leptin resistance. Generally, plasma leptin levels are positively correlated with the proportion of body fat mass; therefore, obese individuals have higher levels of leptin (Considine et al., 1996; Van Heek et al., 1997). However, high leptin levels should result in decreases in body weight based on the known function of leptin. Since approximately 90-95% of obese individuals have high concentrations of leptin, it has been suggested that obesity in most cases is due to leptin resistance (Maffei et al., 1995). Two probable mechanisms cause leptin resistance. One is reduced leptin transport across the blood brain barrier, which decreases sensitivity to peripheral leptin signaling and develops peripheral leptin resistance (Van Heek et al., 1997). The other mechanism is reduced

leptin signaling in target neurons (Hommel et al., 2006). Thus, leptin resistance results in failed regulation of food intake, lipid, and glucose metabolism.

Plasticity of Adipose Tissue

Adipose tissue is not a static organ, but rather has the capability of remodelling itself with the appropriate stimuli. For example, there is some evidence that adipocytes can convert between WAT and BAT phenotypes. In rats, some brown adipocytes can be found in white adipose depots (Cousin et al., 1992). Transforming from brown into WAT occurs after birth (Gemmell, Bell, & Alexander, 1972). Adipocytes have a potential ability to transform depending on different fat pads: WAT has more plastic ability than BAT; in particular, the subcutaneous fat appears more plastic than the internal fat (Guerra, Koza, Yamashita, Walsh, & Kozak, 1998; Prunet-Marcassus et al., 2006). This transformation occurs based on physiological, pharmacological, pathophysiological conditions, and genetic backgrounds (Cousin et al., 1992; Guerra, Koza, Yamashita, Walsh, & Kozak, 1998). In addition, the preadipocytes within adipose tissue are a pool of somatic stem cells (Tomii et al., 2005). Mesenchymal stem cells from adipose tissue show self-renewal and multipotentiality, implying that preadipocytes has ability to convert to multiple cell lines (Guilak et al., 2006; Zuk et al., 2002). Furthermore, adipokines produced by adipose tissue play in role in inflammation and immunity (Hotamisligil et al., 1993; Mohamed-Ali et al., 1997; Sawdey & Loskutoff, 1991), indicating that adipose tissue has enormous plasticity in functional properties. Overall, plasticity in adipose tissue can influence proliferation (Cousin et al., 1993),

differentiation (Klaus, Cassard-Doulcier, & Ricquier, 1991), transdifferentiation, and apoptosis (Prins, Walker, Winterford, & Cameron, 1994).

As stated before, adipose tissue contains multiple cells, including blood, endothelial, adipose precursor, and fibroblasts cells (Geloen, Roy, & Bukowiecki, 1989). Adipose adult stem cells as mentioned above have capacity to convert into multiple cell lineages. In particularly, adipose stem cell is pluripotent stem cell that acts on differentiation into macrophage-like (Charriere et al., 2003), angiogenic (Planat-Benard, Silvestre et al., 2004), osterogenic (Birk et al., 2006), haematopoietic (Corre et al., 2006), neurogenic cells (Safford et al., 2002), cardiomyocytes (Planat-Benard, Menard et al., 2004), and myotubes (Zuk et al., 2002) besides adipogenic cells (Vazquez-Vela et al., 2008; Zuk et al., 2002).

Adipose Depots

Fat depots are metabolically different according to locations. In humans, adipose tissue can generally be divided into subcutaneous and visceral (mesenteric and omental) depots (Vazquez-Vela et al., 2008). In small mammals, such as rats and mice, adipose tissue includes subcutaneous (upper and lower), mesenteric, perigonadal, and retroperitoneal fat masses (Cinti, 2001). Functions of adipose tissue vary depending on different locations. Subcutaneous adipose tissue, as the name implies, is located underneath the skin and maintains body temperature. Visceral adipose fills the room between viscera (Vazquez-Vela et al., 2008) protecting internal organs from mechanical injury. Further, while all depots can produce adipokines, differing depots produce them at varying amounts. For example, IL-6 (Fried, Bunkin, & Greenberg, 1998), PAI-1

(Alessi et al., 1997), and resistin (McTernan et al., 2002) are secreted higher in visceral than in subcutaneous adipose tissue; however, leptin (Van Harmelen et al., 1998) is produced greater in subcutaneous than in visceral fat mass. There are other functional differences besides adipokine production. For example, lipolytic rate is also depot-specific and highly correlating with the size of fat depots. For instance, free fatty acid (FFA) release is also depot dependent with higher rates in upper-body than in lower-body adipose tissue in vivo studies (Jensen & Johnson, 1996; Martin & Jensen, 1991) and more FFA mobilization from subcutaneous, mesenteric, and retroperitoneal fats pads (Tan, Goossens, Humphreys, Vidal, & Karpe, 2004).

Disease risk is related to the location of the fat mass. For example, incidence of insulin resistance and metabolic syndrome are greater with increased visceral fat mass (Bays, 2009; Carey et al., 1997; Lottati, Kolka, Stefanovski, Kirkman, & Bergman, 2009). Further, leptin levels decline upon surgical removal of visceral fat in rat models, thus improving insulin sensitivity in the liver (Barzilai et al., 1999). Thus, the visceral fat pad seems to be particularly problematic for the complications that accompany excess adipose tissue.

Subcutaneous fat depots also contribute to metabolic abnormalities although its role is more controversial. Researchers found that the visceral and deep subcutaneous adipose tissue in obese patients have similar effects on obesity, dyslipidemia, and other chronic diseases (Smith et al., 2001). In addition, most metabolic actions occur in intra-abdominal and subcutaneous depots including deep subcutaneous and posterior subcutaneous depots. In these depots, multiple adipokines are secreted in response to the

pathogenesis of insulin resistance (Abate, Garg, Peshock, Stray-Gundersen, & Grundy, 1995; Cnop et al., 2002; Hube, Birgel, Lee, & Hauner, 1999), glucose intolerance (Weyer, Foley, Bogardus, Tataranni, & Pratley, 2000), cardiovascular risk (Tai, Lau, Ho, Fok, & Tan, 2000), and other metabolic abnormalities, indicating a role for the subcutaneous fat pad in the pathophysiology associated with obesity (Kelley, Thaete, Troost, Huwe, & Goodpaster, 2000).

Pathophysiology of Adipose Tissue

Obesity

Obesity is a state of excessive adipose tissue. Currently, obesity has become a worldwide problem, with approximately 400 million people in the world being classified as obese (World Health Organization, 2006). Obesity increases morbidity as well as rates of diabetes, nonalcoholic fatty liver disease, cardiovascular disease, pulmonary disease, metabolic syndrome, and cancer (Beales et al., 2009). Genetics is unlikely the sole cause for such a significant and rapid increase. Many consider changes in diet and energy expenditure as major causes of the increased obesity rate (Vazquez-Vela et al., 2008). Both hyperplasia (increase in adipocytes cell number) and hypertrophy (increase in adipocytes cell size) contribute to obesity (Spalding et al., 2008). Consequently, increased fat mass results in numerous metabolic complications, including elevated FFAs (Jensen, Haymond, Rizza, Cryer, & Miles, 1989; Roust & Jensen, 1993) and increased inflammatory molecule production (Fontana, Eagon, Trujillo, Scherer, & Klein, 2007). In addition to the metabolic disturbances that accompany obesity, the endocrine function of adipose tissue is also disrupted with obesity. For example, leptin levels are increased

and leptin resistance is observed in obese humans and mouse models (Berryman et al., 2004; Maffei et al., 1995).

Growth Hormone Deficiency and Obesity

Obesity is also influenced by GHD and lower levels of IGF-1. Patients with GHD tend to have obesity, abnormal body composition (Rosen et al., 1993), disorders of lipid metabolism (al-Shoumer, Cox, Hughes, Richmond, & Johnston, 1997; de Boer, Blok, Voerman, Phillips, & Schouten, 1994) and glucose intolerance (Johansson et al., 1995). That is, GHD patients exhibit an increased fat mass (Beshyah et al., 1995), decreased lean mass (De Boer, Blok, Voerman, De Vries, & van der Veen, 1992), reduced body water (Beshyah et al., 1995) and bone mineral mass (Beshyah et al., 1995). Adult patients with GHD are also insulin-resistant (Johansson et al., 1995) due to inhibition of the glucose storage pathway and glycogen synthase activity in peripheral tissues (Hew et al., 1996). A previous study found that the increased fat mass was accumulated at subcutaneous depot on the trunk (De Boer et al., 1992). The subsequent study reveals that increased fat mass is accumulated at upper limbs whereas lean mass is reduced at lower limbs and trunk (Murray et al., 2004). Disorder of lipid includes highly increased total cholesterol, LDL cholesterol, and apolipoprotein B (Colao et al., 1999; de Boer et al., 1994). Consequently, GHD patients appear to suffer from a reduced lifespan (Bates, Van't Hoff, Jones, & Clayton, 1996; Rosen & Bengtsson, 1990).

Obesity and Diabetes

Numerous studies report obesity and type 2 diabetes have a close relationship. Some propose of an “adipo-insulin axis” in which insulin resistance and

hyperinsulinemia may be derived from obesity and/or lead to further obesity (Kahn & Flier, 2000). Insulin has a critical role in glucose and fatty acids metabolism, such as stimulating glucose transport into muscles and adipose tissue, promoting lipogenesis, and inhibiting lipolysis. It still maintains controversial for the causal relationship between obesity and insulin resistance. Possible mechanisms include: (a) insulin secretion is impaired when excess fat mass is deposited (Borkman et al., 1993); (b) enlarged adipocytes are less capable to take up excessive lipids, causing much more lipids to be deposited in ectopic sites, which increases the risk of insulin resistance (Hennes, Dua, & Kissebah, 1997; Wiesenthal et al., 1999); and (c) increased intraportal FFA levels may increase levels of insulin, which causes insulin resistance (Griffin et al., 1999). Further, hypoxia caused by impaired blood flow in enlarged adipose tissue promotes macrophage infiltration which induces insulin resistance (Cinti et al., 2005). In 1998, Ikeda and colleagues further elucidated that fat deposition occurred prior to the onset of hyperinsulinemia, hyperglycemia, and hyperlipidemia in mice, indicating that obesity causes diabetes (Ikeda et al., 1998). Therefore, it is likely that obesity causes insulin resistance which eventually results in type 2 diabetes.

Growth Hormone Receptor Antagonist

Review of Growth Hormone Receptor Antagonist

History and Structure

GH has four α-helices, which are joined by disulfide bridges as mentioned previously. In 1990, Chen et al. discovered three amino acids positioned at 117, 119, and 122 in the third α-helix that play a crucial role in GH action (Chen et al., 1990). It was

noted that the amino acids of bGH positioned at 109-126 consisted of an imperfect arrangement of hydrophobic and hydrophilic residues. The amphipathic α-helix was transformed from Glu-117, Gly-119, and Ala-122 to Leu (E117L), Arg (G119R), and Asp (A122D), thus generating a new "more perfect" amphipathic third helix called bGH-M8. bGH-M8 had a similar affinity to bind to GHR while causing a marked retardation on growth (Chen et al., 1990). This mutated GH inhibits ^{125}I-bGH binding to the liver cell membrane, thus leading to significantly decreased concentrations of IGF-1, elevated GH levels, and a dwarf stature (Chen et al., 1990). Thus, bGH-M8, the mutated GH, was the first reported GHR antagonist (Chen et al., 1990; Harding et al., 1996; Ross et al., 2001).

While bGH-M8 was able to prevent receptor activation and a functional signal transduction (Harding et al., 1996; Ross et al., 2001), it was not clear whether all three amino acids changes were necessary to elicit the antagonistic behavior. In 1991, Chen et al. found that the amino acid at position 119 was the critical amino acid substitution to induce the dwarf phenotype, making the glycine amino acid at position 119 the critical amino acid substitution to induce the dwarf phenotype and antagonistic properties. A single amino acid replacement of glycine 119 for arginine (Arg) resulted in a comparable dwarf stature (Chen, Wight et al., 1991), and alteration at the homologous site in human GH of Gly-120 to Arg (Chen, Chen, Yun, Wagner, & Kopchick, 1994) also resulted in a functional antagonist. Therefore, a single mutated amino acid residue in GH, Gly-119 in mice and Gly-120 in humans, created a significant milestone in GH history as a growth hormone receptor antagonist was now available.

The mutated GH is referred to as growth hormone receptor antagonist (GHR antagonist or GHA). The molecular mass of GHA, approximately ~22kDa, is similar to native GH. Structurally, there is a cleft located in the center of the third α-helix due to Gly being the smallest amino acid and lacking a side chain. However, GHA fills up the cleft by changing the smaller side chain of the glycine reside with an amino acid with a large side group. Apparently, this change of this single cleft is sufficient to render the GH molecule's full antagonistic function (Chen, Wight et al., 1991). Therefore, the alteration of GH structure leads to the failure of functional GHRs. In hGH, any amino acids larger than Ala or Gly (e.g. lysine or arginine) at the position 120 (G120K and G120R mutants) (Chen et al., 1994; Fuh et al., 1992) prevent signal transduction, creating the GH antagonist (Fuh et al., 1992).

Mechanism of Growth Hormone Receptor Antagonist Action

GH binds to two GHRs at two asymmetric sites, forming a GHR/GH/GHR heterotrimeric complex. Site 1 has markedly greater affinity than site 2 (de Vos, Ultsch, & Kossiakoff, 1992). The conformational change of the GHR homodimer due to GH binding induces a functional intracellular signal that results in the expression of IGF-1 as mentioned previously. When GHA competes with GH to bind to GHR, site 1 binding is still proper; however, binding to site 2 is not proper and blocks functional signal transduction in the cell. It is still unclear how GHA binding to site 2 blocks signaling. However, without the proper intracellular signal transduction, intracellular actions due to GH are blocked (Okada et al., 1992).

Pegvisomant

GHA is now a commercially available drug for treating conditions caused by excess GH such as acromegaly and gigantism, but also has the potential to treat other conditions such as diabetes (Flyvbjerg, Bennett, Rasch, Kopchick, & Scarlett, 1999; Goffin et al., 1999), diabetic retinopathy (Flyvbjerg et al., 1999), and nephropathy (Esposito et al., 1996). For the commercial drug, other than replacing glycine120, eight amino acid additional residues are mutated at site 1 in hGH, including H18D, H21N, R167N, K168A, D171S, K172R, E174S, and I179T, improving the competition of GHA with the native GH to bind to the GHR (Cunningham & Wells, 1991; Goffin et al., 1999; Pradhananga, Wilkinson, & Ross, 2002). In order to lengthen the half-life of the GH analog, an addition of *N*-hydroxysuccinimide ester of polyethylene glycol (PEG)-5000, a 5-kDa reagent, is conjugated to primary amino groups to increase the molecular mass (Clark et al., 1996) and prevent excretion by the kidney. These eight amino acids mutations aforementioned plus lysine substitution (the original G120K substitution) and the PEG-5000 form a high potency of GH analog, known as pegvisomant with a trade name Somavert. This drug has an increased half-life and has been successfully used to treat acromegaly (Clark et al., 1996).

Acromegaly

Acromegaly is an adult onset, acquired, and chronic GH disorder caused by the excessive production of GH from a benign pituitary adenoma in most cases. Symptoms include enlargement of the face, extremities, and organs. Other symptoms are related to excess secretion of GH such as lean, soft tissue swelling, hyperhydrosis, diabetes

mellitus, hypertension, sleep apnea, and other conditions (Nabarro, 1987). Again, rheumatologic, neuropathies, cardiovascular, and metabolic tissues are impaired to different degrees. This disease has an insidious onset and slowly progresses for many years. Although acromegaly is very rare, approximately one in 140,000-250,000, rates are increasing with three to four new cases on an annual incidence (Bengtsson & Johannsson, 1998; Orme, McNally, Cartwright, & Belchetz, 1998). Primary causes of mortality are cardiovascular complications and malignancies, such as colonic adenomatous polyps and colonic cancer (Bengtsson, Eden, Ernest, Oden, & Sjogren, 1988; Orme et al., 1998).

Pegvisomant for Treatment of Acromegaly

A major goal in treatment of acromegaly is to decrease IGF-1 levels. Pegvisomant administration is an effective means to decrease IGF-1 levels (Trainer et al., 2000; van der Lely et al., 2001). Numerous clinical data show that the decreasing IGF-1 occurs 2 weeks after beginning pegvisomant treatment; subsequently, IGF-1 maintained a constant, lower levels (Trainer et al., 2000; van der Lely et al., 2001).

As might be expected, pegvisomant also apparently improves symptoms of acromegaly. It improves soft-tissue swelling, excessive perspiration, and fatigue (Trainer et al., 2000). Moreover, this medicine improves metabolic abnormalities, such as lowering serum total cholesterol, LDL, and apo-lipoprotein B levels. In addition, insulin levels decrease and insulin resistance is improved (Parkinson et al., 2002). These changes improve patients' symptoms.

Growth Hormone Receptor Antagonist Mouse Model

Genetic Background of C57Bl/6J

The C57Bl/6J mouse strain is an obese mouse line (Seldin et al., 1994). In previous studies, C57Bl/6J mice tend to exhibit a state of severe obesity, hyperglycemia, and hyperinsulinemia when exposed to a HF, high-sucrose diet, suggesting that this mouse line tends to develop type 2 diabetes (Surwit et al., 1988). In contrast, on a LF diet, mice gain less weight and showed normal levels of glucose and insulin (Surwit et al., 1995). When challenged to a HF diet, their adipose tissue distribution (increased in visceral fat mass and mesenteric regions) is similar to patients with central obesity (Rebuffe-Scrive, Surwit, Feinglos, Kuhn, & Rodin, 1993). Therefore, the C57Bl/6J genetic background strain of mice is considered a good model to study factors such as diet for human progression of obesity, insulin resistance and diabetes.

Physiological Characters of Growth Hormone Receptor Antagonist Mice Line

GHA transgenic mice that express the GHA transgene have been well studied. The majority of studies have utilized mice that have been backcrossed into the C57Bl/6J mice background (99.99% congenic) (Coschigano et al., 2003). The basic physiological characteristics of GHA mice are summarized below in Table 2.

Growth hormone and insulin-like growth factor-1 levels. GHA transgenic mice overexpress GHA, so they maintain low levels of IGF-1 throughout their lifespan. In fact, IGF-1 levels in GHA mice are drastically decreased to approximately 75-80% of nontransgenic mice (Coschigano et al., 2003). Consistent with IGF-1 alteration, IGFBP-3 levels are also reduced to 30% of littermates (Coschigano et al., 2003). Likewise,

because this GH analog has a strong ability to compete with endogenous GH, liver GH receptors and GH binding proteins are elevated in GHA mice (Chen, White et al., 1991). Serum GH levels are significantly increased, which may be attributed to no inhibition by the negative feedback in the hypothalamus-pituitary-GH axis (Chen, White et al., 1991; Trainer et al., 2000).

Body weight. Previous studies show consistently that at least male GHA mice weigh less than counterparts in earlier ages and then catch up to the weight of littermate controls at older ages. For example, GHA mice have been reported to have only 61% of the weight of littermates at 4 weeks of age; however, the male GHA mice catch up with littermate controls in body weight by 44-46 weeks of age (Coschigano et al., 2003). Other studies show other time points in which weight is similar, but in all cases male GHA mice eventually catch up to littermate controls (Magon, 2009). Importantly, gender influences weight gain. Unlike male mice, female GHA mice increase body weight with aging, but at least by 80 weeks of age, have yet to reach the weight of littermate controls (Magon, 2009).

Body composition. In 1992, Okada and colleagues report that GHA has the ability to inhibit mouse 3T3-F442A preadipocyte differentiation and insulin-like response and reduce its lipolytic properties in rat primary adipocytes (Okada et al., 1992). It is therefore assumed that GHA in vivo suppresses the lipolytic action of GH, resulting in obesity. As we expect, GHA mice exhibit a higher percent fat mass relative to nontransgenic controls (Berryman et al., 2004). The fat mass accumulation is gender-specific in GHA mice. While GHA mice exhibited more fat mass in both genders at

earlier ages (6 weeks of age) than counterparts, males start to increase fat mass (at 8 weeks of age) faster than females (at 20 weeks of age). At most ages, absolute fat mass and lean mass in male GHA mice are higher than in female GHA mice (Magon, 2009).

The increase in fat mass in GHA mice is not uniformly distributed among the various depots. Rather, GHA mice preferentially increase absolute and normalized subcutaneous fat mass weight (Berryman et al., 2006); however, there is no significant difference in other depots such as epididymal and retroperitoneal pads (Berryman et al., 2004). Additionally, BAT in the interscapular region (GHR/BP mRNAs located) in GHA mice increases due to repression of GH (Li, Knapp, & Kopchick, 2003). Therefore, different sites of adipose tissues have different changes in response to GH and antagonism of GH.

With regard to fluid mass, both genders of GHA mice have lower absolute fluid than WT counterparts. However, when the fluid weight is normalized to body weight, there is no significant difference between genotypes (De Boer et al., 1992; Magon, 2009). Overall, GHA mice exhibited more fat mass, but less lean mass with lower body fluid.

Insulin and glucose levels. GHA mice maintain slightly lower or normal insulin and glucose levels and normal insulin sensitivity in contrast to littermates (Chen et al., 1994; Chen, White et al., 1991; De Boer et al., 1992). Coschigano et al show that insulin levels in GHA mice is lower in early ages, but gradually increases in later ages. Specifically, insulin levels in GHA mice increased approximately 8-fold from 1 to 11 months of age. Likewise, blood glucose levels at several time points (1, 1.5, 5, and 7 months of age) are significantly lower than WT mice (Coschigano et al., 2003). In

contrast to this result, recent results show that there is no significant difference between GHA mice and nontransgenic mice, or between genders on fasting glucose levels (Magon, 2009).

Food consumption. Male GHA mice display hyperphagia compared to controls. Although GHA and WT mice consume the same amount of food at 2 months of age, male GHA mice consume 43% more than WT mice when normalized to body weight. With aging, the trend decreases: GHA and control mice consume similar food in proportion to their body weight at 8 or 9 months of age (Coschigano et al., 2003). Berryman et al showed that less absolute food weight was consumed by GHA mice from 3 to 5 months of age; however, much more food was consumed when normalized to their body weight (Berryman et al., 2004).

Leptin and adiponectin levels. Since previous studies show that leptin levels are positively related to the quantity of fat mass (Berryman et al., 2004; Considine et al., 1996), it was not surprising that Berryman et al. found that leptin levels were higher in GHA mice than nontransgenic mice. Consistent with this result, female GHA mice displayed higher leptin levels than age-matched littermates (Magon, 2009). Interestingly, the higher level of leptin did not suppress food intake, indicating that there may be some level of leptin resistance in these mice (Berryman et al., 2004). Leptin levels are genotype-and gender-specific; that is, leptin levels are higher in GHA mice than in WT mice, and higher in males than females, reflective of the amount of fat mass in these animals. This significant difference between genotypes occurs after 13 weeks of age.

Like leptin, previous studies revealed that adiponectin is also positively correlated to fat mass (Berryman et al., 2004). Interestingly, Berryman et al show that adiponectin levels are elevated in GHA and $GHR^{-/-}$ obese mice while reduced in lean bGH mice, implicating that there is a positive association between adiponectin and body fat mass in multiple mouse models with altered GH action (Berryman et al., 2004). This is in contrast to what is commonly reported for adiponectin and fat mass (Yamauchi et al., 2001).

Tissue weight. Most absolute organ weights for GHA mice are decreased, especially kidney, liver, and heart compared to counterparts, whereas the brain is similar to littermates (Berryman et al., 2004). Likewise, Coschigano and colleagues reported that gastrocnemius muscle and heart weights are also decreased (Coschigano et al., 2003). Again, when normalized to body weight, almost all organ weights are less than controls (Berryman et al., 2004). It is worthy to note that GHA mice are protected from glomerulosclerosis that causes damage and increases in kidney size (Esposito et al., 1996). In contrast to GHA mice, bGH mice show a severe glomerulosclerosis, presumably resulting from high levels of GH (Chen, Chen, Striker, Striker, & Kopchick, 1997). Female GHA mice have a similar trend in the absolute weight of kidney and liver; however, there is no significant difference in normalized tissue weights in female GHA mice and WT mice. Only the spleen does not show a lower weight in GHA compared to WT mice. In terms of adipose tissue, both the absolute and percent adipose tissue weight is greater in male GHA mice than control groups at both subcutaneous and retroperitoneal depots (Magon, 2009). Also, female GHA mice have more absolute

subcutaneous fat pad in contrast to controls (Magon, 2009; Paolisso, Barbieri, Bonafe, & Franceschi, 2000; Suh et al., 2008).

Lifespan. Excess signaling through the GH/IGF axis has a negative effect on lifespan, and reduced GH and/or IGF-1 action may extend lifespan. The extended lifespan of dwarf mice with blocked GH/IGF-1 pathway may be caused by enhanced stress resistance and reduced age-related diseases (Paolisso, Barbieri, Bonafe, & Franceschi, 2000; Suh et al., 2008). Interestingly, although GHA mice exhibit low levels of IGF-1, GHA mice do not have longer life expectancy, implicating that not all mice with a reduction in GH/ IGF-1 levels exhibit an increase in life expectancy. The lifespan of GHA mice is equal to littermate controls (Coschigano et al., 2003). Though a previous study showed a slight increase in lifespan for female GHA mice, these data do not demonstrate a statistically significant difference (Coschigano et al., 2003). It is still not clear how the GH/IGF-1 axis influences life expectancy, a possible explanation includes that GHA mice have no significant improvement in insulin sensitivity, a factor repeatedly correlated with improvements in longevity (Coschigano et al., 2003).

Table 2

Summary GHA Mouse Data from Studies Using Standard Chow Diets

Reference	Age	Gender	Food intake	Body weight	Body composition	Tissue weight	Glucose and insulin	Leptin and adiponectin level
Li et al., 2003	10-52 wk	♂♀	NR	♂♀ = WT posterior	♂♀ ↑ Iat	• ♂ iWAT and iBAT ↑ • ♂ > ♀ in iAT • ♂♀ ↑ in normalized epididymal WAT	NR	NR
Coschigano et al., 2003	4wk-death	♂♀	Consumed more food than WT in normalized food at earlier ages and consumed = WT at later ages	♂ increase BW with aging and reached the controls by 44-46 week	NR	• Absolute weight of all organs significantly ↓ compare to controls except Epi fat • Gastrocnemius muscle and heart weights↓; Epi. fat mass↑	• Insulin levels ↑ with age • GHA < littermates in glucose levels	NR

Table 2 (Continued)

Reference	Age	Gender	Food intake	Body weight	Body composition	Tissue weight	Glucose and insulin	Leptin and adiponectin level
Berryman et al., 2004	3-6m	♂	Consumed less absolute food; higher food consumption when normalized to BW	< controls	• Higher percent of fat mass • Absolute weight > controls in Sc fat; = WT mice in Epi and retroperitoneal pads • ↑ Sc fat in Normalized data	• Normalized organ weights ↓ but heart = WT • Absolute brain = controls, kidney, liver and heart ↓	NR	Leptin ↑ Adiponectin ↑
Magon, 2009	6-80wk	♂♀	NR	♂ catch up WT while ♀ do not	• Absolute fat mass: ↑ in ♂♀; ♂ > ♀ and ♂ gained earlier • Normalized fat mass: ♂♀ > WT • ♂♀ have lower absolute lean mass • ♂♀ have low absolute fluid mass • ♂♀ have low absolute fluid mass, but NS when normalized to BW	• ♂ ↑ in absolute and percent fat pads on Sc and retroperitoneal • ♀ ↑ absolute fat mass particularly in Sc • ♂ ↓ absolute and percent • organ weights except for spleen • ♀ ↓ absolute organ weights besides kidney and liver; NS in percent of tissue weight in ♀	NS in glucose	• ♂ ↑ earlier • ♂ >♀ in leptin levels

Note. Standard rodent chow: Prolab RMH 3000, Brentwood, NJ; 14% of k calories from fat, 16% from protein and 60% from carbohydrates.
Epi: Epididymal fat pat; Sc: subcutaneous fat pad; iAT: interscapular adipose tissue; iBAT: interscapular BAT; iWAT: interscapular WAT; iAT = iBAT + iWAT. WT: wild type mice; BW: body weight. NR: not reported. NS: no significantly statistical difference compared to controls.

Summary

The GH/IGF-1 axis exerts a profound impact on regulating lipid, carbohydrate, and mineral metabolism. Overall, GH improves longitudinal growth, growth of lean mass while decreasing fat mass, and promotes retention of body fluid. GH in three mouse lines, transgenic bGH, transgenic GHA, and gene-disrupted $GHR^{-/-}$, exhibit a distinct effect on body composition. Both GHA and $GHR^{-/-}$ mice are dwarf with excess fat mass, normal or lower glucose and insulin levels. Unlike GHA and $GHR^{-/-}$, bGH mice are giant, have more lean mass with less fat mass, and higher levels of glucose and insulin. bGH, $GHR^{-/-}$, and GHA mice have shorter, longer, and unchanged lifespan, respectively, compared to WT controls. Recent reports in our lab detected differences in body composition dependent on gender and genotype throughout 80 weeks of age in GHA mice. GHA mice are obese since early life (6 weeks of age) and accumulate excess fat in the subcutaneous region. Male GHA mice have greater body weights, leptin levels, and fat mass than females. Previous studies revealed that $GHR^{-/-}$ mice were more susceptible to diet-induced obesity while bGH were protected from gaining excess fat mass and an incomplete resistance to diet-induced obesity. These studies did not address gender differences and did not look at the impact of GHA on susceptibility to diet-induced obesity. Thus, the purpose of this study is to investigate the susceptibility to diet-induced obesity in male and female GHA mice as compared to control mice.

CHAPTER 3: MATERIALS AND METHODS

Previous studies have studied the susceptibility of diet-induced obesity in mice with altered GH function (bGH and $GHR^{-/-}$ mice). The primary aim in this study was to explore the susceptibility to diet-induced obesity and diabetes in another mouse model with altered GH function, GHA transgenic mice. Measurement of body weight, body composition, food intake, and blood glucose were recorded and analyzed in order to understand the diet's impact on obesity and diabetes.

Animals

The GHA mice used in this study had been described previously (Chen, Wight et al., 1991) and had been backcrossed into the C57Bl/6J background strain. The C57Bl/6J strain of mice has been shown to be susceptible to obesity when fed a HF diet (Surwit et al., 1995). Twenty male and 20 female GHA C57Bl/6J mice were used for this study. The same age-matched 44 controls were treated in the same manner as the GHA mice. Ten-week old mice were genotyped at Edison Biotechnology Institute of Ohio University to ensure that the mice are properly identified. These mice then were divided into two groups which were fed a HF or a LF diet. Two to 4 mice were housed per cage in a room with controlled light (12h light/dark circle) and controlled temperature (22 ± 2 °C). All procedures were approved by the Ohio University Institutional Animal Care and Use Committee; all activities in this study abide by federal, state and local law and policies.

Dietary Manipulation

One group of 11 GHA male mice and one group of 12 male WT mice were provided a HF diet; this HF diet consisted of 20g fat/100g (19g butter oil and 1g soybean

oil), which resulted in 4.54kcal/g energy. The other 9 GHA male mice and 12 male WT mice were given a LF diet that had 3g butter oil and 1g soybean oil/100g and offered 3.8kcal/g energy. All experimental diets were pelleted, fully nutritional and were provided by Dyets (Bethlehem, PA). Females were similarly divided into four groups: HF GHA (n = 9), LF GHA (n = 11), HF WT (n = 9) and LF WT (n = 11). Overall, 84 mice were divided into eight groups according to the feeding regimen as described above. Mice were maintained on these diets and monitored for 11 weeks.

Measurements

It was necessary to monitor various parameters relative to obesity and diabetes during the dietary manipulation. A timeline for the study and these experimental measurements were provided in Figure 4 and Figure 5. Timing relative to measurements of food consumption, weight, body composition, and glucose (blood glucose, glucose tolerance test [GTT]) were included.

Food Consumption

Food intake and kilocalories consumed were calculated weekly during the 11-week period. The food consumed was calculated by subtracting the remaining food amount from the originally added amount at the beginning of every week. Calories consumed were calculated based on the known energy content of these defined diets. Food intake and calorie intake were divided by the number of mice in the same cage, to represent the food consumption for each mouse.

Weight Gain and Body Composition Measurement

Measurement of weight and body composition was done weekly. A Mettler

Toledo PL 202-S balance was used to measure the body weight. All mice were measured twice before conducting body composition measurements; all data were recorded to two decimal places. Averages of the data were calculated and included in all analyses.

For body composition, a desktop Minispec system, a custom-designed rodent quantitative nuclear magnetic resonance (NMR) machine, was used to measure body composition in live, awake mice as previously described (Palmer et al., 2009). Each mouse was put in the sample tube and inserted in the NMR's chamber maintained at 37°C. Every mouse was touched to excrete urine and feces prior to every measurement in order to minimize the measure error. All data, such as fat, lean, and fluid mass, were recorded. Measurements were started prior to the initiation of the HF diet in 10-week old mice till the end of the study (21-week old). This study design is to mimic the previous feeding study using the exact same diet and aged mice (Berryman et al., 2006) and to compare our results to the male bGH and $GHR^{-/-}$ mice.

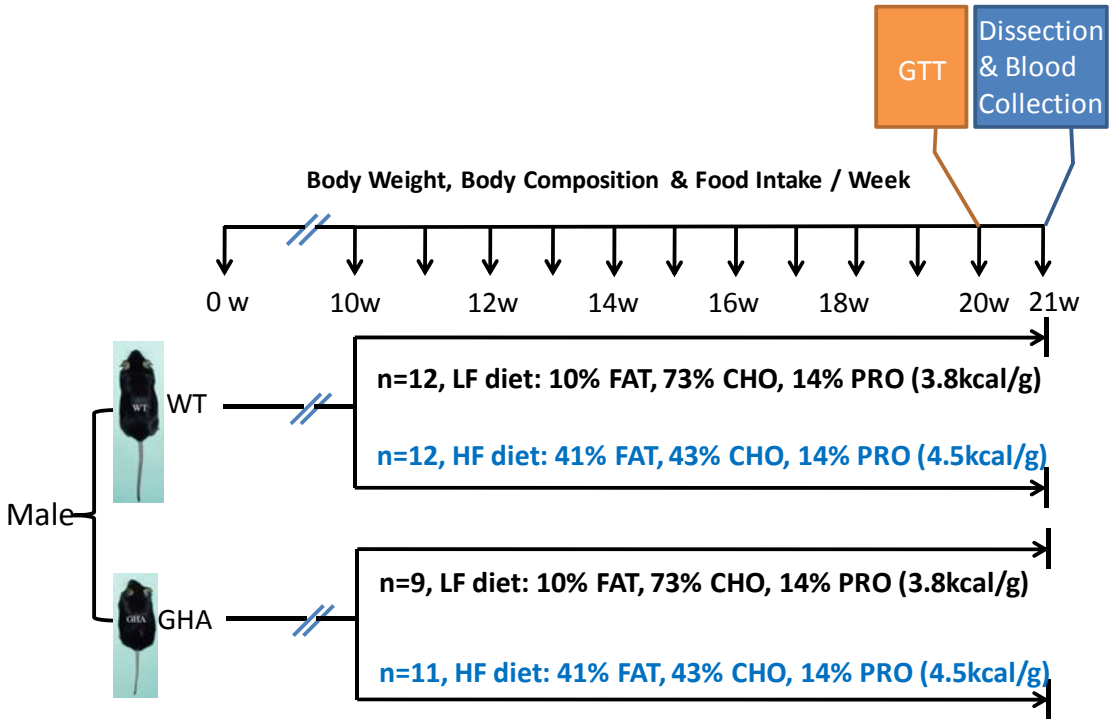


Figure 4. Male mice feeding manipulation during the 11-week diet study.

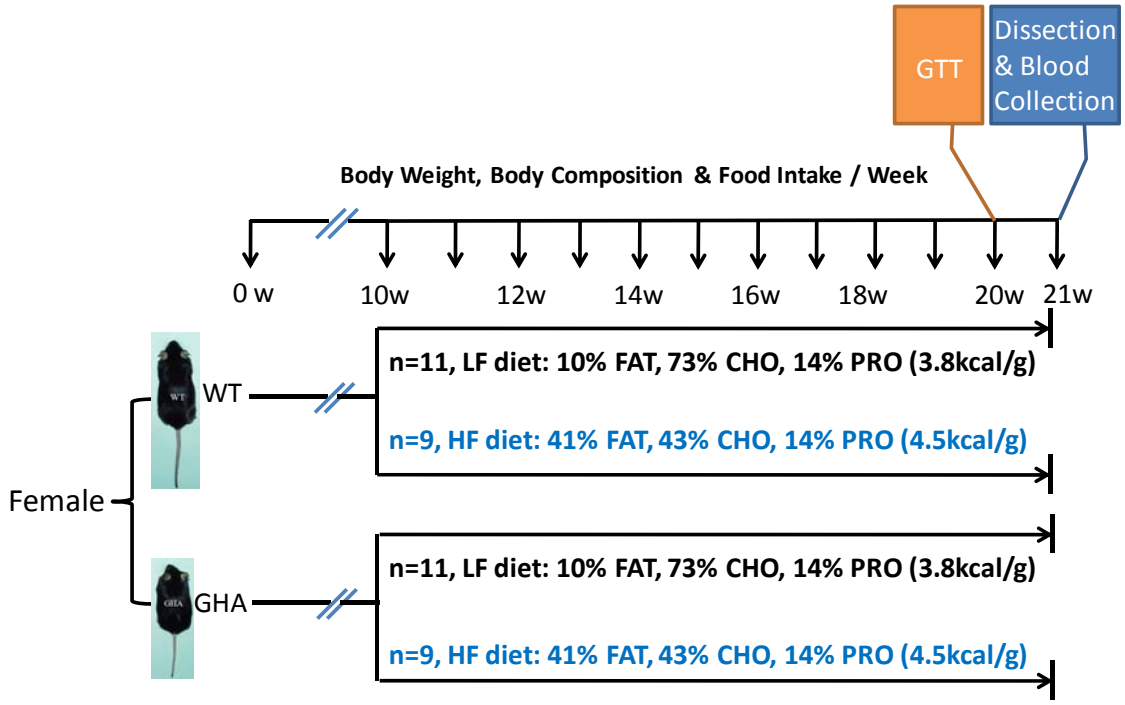


Figure 5. Female mice feeding manipulation during the 11-week diet study.

Glucose Tolerance Test

A glucose tolerance test was done at 20 weeks of the feeding study. Measurement begun at 10:00 am prior to fasting 13 hours for all mice. For this test, glucose measurements were done before and after injection of a 15% glucose solution in phosphate buffered saline according to 0.01mL/g body weight. The glucose solution was injected to each mouse's interperitoneal cavity and additional blood glucose readings were taken at 20-, 60-, 90-, and 120-minutes after the injection. The One Touch Life Scan Glucometer, as mentioned previously, was used to test the glucose levels.

Area Under the Curve

Previous studies summarized that area under the curve (AUC) is a more efficient means to represent glucose tolerance status (Potteiger, Jacobsen, & Donnelly, 2002). Specifically, in this study, values of positive AUC (y = 0) were used to analyze glucose tolerance. A free calculation tool on the website http://amchang.net/StatTools/AUC_Pgm.php was used to calculate AUC (y = 0) using the glucose concentrations from the five time points during the process of GTT.

Tissue Weights and Distribution of Fat

At the end of the 11-week feeding study, all mice were sacrificed by cervical dislocation. Organs were collected and weighed. Tissues that were collected included 5 distinct fat depots (inguinal, retroperitoneal, epididymal, mesenteric, and BAT), kidney, liver, heart, spleen, muscle, and lung. All tissues were flash frozen in liquid nitrogen and stored at -80° C for later use.

Note

One of the LF-fed GHA mice had yellow malformed teeth, which drastically affected eating. Compared to the others in the same group, this mouse showed a very low body weight with low indices of body composition. Therefore, data for this mouse were excluded from all data analyses. In addition, a wrong dose of glucose injection occurred in another male LF fed GHA mouse during the GTT, so data for this mouse were not included in the analyses.

Statistical Analysis

All measurement data including food intake, body weight, body composition, blood glucose (AUC, y = 0), and tissue weights were analyzed using the Statistical Package for the Social Sciences software (SPSS version 17.0, Chicago, IL). Eight groups were reported as HF-GHA male (HF diet, GHA male mice), HF-GHA female (HF diet, GHA female mice), LF-GHA male (LF diet, GHA male mice), LF-GHA female (LF diet, GHA female mice), HF-WT male (HF diet, WT male mice), HF-WT female (HF diet, WT female mice), LF-WT male (LF diet, WT male mice), and LF-WT female (LF diet, WT female mice). All the variables and group means were calculated as mean ± standard error of the mean (SEM). Univariate three-way ANOVA (2 x 2 x 2) was used to identify the effects of genotype, gender and diet as well as the interactions among them on the variables mentioned above. Body weights and body composition were conducted by three-way repeated ANOVA. For repeated measures, three-way ANOVA was used to identify differences at each time point, and paired t-test was used to examine differences between the beginning and end time points. Pearson correlation was used to analyze the relationship between fat mass (absolute and percent fat mass) and AUC/fasting glucose levels. Tissue/organ weights were analyzed by using three-way ANOVA. The result of $p < .05$ was used as cutoff for statistical significance.

CHAPTER 4: RESULTS

This study assessed the impact of LF versus HF feeding on male and female GHA mice relative to littermate controls. Body weight, body composition, and food consumption were measured weekly from 10- to 21-week of age. Glucose tolerance tests and a glucose measurement were taken at 20 weeks of age. Mice were sacrificed at 21 weeks of age and tissue weights were recorded. All data are shown in two different ways to better reveal the genotype differences as well as the gender differences although statistics were run on the entire data set. That is, for most data, graphs are first presented to reveal genotype differences such that groups of the same gender are plotted together (so male GHA LF/HF versus male WT LF/HF on one graph and female GHA LF/HF versus female WT LF/HF on another graph). In addition, a second graph is provided to show the data set with both genders and dietary treatments on one graph, but genotypes separated into separate graphs (WT male and female LF/HF on one graph with GHA male and female LF/HF on the other graph).

Body Weight

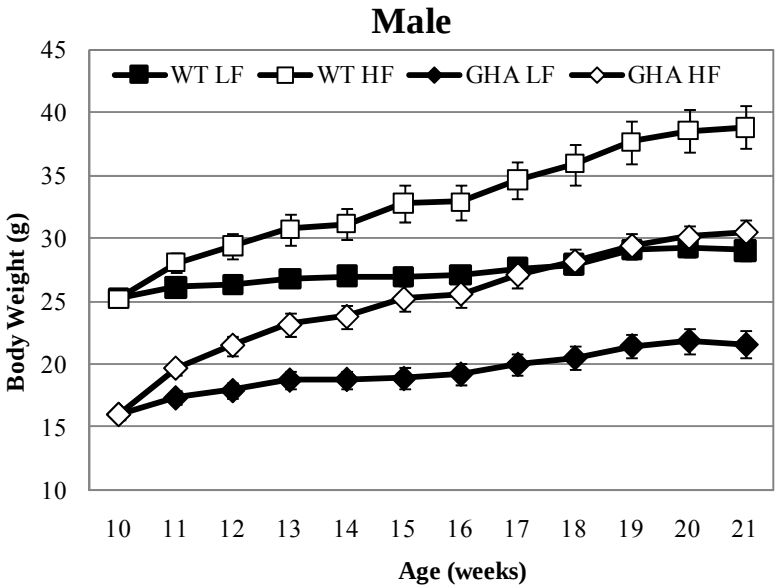
All mice showed an increased trend in body weight during the 11-week feeding study. The mean body weight of all GHA mice was less than the age-, gender-, and diet-controlled WT mice throughout the entire study (see Figures 6a and 6b). This substantial difference can be seen at the first and the last points of measurement (see Table 3 and Appendix D). At the beginning of the feeding study, all male and female GHA mice were approximately 63-67% of diet- and gender-controlled WT littermates. By the end of the feeding study, the body weights of all mice increased and the relative values of

GHA mice increased to 73-79% of gender- and diet-matched WTs (see Table 3 and Appendix D; see Figures 6a and 6b). Overall, the trends of mean body weight between different genotypes were parallel on the same diet. HF fed animals weighed more than their LF fed counterparts. In contrast to HF, LF fed groups had only a modest increase in body weight.

Regarding gender differences, all female mice weighed less than genotype- and diet-controlled males from the beginning to the end of this diet study (see Table 4 and Appendix E; see Figures 7a and 7b). Again, a parallel trend between genotype- and diet-controlled males and females was noticed. Furthermore, male HF fed GHA and WT mice showed a steeper trend in weight gain than the diet- and genotype-matched females. Interestingly, in female GHA mice, there was no notable difference between the weight gain trends in HF and LF diet feeding.

Three-way repeated ANOVA revealed significant main effect for genotype ($F(1,76) = 135.360$, $p = 1.49\text{E-}18$), gender ($F(1,76) = 152.489$, $p = 7.57\text{E-}20$), and diet ($F(1,76) = 47.564$, $p = 1.37\text{E-}09$) as well as gender x diet interaction ($F(1,76) = 5.824$, $p = .018218$) (see Appendix B). A three-way ANOVA for the data from each time point revealed significant differences between gender, genotype, and diet for all weeks except at the beginning of the feeding study on week 10 ($p > .05$, see Appendix C). Thus, the randomly chosen groups started the feeding study at similar weights, as one would hope.

6a



6b

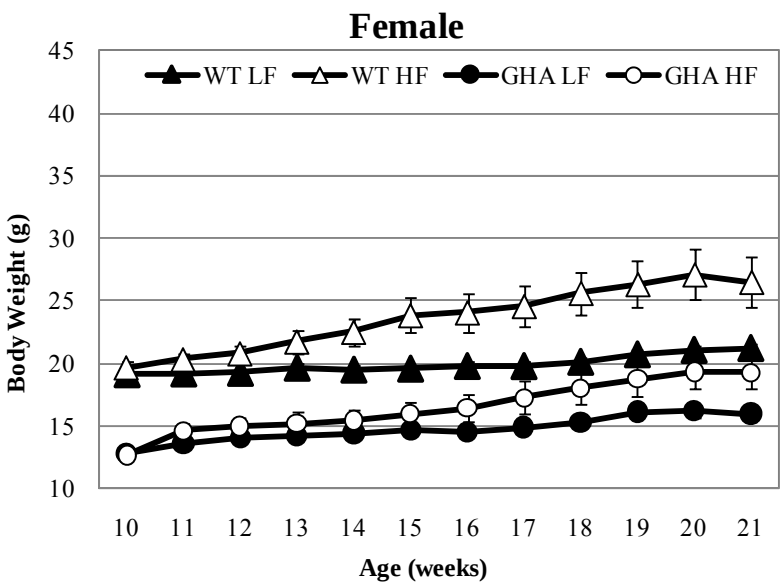


Figure 6. Body weights of male and female mice over the 10- to 21-week feeding study. Data are expressed as mean ± SEM. WT male on LF (n = 12), WT male on HF (n = 12), GHA male on LF (n = 9), GHA male on HF (n = 11), WT female on LF (n = 11), WT female on HF (n = 9), GHA female on LF (n = 11), GHA female on HF (n = 9).

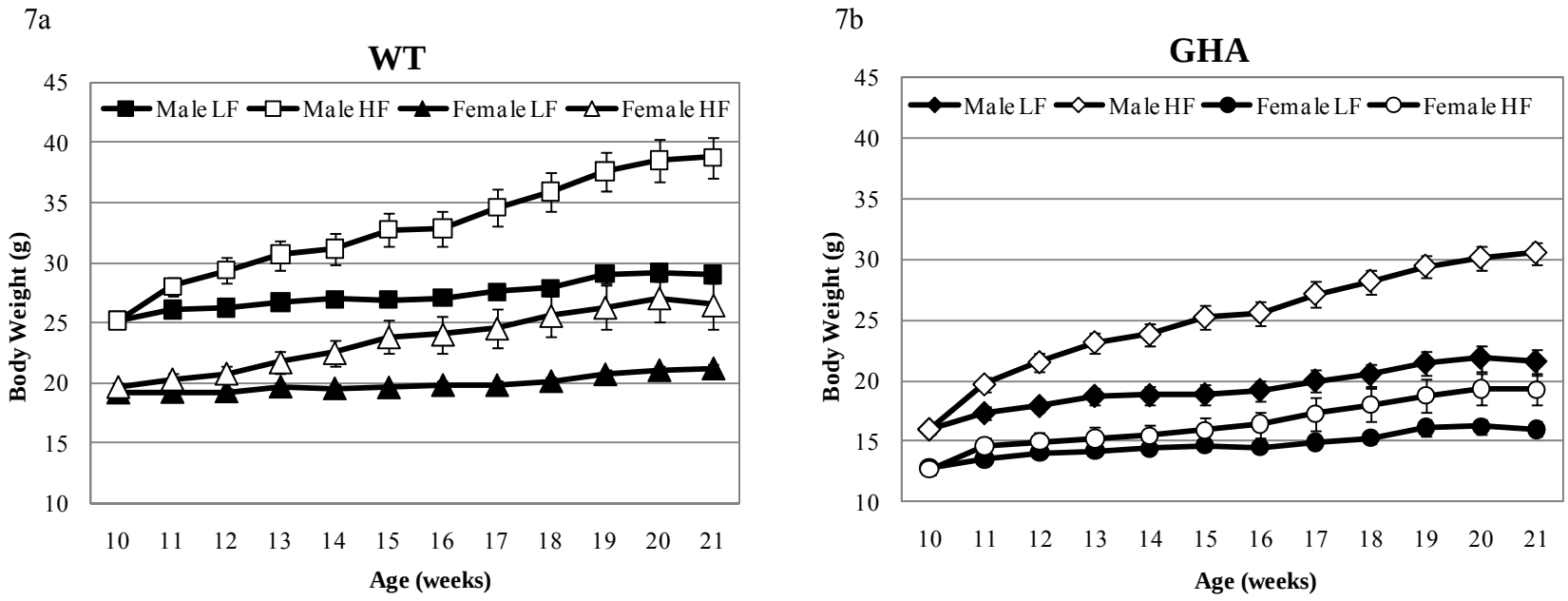


Figure 7. Body weights of GHA and WT mice over the 10- to 21-week feeding study. Data are expressed as mean ± SEM. WT male on LF (n = 12), WT male on HF (n = 12), GHA male on LF (n = 9), GHA male on HF (n = 11), WT female on LF (n = 11), WT female on HF (n = 9), GHA female on LF (n = 11), GHA female on HF (n = 9).

Table 3

Genotype Comparison: Percent Values in Body Weight and Body Composition of GHA Mice as Compared to WT Controls of the Same Diet and Gender at the Beginning and End of the Feeding Study

Genotype Comparison	Body Weight		Absolute Weight						Normalized Weight					
			Fat		Lean		Fluid		Fat		Lean		Fluid	
	10 wk	21 wk	10 wk	21 wk	10 wk	21 wk	10 wk	21 wk	10 wk	21 wk	10 wk	21 wk	10 wk	21 wk
Male LF GHA vs. WT	63%	74%	210%	182%	57%	64%	80%	82%	332%	243%	91%	87%	126%	112%
Female LF GHA vs. WT	67%	75%	171%	190%	61%	65%	72%	82%	252%	244%	91%	87%	101%	109%
Male HF GHA vs. WT	64%	79%	**229%**	**102%**	58%	69%	83%	92%	**356%**	**133%**	91%	87%	130%	117%
Female HF GHA vs. WT	64%	73%	**142%**	**102%**	59%	65%	67%	68%	**217%**	**146%**	92%	88%	105%	96%

Note. Bold values highlight drastic changes between the beginning (10 weeks) and end (21 weeks) of the feeding study. (See supporting statistics provided in Appendix D.)

Table 4

Gender Comparison: Percent Values in Body Weight and Body Composition of Female Versus Male Mice of the Same Diet and Genotype at the Beginning and End of the Feeding Study

Gender Comparison	Body Weight		Absolute Weight						Percent Weight					
			Fat		Lean		Fluid		Fat		Lean		Fluid	
	10 wk	21wk	10 wk	21 wk	10 wk	21 wk	10 wk	21 wk	10 wk	21 wk	10 wk	21 wk	10 wk	21 wk
WT LF Female vs.Male	76%	73%	**95%**	**60%**	73%	75%	104%	78%	**128%**	**85%**	97%	102%	137%	108%
GHA LF Female vs.Male	**80%**	**41%**	**78%**	**63%**	78%	76%	87%	79%	**97%**	**86%**	98%	102%	109%	105%
WT HF Female vs.Male	78%	68%	**123%**	**49%**	76%	76%	104%	88%	**154%**	**66%**	97%	112%	132%	128%
GHA HF Female vs.Male	79%	63%	**76%**	**49%**	77%	71%	83%	65%	**94%**	**73%**	98%	114%	106%	105%

Note. Bold values highlight drastic changes between the beginning (10 weeks) and end (21 weeks) of the feeding study. (See supporting statistics in Appendix E.)

Body Composition

Absolute Fat Mass

Three-way repeated ANOVA revealed significant differences in gender ($F(1,76) = 26.189$, $p = .000002$) and diet ($F(1,76) = 51.243$, $p = 4.4E\text{-}10$) but not genotype ($F(1,76) = 3.897$, $p = .052$) in absolute fat mass. Three-way repeated ANOVA also revealed a significant difference in gender x diet interaction ($F(1,76) = 8.589$, $p = .004$ (see Appendix F). Significant differences between week 10 and 21 of the feeding study in each group were shown by using paired *t*-test (see Appendix G).

Regarding genotype differences, only LF fed GHAs were statistically larger in absolute fat mass than age-, diet-, and gender-controlled WT mice throughout the entire feeding period, unlike HF fed GHA and WT counterparts that showed no statistical difference in absolute fat mass (see Figures 8a and 8b). Thus, despite their dwarf size, the male GHA LF males were relatively obese as compared to the WT littermates on the same diet. The mean absolute fat mass of male HF fed GHA mice was greater than gender- and diet-controlled WT mice at the beginning of the feeding study; however, by the end of the feeding study, there was no significant difference in fat mass on the HF fed. WT males caught up with the GHA males by 21 weeks on the HF diet. Absolute fat mass of female HF GHA and WT mice showed a similar trend. In contrast, both genders in LF groups did not have large increases; rather, they showed a slight increase (see Figures 8a and 8b). The gap in absolute fat mass between different genotypes decreased from 10 weeks to 21 weeks. For example, at the beginning of the feeding study, male GHA HF

mice were 229% of WT controls in fat mass versus slightly increased fat mass by 21 weeks (102%) (see Table 3 and Appendix D).

With respect to gender differences, males had greater absolute fat mass than females. HF fed males of both genotypes had markedly higher absolute fat mass than HF fed females. However, LF fed GHA and WT males had relatively greater absolute fat mass than females compared to HF diet (see Figure 9a and 9b). Overall, females of both genotypes had a lower fat mass gain as compared to males from 76-123% at 10 weeks of age to 49-63% at the end of diet study as compared to their male genotype controls (see Table 4). Again, the gap of absolute fat mass between female GHAs on different diets was small. Three-way ANOVA for each time point are provided in Appendix H. Genotype differences were only seen from week 10 to 14 during the feeding study, while gender and diet differences were seen in most time periods except at the beginning of the study.

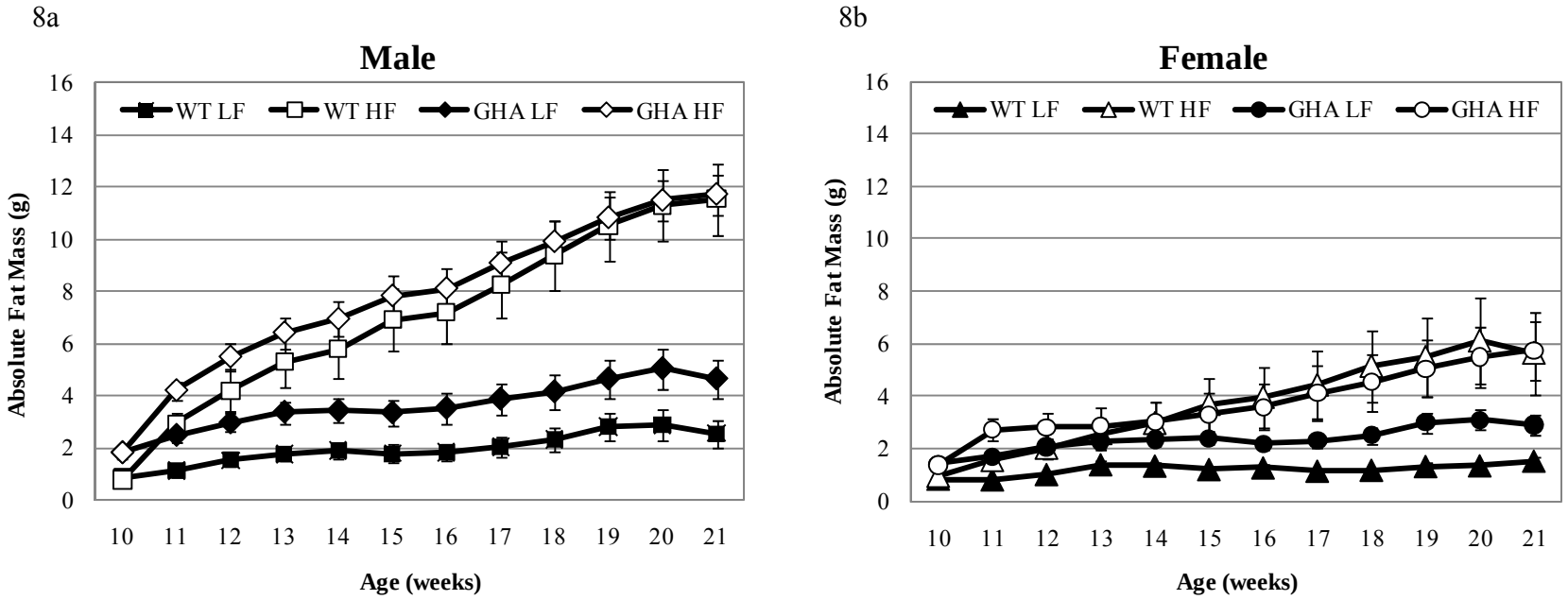


Figure 8. Absolute fat mass of male and female mice over the 10- to 21-week feeding study. Data are expressed as mean ± SEM. WT male on LF (n = 12), WT male on HF (n = 12), GHA male on LF (n = 9), GHA male on HF (n = 11), WT female on LF (n = 11), WT female on HF (n = 9), GHA female on LF (n = 11), GHA female on HF (n = 9).

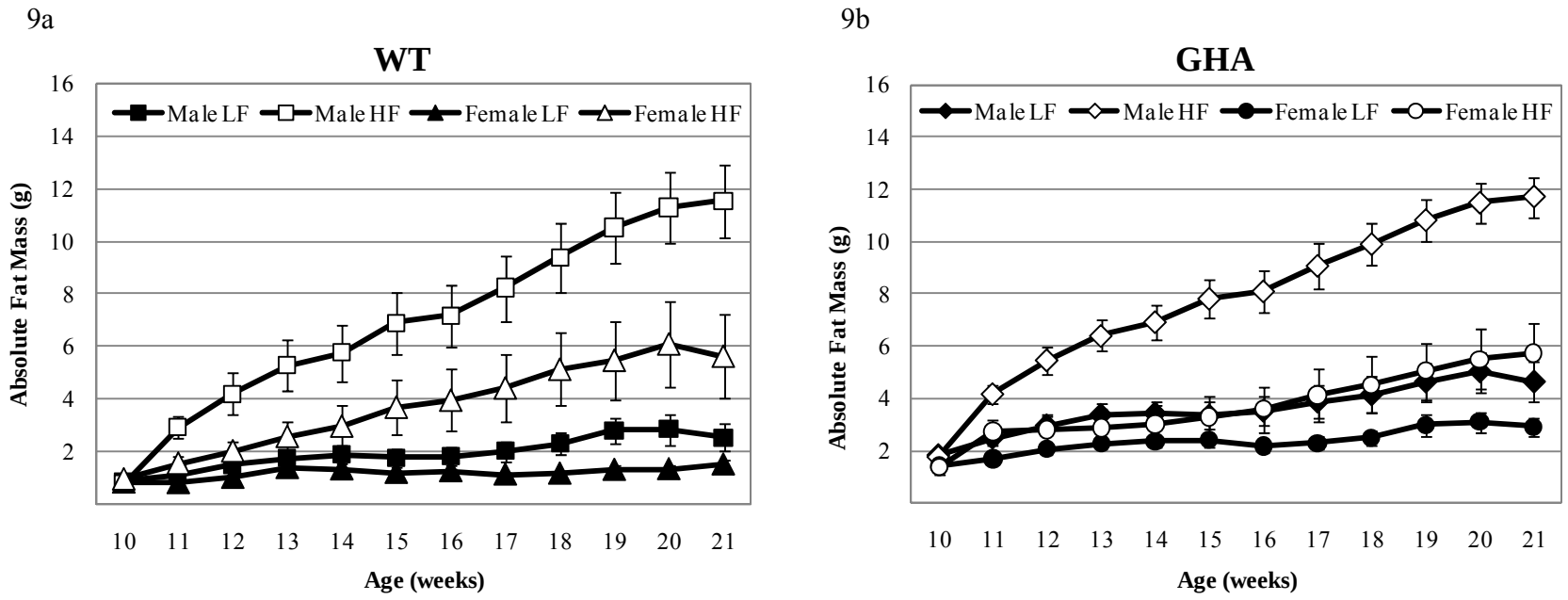


Figure 9. Absolute fat mass of GHA and WT mice over the 10- to 21-week feeding study. Data are expressed as mean ± SEM. WT male on LF (n = 12), WT male on HF (n = 12), GHA male on LF (n = 9), GHA male on HF (n = 11), WT female on LF (n = 11), WT female on HF (n = 9), GHA female on LF (n = 11), GHA female on HF (n = 9).

Normalized Fat Mass

Because the GHA mice are dwarf, it is important to normalize the values to body weight to determine the relative proportion of fat mass to total body weight. Unlike absolute fat mass in which GHA mice were similar to WT mice, GHAs were statistically larger in percent fat mass than WTs at all time points (see Figures 10a and 10b); thus, a larger proportion of body weight in GHA mice is due to fat mass. Again, trends of percent fat mass between GHA mice and WT controls were parallel. Consistent with absolute fat mass, GHA mice showed a lower percent fat mass gain than gender- and diet-matched WT littermate mice during the feeding period. In other words, the gap in percent fat mass between GHA and diet- and gender-matched counterparts decreased (see Table 3 and Appendix D). The greatest change in normalized fat mass was seen in male HF GHAs as compared to HF WT littermates, which decreased from 356% at 10 weeks to 133% by 21 weeks of age.

Significant differences in genotype ($F(1,76) = 45.863$, $p = 2.35E\text{-}09$), gender ($F(1,76) = 10.491$, $p = .001780$), diet ($F(1,76) = 45.055$, $p = 3.04E\text{-}09$), and gender x diet interaction ($F(1,76) = 4.299$, $p = .041524$) were noticed by three-way repeated ANOVA (see Appendix I). Furthermore, there were statistical differences in genotypes for each specific time point using three-way ANOVA ($p < .001$) (see Appendix J).

Regarding gender differences, females had less percent fat mass than the males when fed the same diet (see Figures 11a and 11b). Overall, all females had less change in percent fat mass as compared to genotype- and diet-matched males during the entire feeding study. Females in the HF fed WT group had the lowest gain in percent fat mass,

as compared to their diet- and genotype-controlled male mice, which decreased from 154% to 66% of the percent fat mass by the end of the study (see Table 4 and Appendix E). No gender difference was seen in LF fed WT mice. The notable difference between female GHAs on both diets was not observed until the later stages of this feeding study (see Figures 11a and 11b).

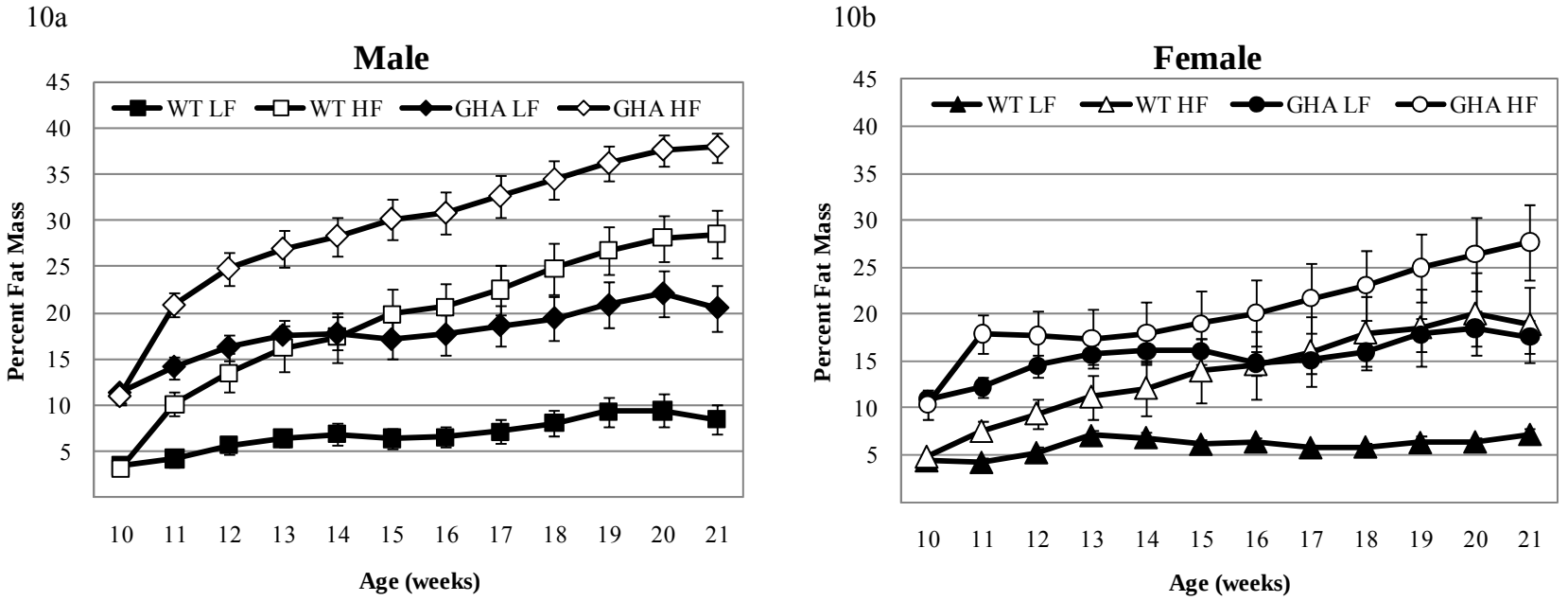
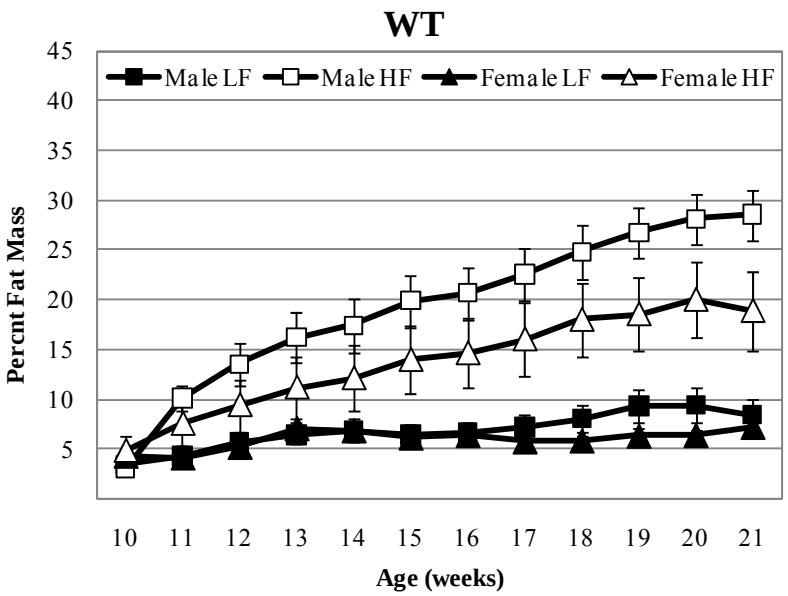


Figure 10. Percent fat mass of male and female mice over the 10- to 21-week feeding study. Data are expressed as mean ± SEM. WT male on LF (n = 12), WT male on HF (n = 12), GHA male on LF (n = 9), GHA male on HF (n = 11), WT female on LF (n = 11), WT female on HF (n = 9), GHA female on LF (n = 11), GHA female on HF (n = 9).

11a



11b

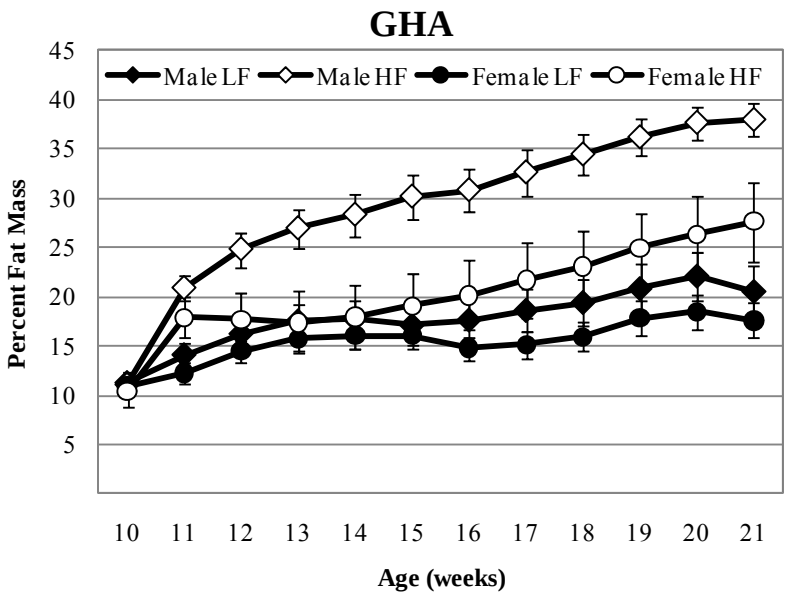


Figure 11. Percent fat mass of GHA and WT mice over the 10- to 21-week feeding study. Data are expressed as mean ± SEM. WT male on LF (n = 12), WT male on HF (n = 12), GHA male on LF (n = 9), GHA male on HF (n = 11), WT female on LF (n = 11), WT female on HF (n = 9), GHA female on LF (n = 11), GHA female on HF (n = 9).

Lean Mass

There were significant differences in gender ($F(1,76) = 499.867$, $p = 3.70E-35$), genotype ($F(1,76) = 1162.652$, $p = 8.17E-48$), diet ($F(1,76) = 21.751$, $p = .000013$), and gender x genotype interaction ($F(1,76) = 24.746$, $p = .000004$) by three-way repeated ANOVA (see Appendix K).

Regarding genotype differences, GHA mice had less absolute lean mass than WT mice (see Figures 12a and 12b). Mean absolute lean mass of GHA mice were around only half of WT controls (57-61 %) at the onset of diet study. GHA mice also only slightly increased absolute lean mass as compared to littermate controls (64-69%) by week 21 (see Table 3 and Appendix D). All trends in GHA and gender- and diet-controlled genotype littermate mice were parallel and slightly ascended throughout the feeding period. Again, genotype differences at specific time points throughout the entire feeding study were detected by three-way ANOVA (see Appendix L).

Regarding gender differences, males had greater absolute lean mass than females (see Figures 13a and 13b). All groups maintained relatively stable trends during the diet study, with only a modest increase. Females of both genotypes had no marked change in absolute lean mass from approximately 73-78% of control males at 10 weeks to around 71-76% by the end of the feeding. Overall, female GHA animals had very little change in absolute lean mass, unlike male GHAs.

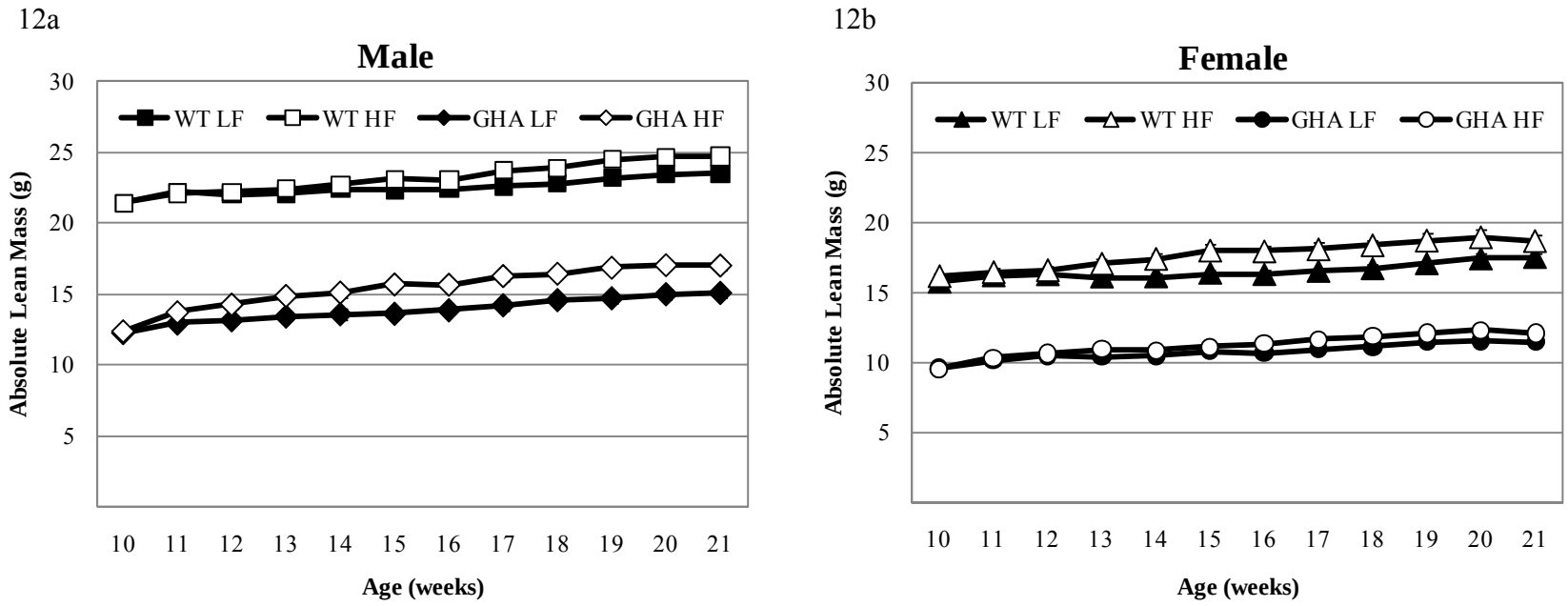
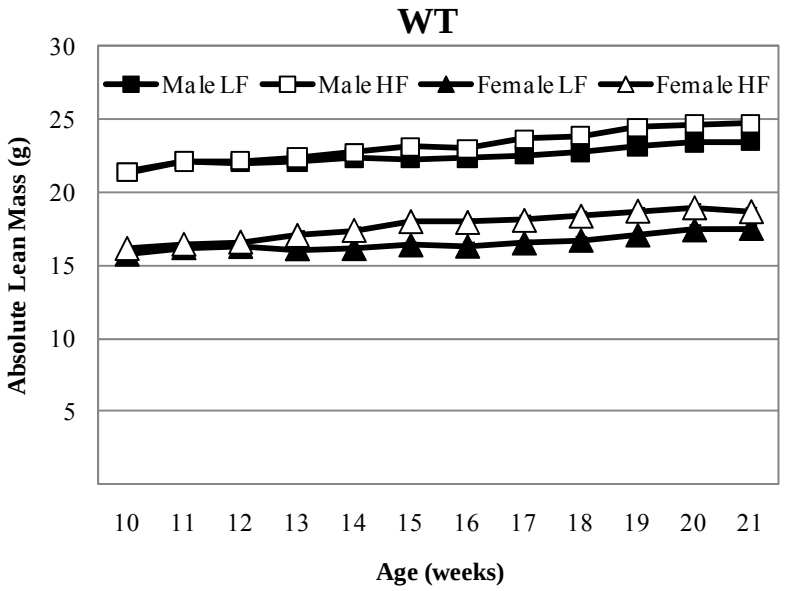


Figure 12. Absolute lean mass of male and female mice over the 10- to 21-week feeding study. Data are expressed as mean ± SEM. WT male on LF (n = 12), WT male on HF (n = 12), GHA male on LF (n = 9), GHA male on HF (n = 11), WT female on LF (n = 11), WT female on HF (n = 9), GHA female on LF (n = 11), GHA female on HF (n = 9).

13a



13b

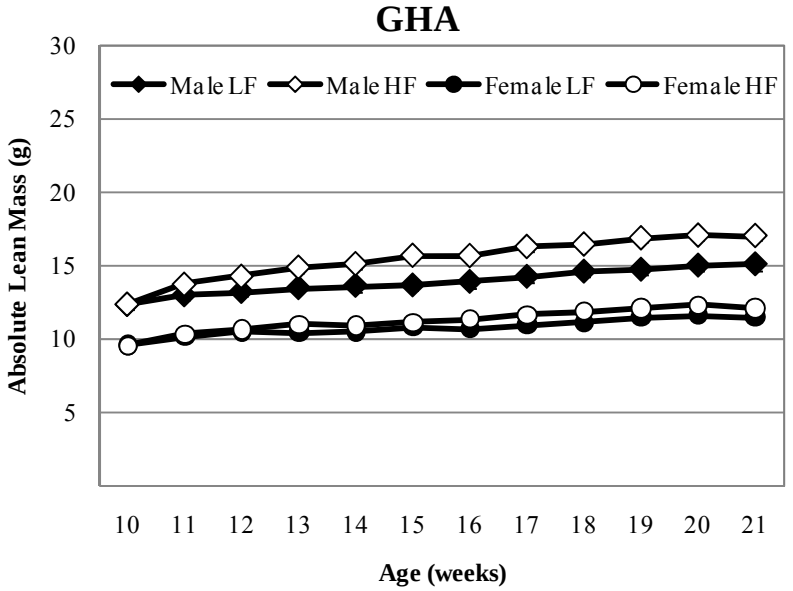


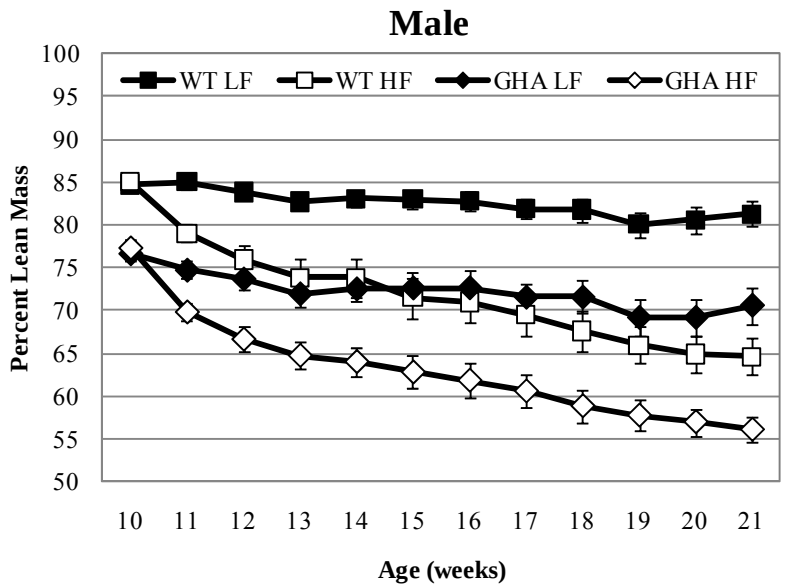
Figure 13. Absolute lean mass of GHA and WT mice over the 10- to 21-week feeding study. Data are expressed as mean ± SEM. WT male on LF (n = 12), WT male on HF (n = 12), GHA male on LF (n = 9), GHA male on HF (n = 11), WT female on LF (n = 11), WT female on HF (n = 9), GHA female on LF (n = 11), GHA female on HF (n = 9).

Normalized Lean Mass

As for genotyped differences, and in contrast to absolute lean mass, the percent lean mass gradually declined for all groups except female LF fed WTs, which fluctuating slightly (see Figures 14a and 14b). All GHA mice were lower in percent lean mass than WT mice. When gender- and diet-controlled WT mice were compared to all GHA mice, percent lean mass did not change (see Table 3). Again, percent lean mass in all HF groups decreased more sharply than LF groups, most likely because of the gain in fat mass. Three-way repeated ANOVA revealed significant differences in gender ($F(1,76) = 7.771$, $p = .006704$), genotype ($F(1,76) = 59.192$, $p = 4.24E\text{-}11$), diet ($F(1,76) = 42.042$, $p = 8.09E\text{-}09$), and gender x diet interaction ($F(1,76) = 4.500$, $p = .037159$) but not for other interactions (see Appendix M). Three-way ANOVA also confirmed genotype differences at each week ($p < .001$) and gender differences each week except for 11 weeks of age (see Appendix N). Although there was no significant difference in percent lean mass of female LF fed WTs between at the onset and the conclusion of feeding study, all other groups showed statistically significant differences by the end of the study by paired *t*-test (see Appendix G).

Regarding gender differences, females were greater in percent lean mass than male mice, most likely because of their reduced fat mass as compared to males. In addition, there was no gender difference in LF fed GHAs and WT mice (see Figure 15a and 15b or Appendix E).

14a



14b

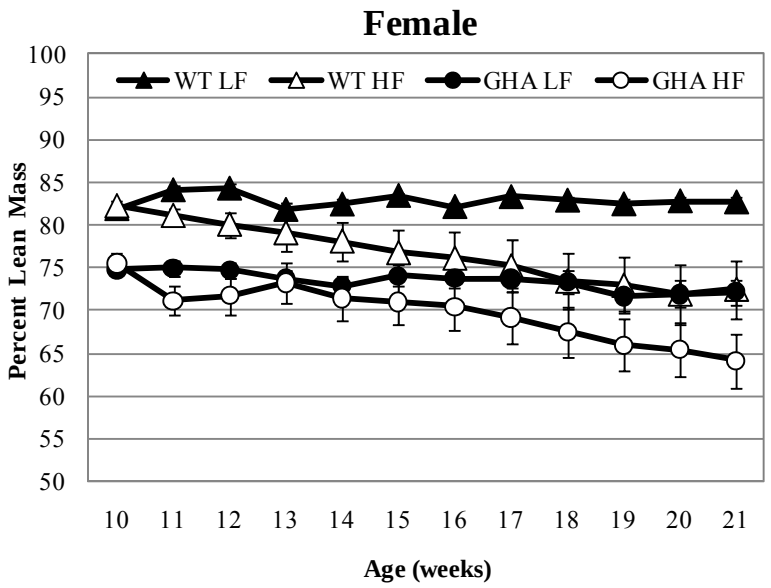
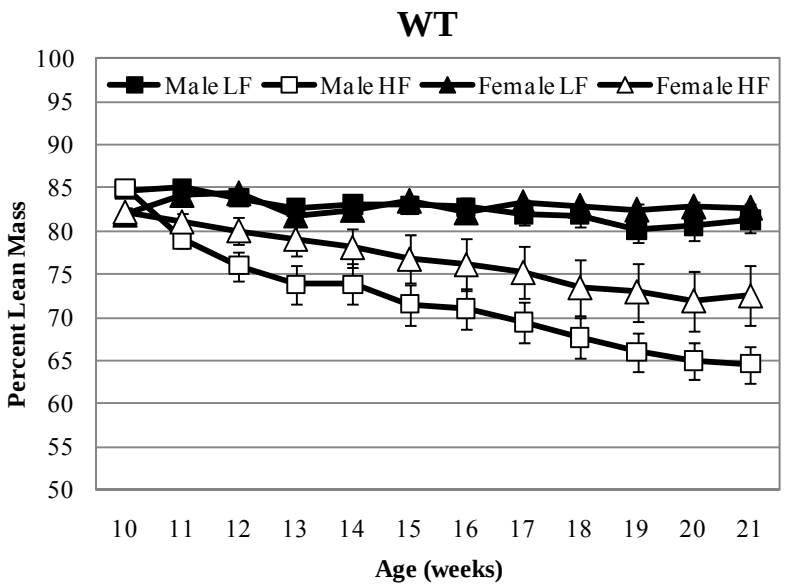


Figure 14. Percent lean mass of male and female mice over the 10- to 21-week feeding study. Data are expressed as mean ± SEM. WT male on LF (n = 12), WT male on HF (n = 12), GHA male on LF (n = 9), GHA male on HF (n = 11), WT female on LF (n = 11), WT female on HF (n = 9), GHA female on LF (n = 11), GHA female on HF (n = 9).

15a



15b

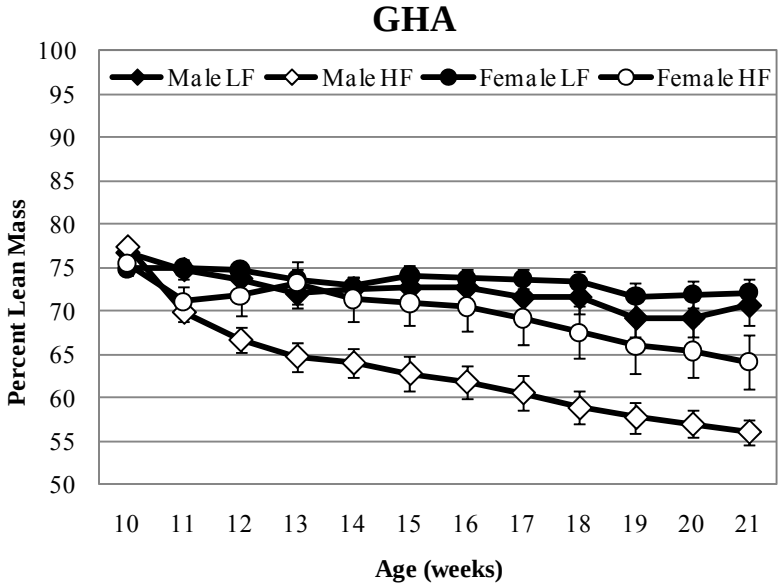


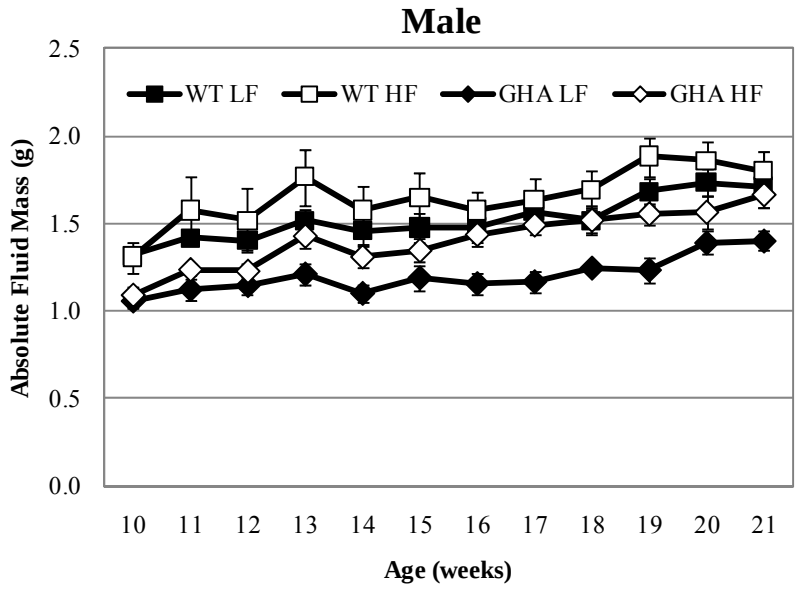
Figure 15. Percent lean mass of GHA and WT mice over the 10- to 21-week feeding study. Data are expressed as mean ± SEM. WT male on LF (n = 12), WT male on HF (n = 12), GHA male on LF (n = 9), GHA male on HF (n = 11), WT female on LF (n = 11), WT female on HF (n = 9), GHA female on LF (n = 11), GHA female on HF (n = 9).

Fluid Mass

When comparing different genotypes in absolute fluid mass, GHA mice had a lower mean absolute fluid mass than WT littermate mice (see Figures 16a and 16b). GHA and diet- and gender-matched WT mice showed parallel trends with fluctuating values. GHA mice had slightly increased fluid mass (1-10%) versus WT controls by the end of feeding study (see Table 3 and Appendix D). No significant changes were seen between week 10 and 21 of the diet study in either female HF or LF fed WT mice ($p > .05$, see Appendix G). Three-way repeated ANOVA revealed significant differences in each group with differences in gender ($F(1,76) = 30.815$, $p = 3.98E\text{-}07$), genotype ($F(1,76) = 41.012$, $p = 1.14E\text{-}08$), and diet ($F(1,76) = 8.446$, $p = .004791$) but not for any interactions (see Appendix O). Genotype differences were present at each time point in three-way ANOVA (see Appendix P).

Concerning gender differences, female mice had lower absolute fluid mass than male mice (see Figures 17a and 17b). Female WT mice and female GHA mice decreased in fluid versus males (week 10 and 21, see Table 4 and Appendix E). Three-way ANOVA revealed gender differences each week, except at the beginning of the feeding study during weeks 10 and 11(see Appendix P).

16a



16b

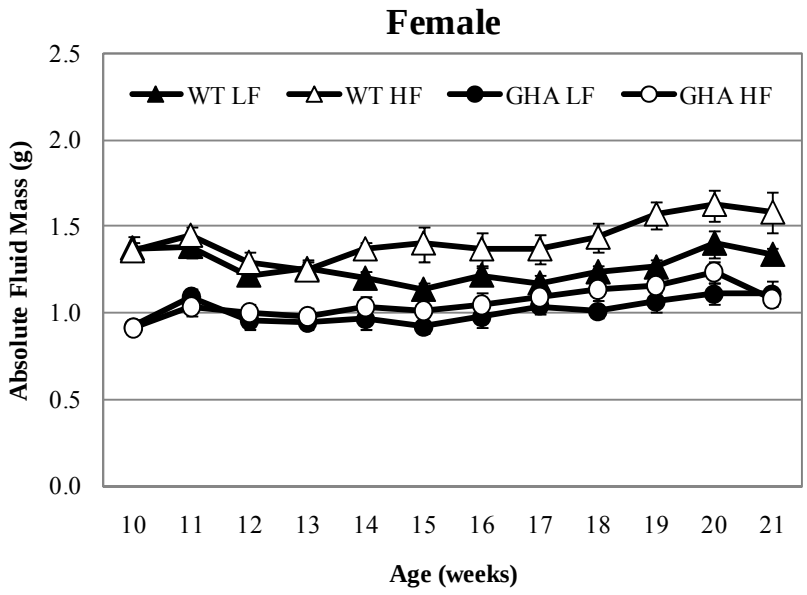
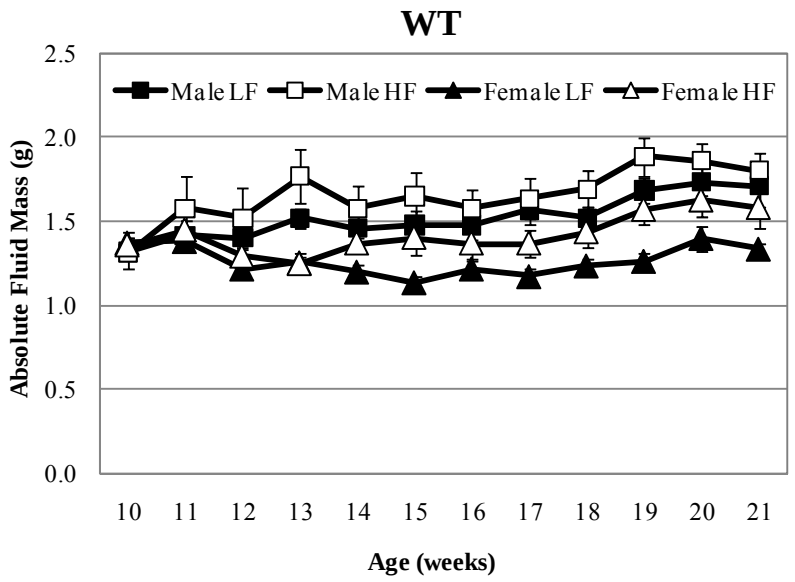


Figure 16. Absolute fluid mass of male and female mice over the 10- to 21-week feeding study. Data are expressed as mean ± SEM. WT male on LF (n = 12), WT male on HF (n = 12), GHA male on LF (n = 9), GHA male on HF (n = 11), WT female on LF (n = 11), WT female on HF (n = 9), GHA female on LF (n = 11), GHA female on HF (n = 9).

17a



17b

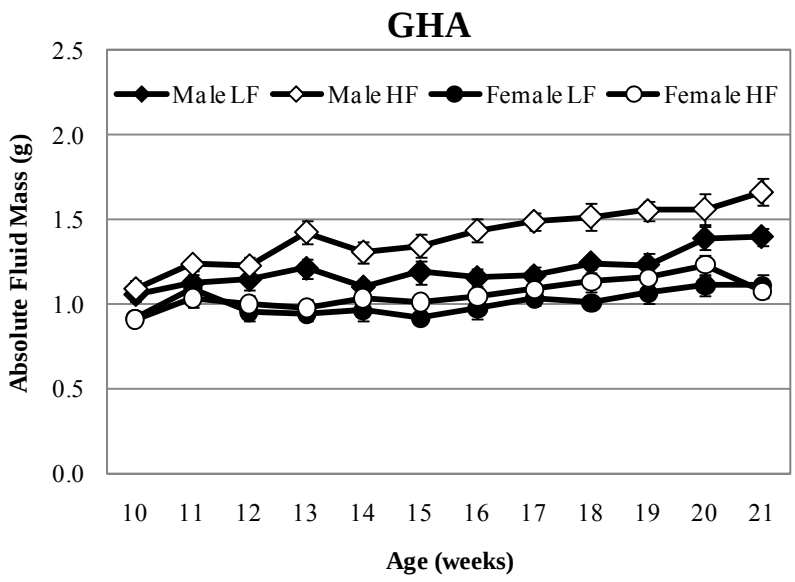


Figure 17. Absolute fluid mass of GHA and WT mice over the 10- to 21-week feeding study. Data are expressed as mean ± SEM. WT male on LF (n = 12), WT male on HF (n = 12), GHA male on LF (n = 9), GHA male on HF (n = 11), WT female on LF (n = 11), WT female on HF (n = 9), GHA female on LF (n = 11), GHA female on HF (n = 9).

Normalized Fluid Mass

Three-way repeated ANOVA revealed significant differences in gender ($F(1,76) = 40.721$, $p = 1.25E-08$), genotype ($F(1,76) = 19.629$, $p = .000031$), and diet ($F(1,76) = 10.138$, $p = .002106$) but not for any interactions (see Appendix Q). No significant changes were seen in male LF WTs, male LF GHAs, and female LF GHAs between the beginning and the end of the feeding study (in weeks 10 and 21, $p > .05$, see Appendix G). However, GHA animals showed greater percent fluid mass than WT counterparts during the study. All mice showed fluctuating trends in percent fluid mass (see Figures 18a and 18b). Again, statistically significant genotype differences at specific time points were seen and are provided in Appendix S.

When comparing females to males, all female mice were slightly and statistically greater in percent fluid mass than male mice (see Figures 19a and 19b). Modest changes were seen in percent fluid mass when comparing females to males between week 10 and 21 of the regimen study (see Table 4). Again, apart from week 13 of the study, gender differences for every week were present by three-way ANOVA (see Appendix S).

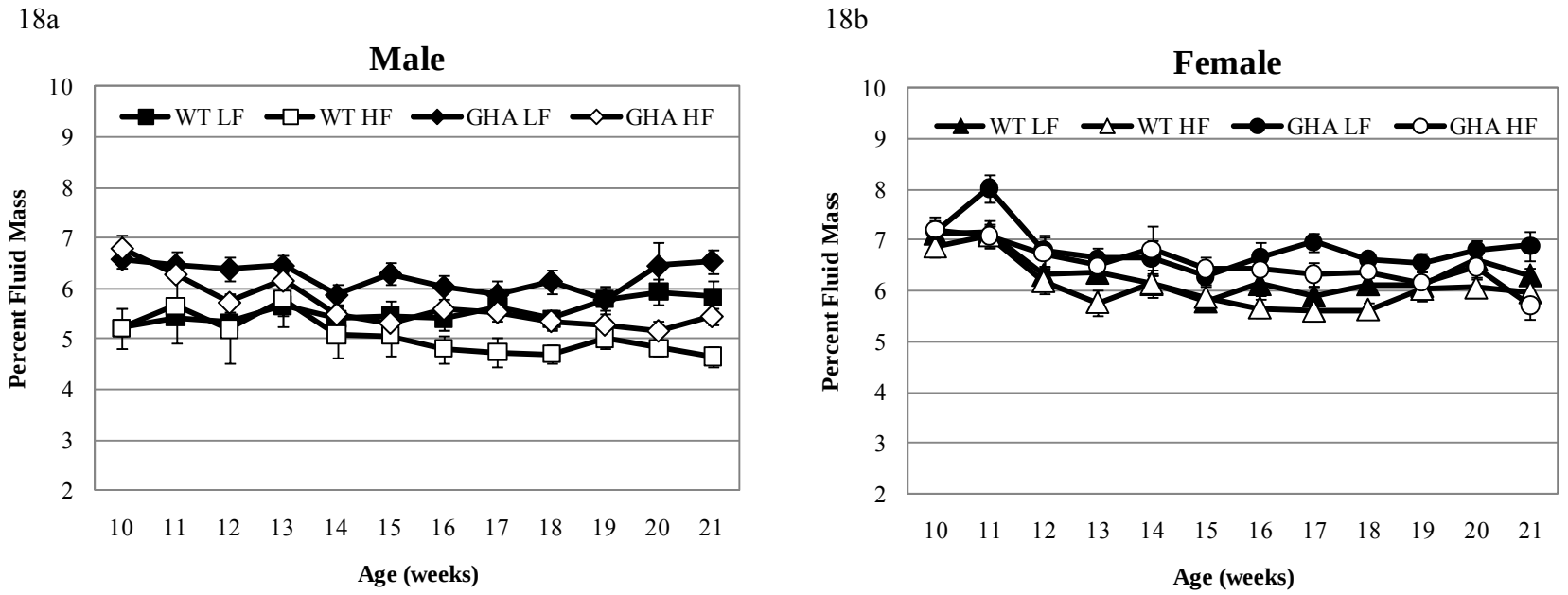
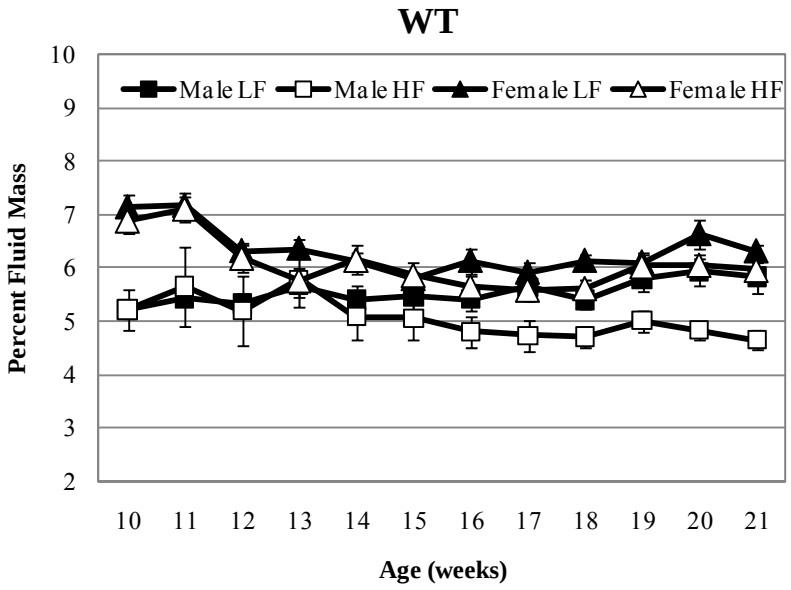


Figure 18. Percent fluid mass of male and female mice over the 10- to 21-week feeding study. Data are expressed as mean ± SEM. WT male on LF (n = 12), WT male on HF (n = 12), GHA male on LF (n = 9), GHA male on HF (n = 11), WT female on LF (n = 11), WT female on HF (n = 9), GHA female on LF (n = 11), GHA female on HF (n = 9).

19a



19b

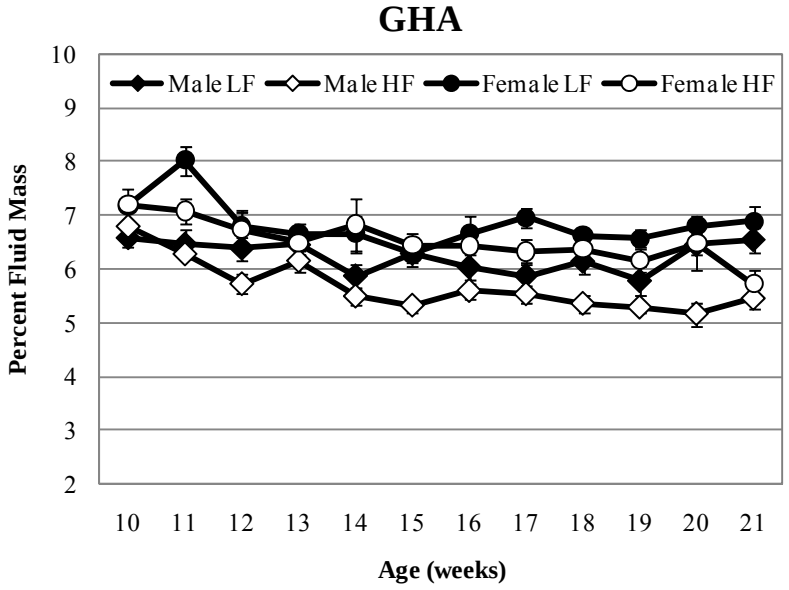


Figure 19. Percent fluid mass of GHA and WT mice over the 10- to 21-week feeding study. Data are expressed as mean ± SEM. WT male on LF (n = 12), WT male on HF (n = 12), GHA male on LF (n = 9), GHA male on HF (n = 11), WT female on LF (n = 11), WT female on HF (n = 9), GHA female on LF (n = 11), GHA female on HF (n = 9).

Food Consumption

Energy Consumption

As metabolic cages weren't available, food consumption data were collected for a cage containing several mice and then divided to determine food consumed per mouse. Thus, the data for food consumption was not as accurate as it could have been and because of this, we only considered consumption over the entire 11-week feeding study time period instead of weekly measurements. This limitation should be considered when interpreting these results, because the data for individual mice provide only an estimate of energy consumption. Despite this, three-way ANOVA revealed significant differences in genotype ($F(1,76) = 22.926$, $p = .000008$), gender ($F(1,76) = 113.712$, $p = 9.35E\text{-}17$), diet ($F(1,76) = 314.480$, $p = 9.91E\text{-}29$), gender x diet ($F(1,76) = 11.771$, $p = .000976$), and genotype x gender x diet ($F(1,76) = 23.225$, $p = .000007$) (see Appendix T).

When comparing different genotypes, male LF fed GHA mice consumed significantly less energy (see Figures 20a and 20b) than the same diet fed male WT mice while HF fed male GHA and WT animals consumed equal amounts of energy. However, female GHAs consumed similar levels to WT mice when fed the LF diet, but less when fed the HF diet. Regardless of gender or genotype, all mice consumed more energy on the HF diet than the LF diet.

Regarding gender differences, male mice ingested more energy than female mice, when ignoring diets and genotypes (see Figures 21a and 21b). Female both genotypes mice consumed less energy than male mice in HF diet while only female WT showed the same trend but not in GHA when fed a LF diet.

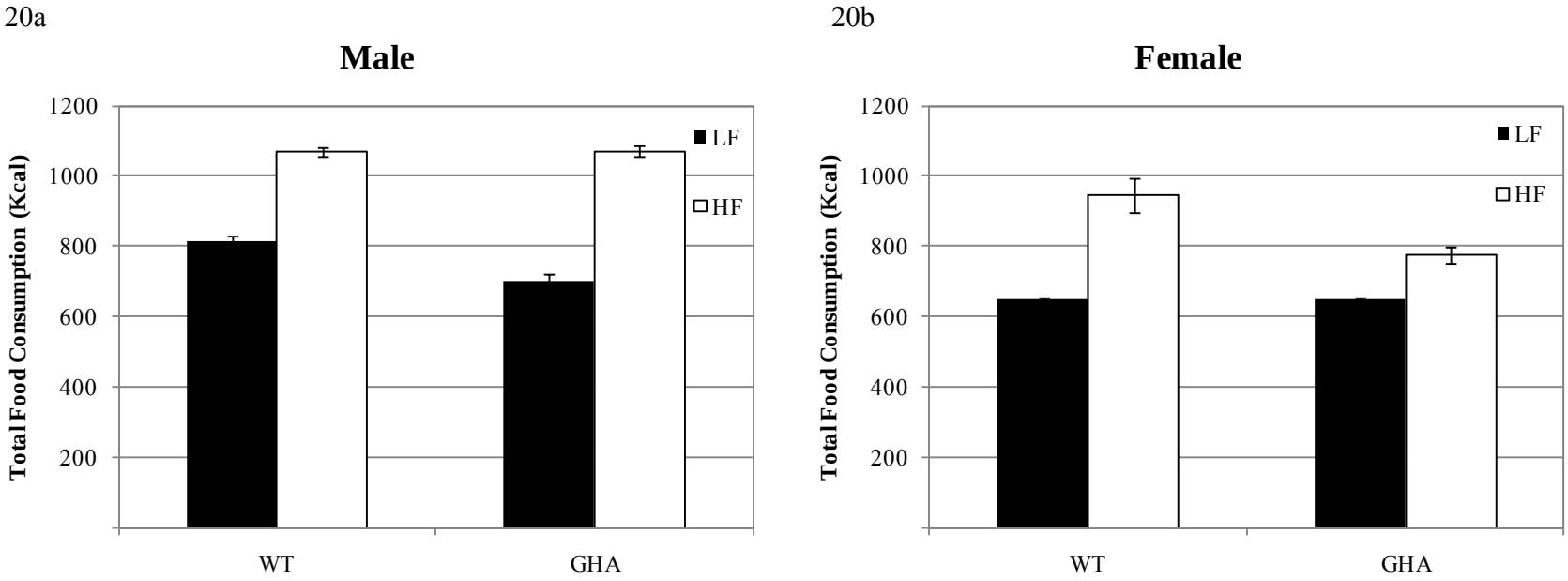


Figure 20. Energy consumption of male and female mice over the 10- to 21-week feeding study. Data are expressed as mean ± SEM. WT male on LF (n = 12), WT male on HF (n = 12), GHA male on LF (n = 9), GHA male on HF (n = 11), WT female on LF (n = 11), WT female on HF (n = 9), GHA female on LF (n = 11), GHA female on HF (n = 9).

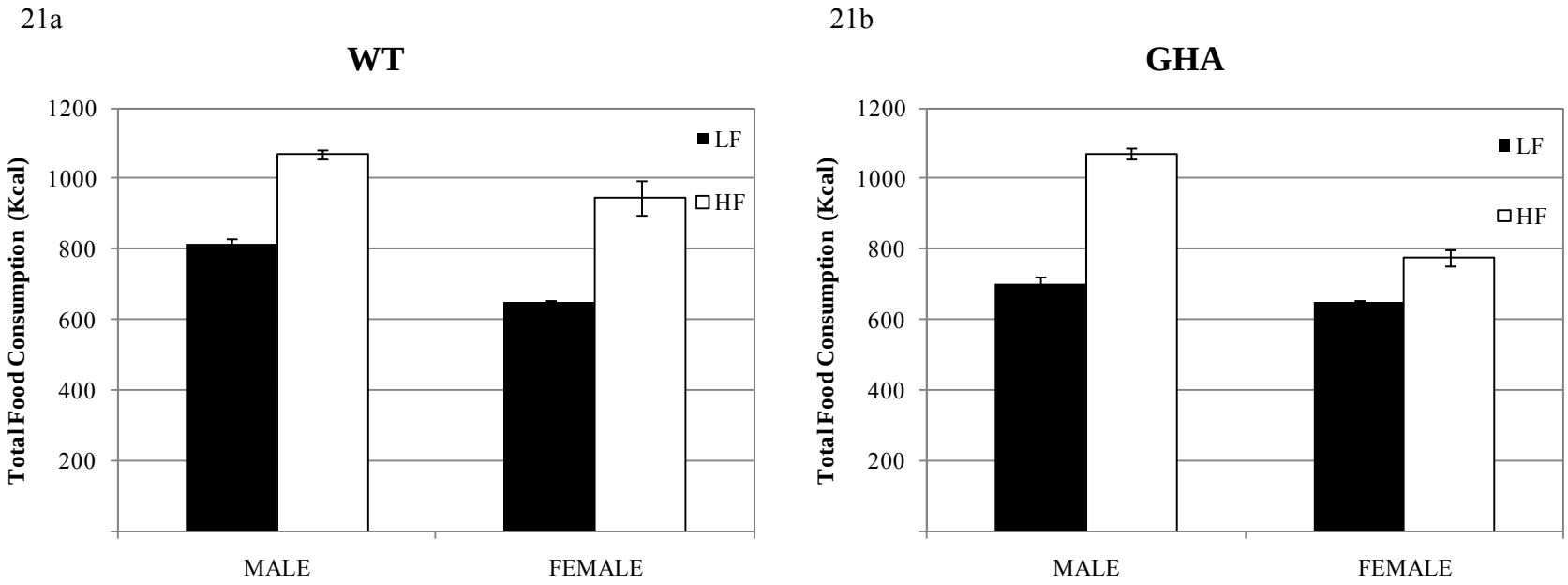


Figure 21. Energy consumption of GHA and WT mice over the 10- to 21-week feeding study. Data are expressed as mean ± SEM. WT male on LF (n = 12), WT male on HF (n = 12), GHA male on LF (n = 9), GHA male on HF (n = 11), WT female on LF (n = 11), WT female on HF (n = 9), GHA female on LF (n = 11), GHA female on HF (n = 9).

Blood Glucose and Glucose Tolerance Test

At week 20, fasting glucose levels were taken. Three-way ANOVA revealed significant differences in genotype ($F(1,75) = 13.294$, $p = .000489$), and diet ($F(1,75) = 21.885$, $p = .000013$); however, no significant differences were seen in gender or in any interactions (see Appendix U). Regarding genotype difference, GHAs were lower in fasting glucose than WT controls at least on the HF diet (see Figures 22a and 22b). With regard to gender differences, no significant difference between female and male mice in blood glucose levels were seen in either the HF or LF diet groups (see Figures 23a and 23b). As expected, mice in the HF diet had higher glucose levels than in the LF feeding.

As previously mentioned, glucose tolerance tests reveals how the body responds to a glucose challenge. Area under the curve data (AUC) summarize the glucose readings over the entire test and provide a gauge of the level of glucose tolerance. No significant difference was revealed by three-way ANOVA in gender ($F(1,75) = 3.348$, $p = .071241$), genotype ($F(1,75) = .157$, $p = .693130$), or diet ($F(1,75) = .961$, $p = .330189$); however, statistical differences in genotype x diet ($F(1,75) = 6.687$, $p = .011653$) and gender x genotype x diet ($F(1,75) = 4.443$, $p = .038388$) were noticed (see Appendix V).

Regarding genotype difference, as mentioned above, no significant difference was seen ($p < .05$, see Figures 24a and 24b). Male GHA mice had less AUC trend when fed a HF diet as compared to WTs while an opposite trend was seen in LF fed diet in which GHA mice were greater in AUC than the same gender WT controls. No genotype or diet differences were seen in female mice.

Likewise, no statistical difference was noticed between different genders when diets and genotypes were calculated ($p < .05$, see Figures 25a and 25b). Only female WT on the HF diet showed a lower trend in AUC than diet- and genotype-matched male controls.

Correlations between fasting glucose levels or AUC and absolute fat mass or percent fat mass were analyzed to determine if these glucose parameters could be explained by the level of adiposity. Pearson correlations revealed fasting glucose levels were positively correlated with absolute fat mass and percent fat mass respectively ($r(83) = .447$, $p = .000023$ and $r(83) = .297$, $p = .006409$). Similarly, positive relationships between AUC and fat mass (absolute and percent) existed ($r(83) = .286$, $p = .008871$ and $r (83) = .264$, $p = .016010$, respectively) (see Table 5).

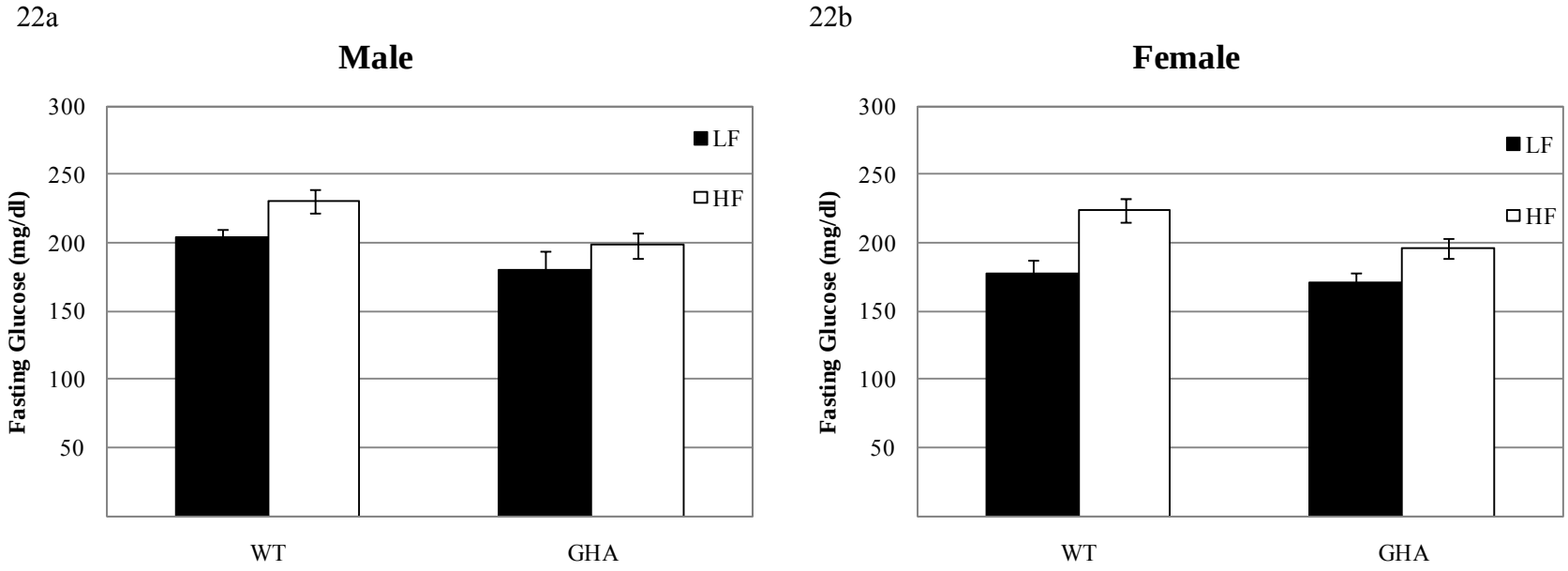


Figure 22. Fasting glucose of male and female mice at week 20 of feeding study. Data are expressed as mean ± SEM. WT male on LF (n = 12), WT male on HF (n = 12), GHA male on LF (n = 9), GHA male on HF (n = 11), WT female on LF (n = 11), WT female on HF (n = 9), GHA female on LF (n = 11), GHA female on HF (n = 9).

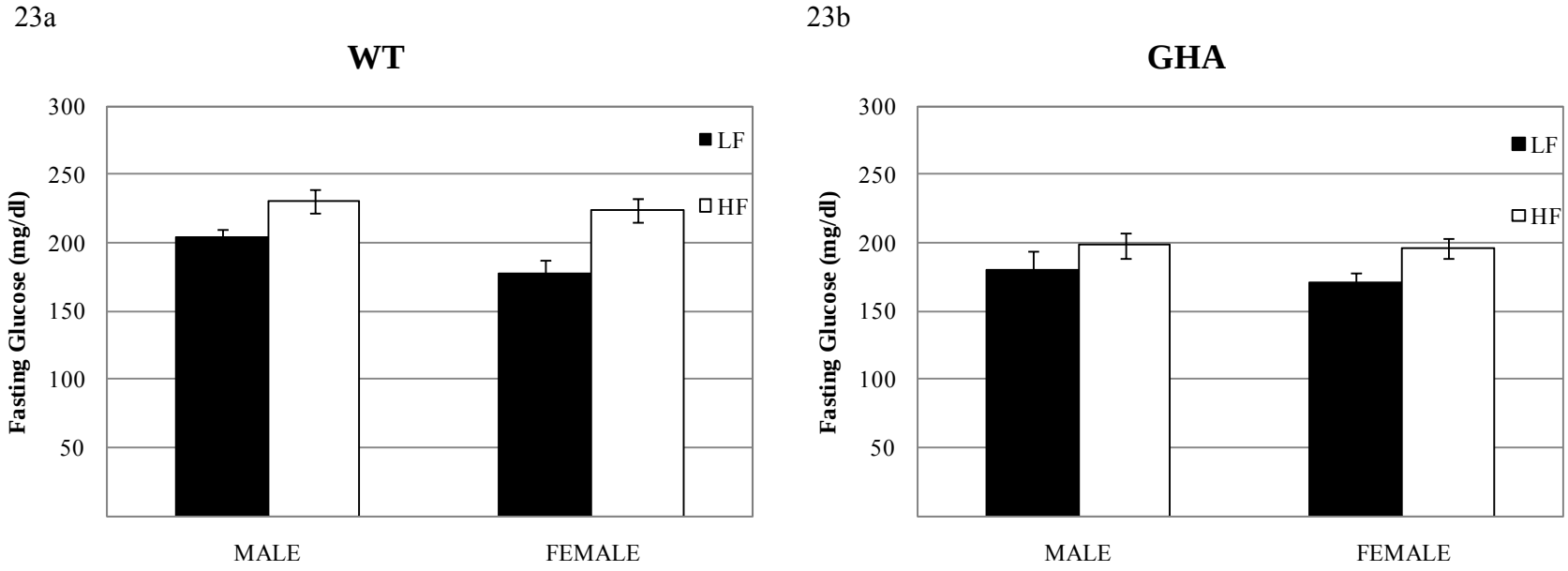


Figure 23. Fasting glucose of GHA and WT mice at week 20 of feeding study. Data are expressed as mean ± SEM. WT male on LF (n = 12), WT male on HF (n = 12), GHA male on LF (n = 9), GHA male on HF (n = 11), WT female on LF (n = 11), WT female on HF (n = 9), GHA female on LF (n = 11), GHA female on HF (n = 9).

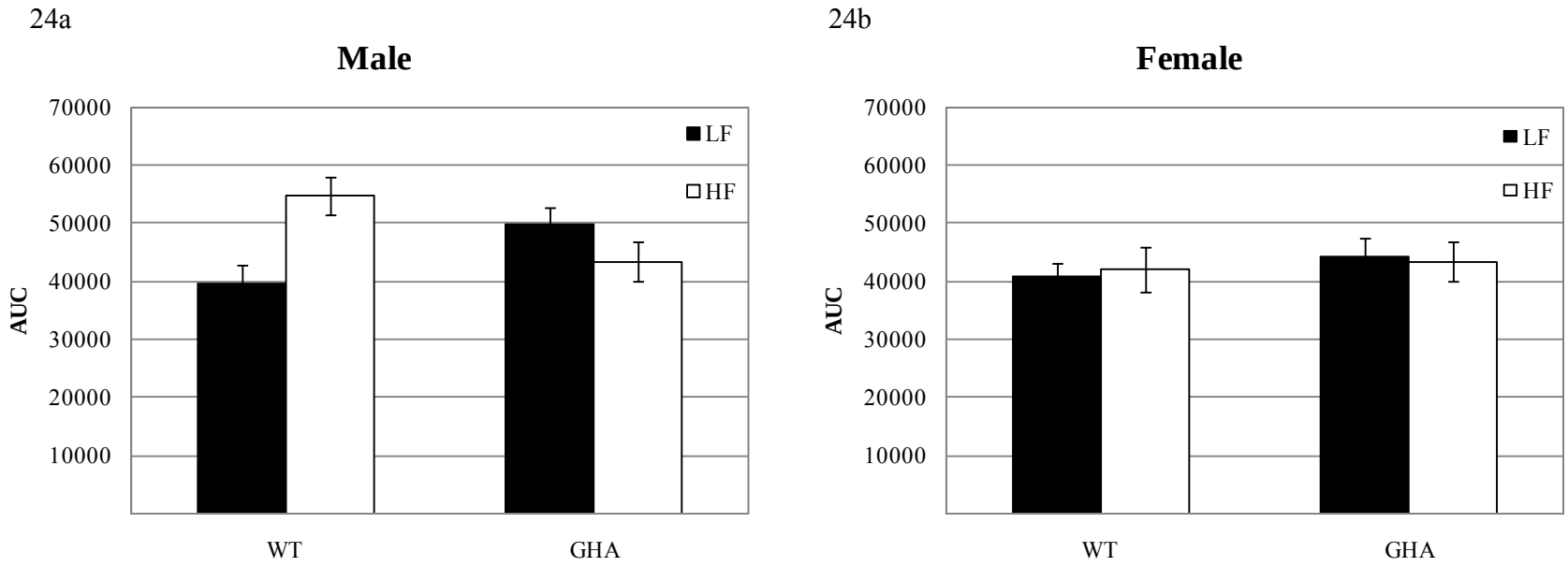


Figure 24. Area under the curve of male and female mice over the 10- to 21-week feeding study. Data are expressed as mean ± SEM. WT male on LF (n = 12), WT male on HF (n = 12), GHA male on LF (n = 9), GHA male on HF (n = 11), WT female on LF (n = 11), WT female on HF (n = 9), GHA female on LF (n = 11), GHA female on HF (n = 9).

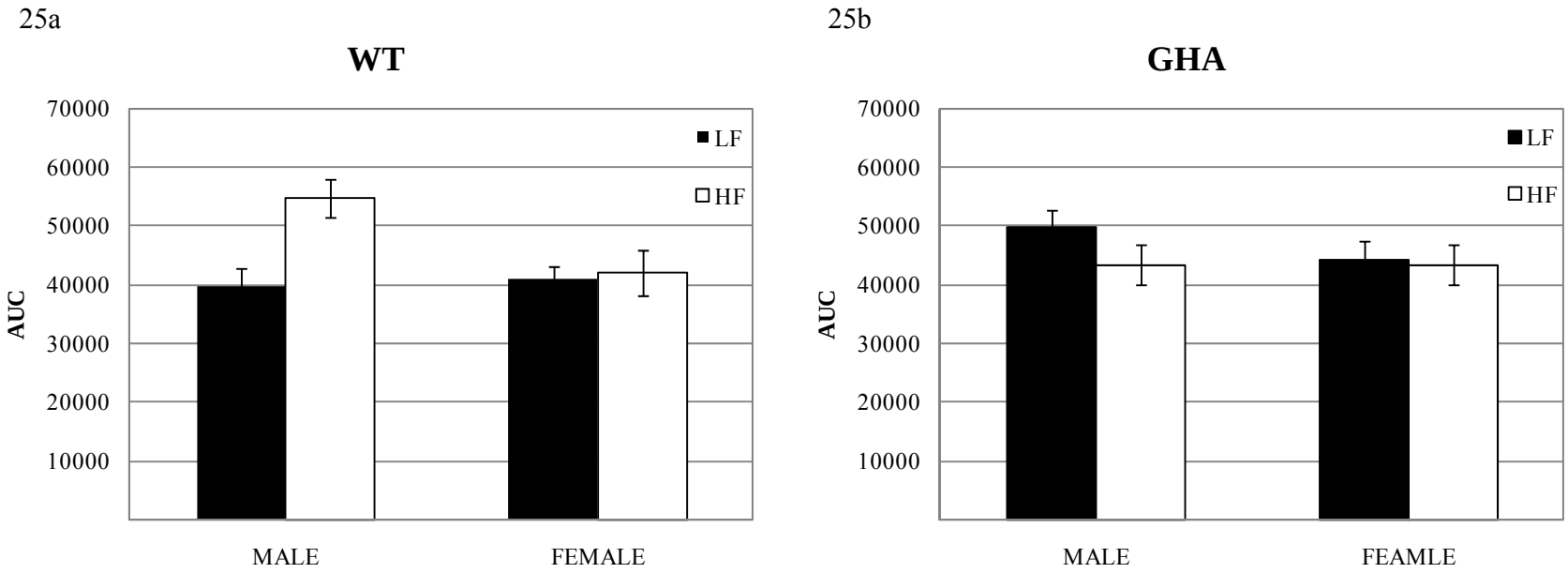


Figure 25. Area under the curve of GHA and WT mice over the 10- to 21-week feeding study. Data are expressed as mean ± SEM. WT male on LF (n = 12), WT male on HF (n = 12), GHA male on LF (n = 9), GHA male on HF (n = 11), WT female on LF (n = 11), WT female on HF (n = 9), GHA female on LF (n = 11), GHA female on HF (n = 9).

Table 5

Correlation of Glucose/AUC and Absolute Fat/Percent Fat Mass

		Glucose	AUC	Absolute Fat	Percent Fat
Glucose	Pearson Corrlation	1	.379**	**.447****	**.297***
	Sig . (2-tailed)		.000417	**.000023**	**.006409**
AUC	Pearson Corrlation	.379**	1	**.286****	**.264***
	Sig . (2-tailed)	.000417		**.008871**	**.016010**
Absolute Fat	Pearson Corrlation	**.447****	**.286****	1	.919*
	Sig . (2-tailed)	**.000023**	**.008871**		.000000
Percent Fat	Pearson Corrlation	**.297****	**.264***	.919**	1
	Sig . (2-tailed)	**.006409**	**.016010**	.000000	

Note. ** Correlation is significant at the 0.01 level (2-tailed).
*Correlation is significant at the 0.05 level (2-tailed).
Bold values represent significant difference.

Absolute Tissue/Organ Weights

Data from five fat depots (subcutaneous, perigonadal, retroperitoneal, mesenteric, and BAT) and six organs (liver, kidney, spleen, heart, muscle, and lung) were analyzed by three-way ANOVA to compare differences in gender, genotype, diet, and interactions. There were significant differences in gender, diet, and gender x diet interaction in all fat depots (see Table 6). In addition, the perigonadal depot had a significant interaction between genotype x diet. Interestingly, genotype differences were not seen for retroperitoneal, mesenteric, or BAT depots. Only subcutaneous and perigonadal fat masses were significantly different between genotypes (see Table 6) with GHA mice having more subcutaneous fat mass but less perigonadal fat mass than WT littermate mice (see Figures 26a and 26b). In fact, the subcutaneous pad in all GHAs was the largest among all fat pads dissected even including WT controls. In contrast, perigonadal mass was the smallest pad for GHA mice when compared to WT mice.

Concerning gender differences, male mice were greater in all absolute fat masses than female mice (see Figures 27a and 27b). Not surprisingly, fat depots in HF groups were greater than LF groups.

Significant differences were noticed in absolute organ weights of all organs with regard to gender, genotype, diet, and there was a significant interaction for spleen (gender x genotype) as well as for liver (gender x diet) (see Table 6). GHA mice were lower in each absolute organ weights than same gender and diet controlled WT mice (see Figures 28a and 28b).

As expected, females had lower organ weights than males, due to the females' lower body weights (see Figures 29a and 29b). Consistently, organ weights of females were less than genotype- and diet-matched male animals with the exception of spleen.

Table 6

Factorial ANOVA Results of Absolute Tissue/Organ Weight in GHA and WT Mice

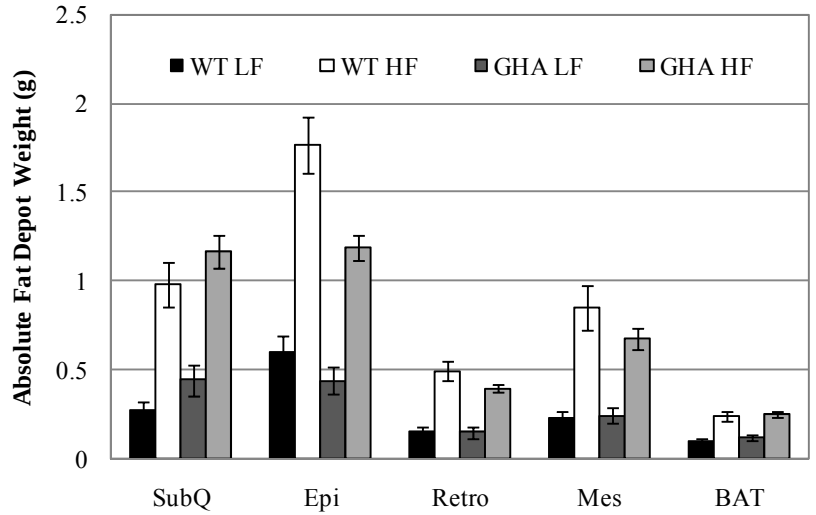
Absolute Wt	Gender	Genotype	Diet	Gender x Genotype	Gender x Diet	Genotype x Diet	Gender x Genotype x Diet
Depots							
SubQ	$F(1,76) = 23.32$, **$p < .001$**	$F(1,76) = 6.48$, **$p < .05$**	**$F(1,76) = 60.47$, $p < .001$**	$F(1,76) = .13$, $p > .05$	**$F(1,76) = 8.18$, $p < .05$**	$F(1,76) = .13$, $p > .05$	$F(1,76) = .08$, $p > .05$
Peri	**$F(1,76) = 51.50$, $p < .001$**	**$F(1,76) = 12.00$, $p < .001$**	**$F(1,76) = 69.58$, $p < .001$**	$F(1,76) = .92$, $p > .05$	**$F(1,76) = 11.45$, $p < .05$**	**$F(1,76) = 5.26$, $p < .05$**	$F(1,76) = .21$, $p > .05$
Retro	**$F(1,75) = 76.82$, $p < .001$**	$F(1,75) = .95$, $p > .05$	**$F(1,75) = 66.38$, $p < .001$**	$F(1,75) = .73$, $p > .05$	**$F(1,75) = 16.84$, $p < .001$**	$F(1,75) = 2.59$, $p > .05$	$F(1,75) = .54$, $p > .05$
Mes	**$F(1,76) = 40.48$, $p < .001$**	$F(1,76) = 1.15$, $p > .05$	**$F(1,76) = 46.88$, $p < .001$**	$F(1,76) = .17$, $p > .05$	**$F(1,76) = 14.65$, $p < .001$**	$F(1,76) = 1.93$, $p > .05$	$F(1,76) = .61$, $p > .05$
BAT	**$F(1,76) = 107.91$, $p < .001$**	$F(1,76) = .16$, $p > .05$	**$F(1,76) = 64.54$, $p < .001$**	$F(1,76) = 1.57$, $p > .05$	**$F(1,76) = 24.65$, $p < .001$**	$F(1,76) = .19$, $p > .05$	$F(1,76) = .97$, $p > .05$

Table 6 (continued)

Absolute Wt	Gender	Genotype	Diet	Gender x Genotype	Gender x Diet	Genotype x Diet	Gender x Genotype x Diet
Organs							
Liver	$F(1,76) = 32.10$, **$p < .001$**	$F(1,76) = 20.20$, **$p < .001$**	$F(1,76) = 26.84$, **$p < .001$**	$F(1,76) = .01$, $p > .05$	$F(1,76) = 9.03$, **$p < .05$**	$F(1,76) = 2.25$, $p > .05$	$F(1,76) = .04$, $p > .05$
Kidney	$F(1,76) = 228.37$, **$p < .001$**	$F(1,76) = 319.20$, **$p < .001$**	$F(1,76) = 17.94$, **$p < .001$**	$F(1,76) = 2.05$, $p > .05$	$F(1,76) = 1.50$, $p > .05$	$F(1,76) = .25$, $p > .05$	$F(1,76) = .00$, $p > .05$
Spleen	$F(1,76) = 4.61$, **$p < .05$**	$F(1,76) = 51.50$, **$p < .001$**	$F(1,76) = 28.17$, **$p < .001$**	$F(1,76) = 9.01$, **$p < .05$**	$F(1,76) = .58$, $p > .05$	$F(1,76) = 1.48$, $p > .05$	$F(1,76) = .00$, $p > .05$
Heart	$F(1,76) = 45.46$, **$p < .001$**	$F(1,76) = 149.02$, **$p < .001$**	$F(1,76) = 38.28$, **$p < .001$**	$F(1,76) = .35$, $p > .05$	$F(1,76) = .01$, $p > .05$	$F(1,76) = 1.92$, $p > .05$	$F(1,76) = .57$, $p > .05$
Muscle	$F(1,76) = 151.82$, **$p < .001$**	$F(1,76) = 358.72$, **$p < .001$**	$F(1,76) = 20.92$, **$p < .001$**	$F(1,76) = 2.32$, $p > .05$	$F(1,76) = .11$, $p > .05$	$F(1,76) = .54$, $p > .05$	$F(1,76) = 2.31$, $p > .05$
Lung	$F(1,76) = 74.13$, **$p < .001$**	$F(1,76) = 70.34$, **$p < .001$**	$F(1,76) = 12.82$, **$p < .001$**	$F(1,76) = 2.44$, $p > .05$	$F(1,76) = 1.76$, $p > .05$	$F(1,76) = 1.74$, $p > .05$	$F(1,76) = 1.46$, $p > .05$

Note. Bold values represent significant difference of absolute tissue/organ weights. SubQ: subcutaneous; Peri: epididymal in males and parametrial in females; Retro: retroperitoneal; Mes: mesenteric; and BAT: brown adipose tissue.

26a



26b

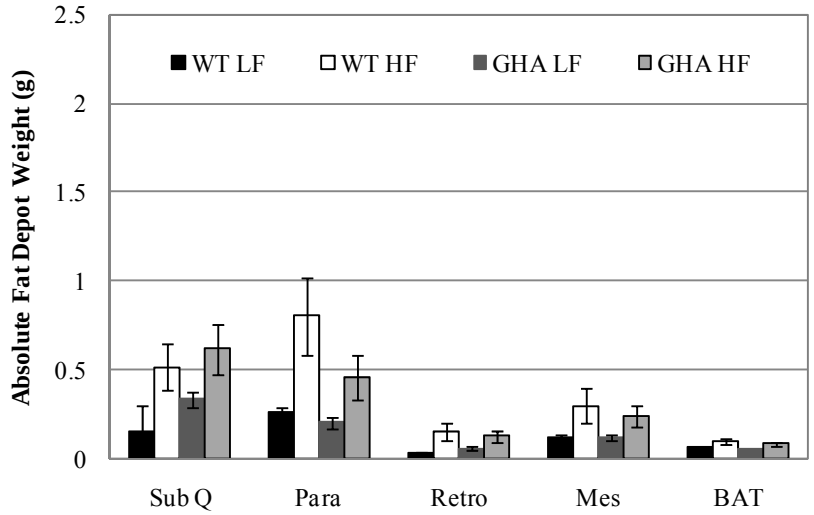
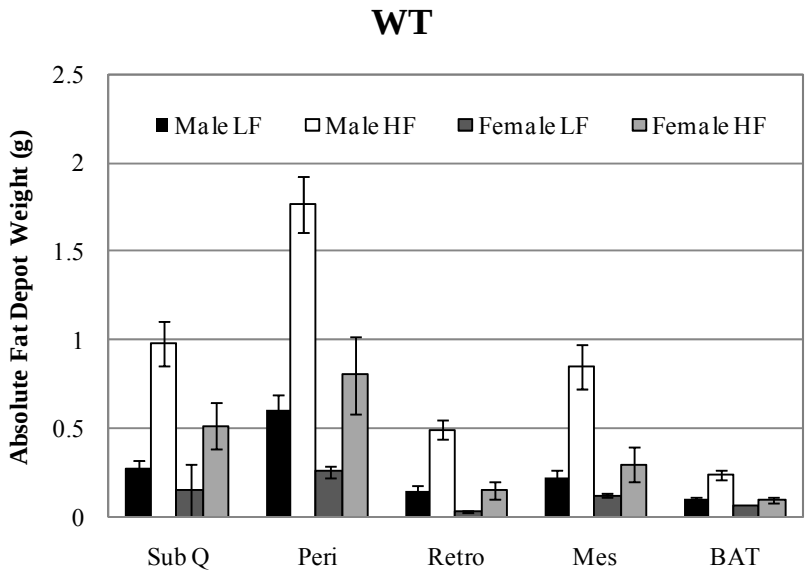


Figure 26. Absolute fat depot weights of male and female mice over the 10- to 21-week feeding study. Data are expressed as mean ± SEM. WT male on LF (n = 12), WT male on HF (n = 12), GHA male on LF (n = 9), GHA male on HF (n = 11), WT female on LF (n = 11), WT female on HF (n = 9), GHA female on LF (n = 11), GHA female on HF (n = 9).

27a



27b

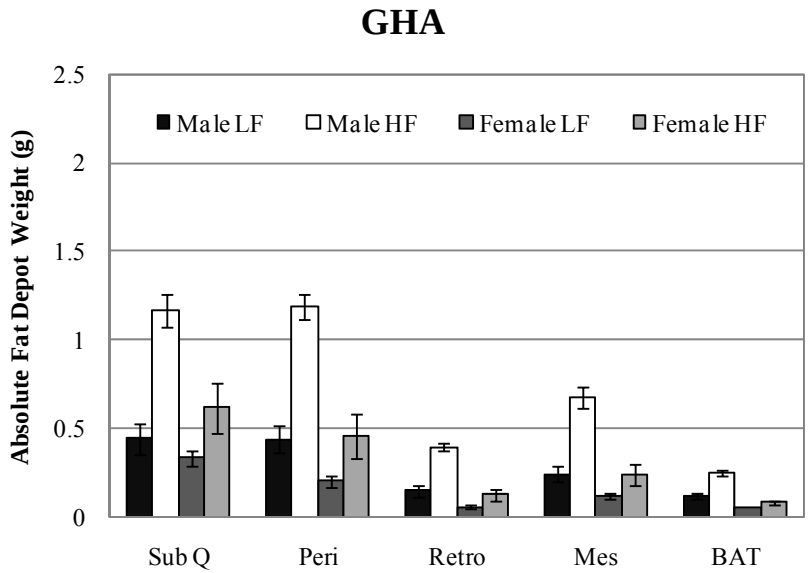
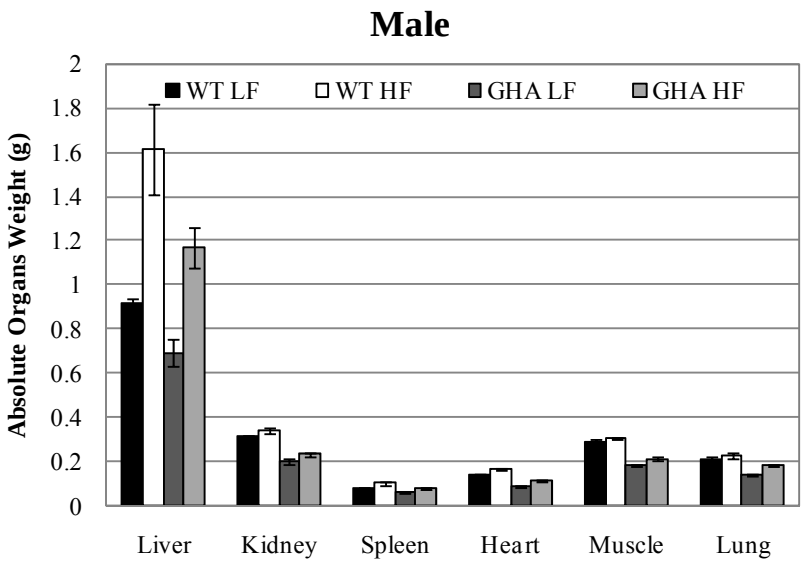


Figure 27. Absolute fat depot weights of GHA and WT mice over the 10- to 21-week feeding study. Data are expressed as mean ± SEM. WT male on LF (n = 12), WT male on HF (n = 12), GHA male on LF (n = 9), GHA male on HF (n = 11), WT female on LF (n = 11), WT female on HF (n = 9), GHA female on LF (n = 11), GHA female on HF (n = 9).

28a



28b

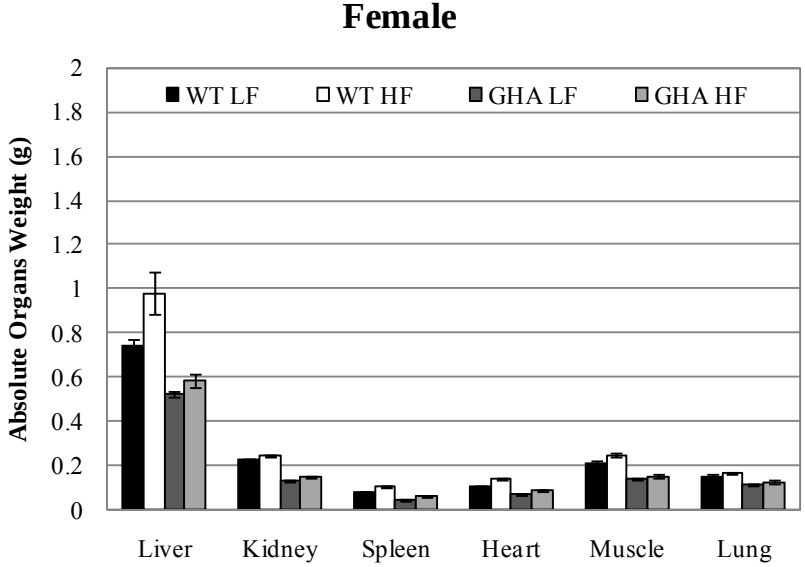
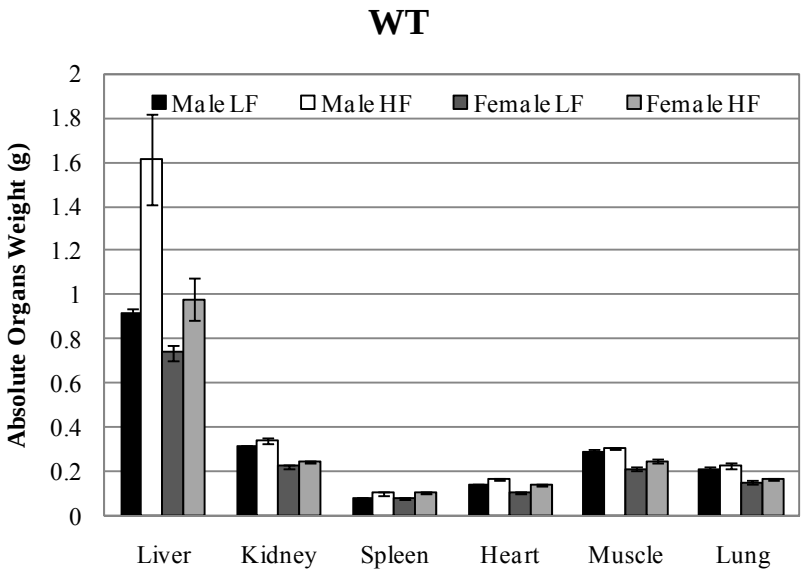


Figure 28. Absolute organ weights of male and female mice over the 10- to 21-week feeding study. Data are expressed as mean ± SEM. WT male on LF (n = 12), WT male on HF (n = 12), GHA male on LF (n = 9), GHA male on HF (n = 11), WT female on LF (n = 11), WT female on HF (n = 9), GHA female on LF (n = 11), GHA female on HF (n = 9).

29a



29b

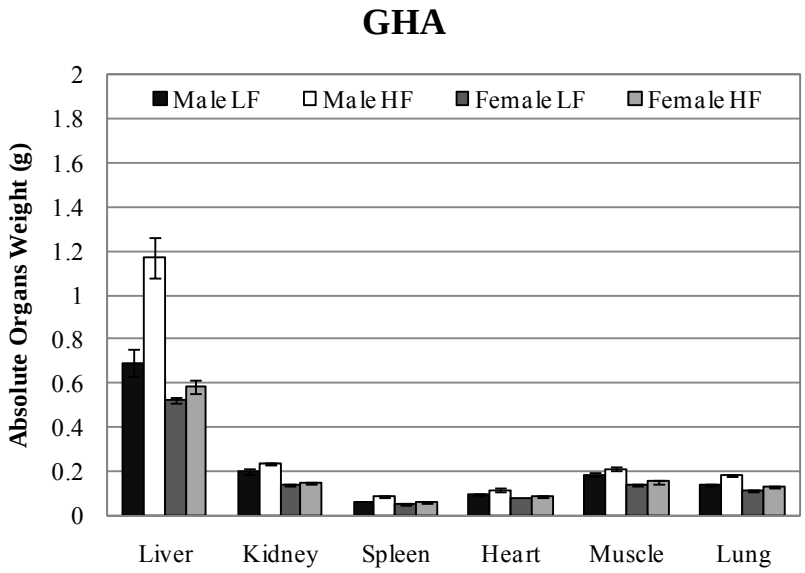


Figure 29. Absolute organ weights of GHA and WT mice over the 10- to 21-week feeding study. Data are expressed as mean ± SEM. WT male on LF (n = 12), WT male on HF (n = 12), GHA male on LF (n = 9), GHA male on HF (n = 11), WT female on LF (n = 11), WT female on HF (n = 9), GHA female on LF (n = 11), GHA female on HF (n = 9).

Normalized Tissue/Organ Weights

Because of the difference in body weight, it was valuable to view the differences in tissue mass relative to body weight. Three-way ANOVA revealed statistical differences in gender and diet for all fat depots as well as significant differences in genotype for subcutaneous, retroperitoneal, and BAT fat depots. Several depots, but not all depots, also showed significant interactions (see Table 7).

Regarding genotype differences, all percent depot weights in GHA mice were greater, except the perigonadal depot (see Figures 30a and 30b). Consistent with absolute subcutaneous fat depot, GHA mice showed a markedly greater normalized subcutaneous fat depot than WT littermates ($p < .05$). Likewise, the subcutaneous pad was the biggest, while perigonadal was the smallest when comparing GHAs to diet- and gender- matched WT siblings. Interestingly, GHA mice had greater percent BAT fat than WT littermates.

Regarding gender difference in normalized depot weights, all female mice had lower fat depot weights than male mice (see Figures 31a and 31b). Moreover, HF fed mice had greater fat depots.

GHAs showed a statistically lower percent organ weights than WT counterparts except liver and lung ($p > .05$, see Table 7 or Figures 32a and 32b). Again, as compared to diet- and gender-controlled WT mice, GHAs overall had less normalized organ weights except for the liver and spleen in male LF feeding.

Regarding gender differences, male mice were lower or equal in percent organ weight than female mice (see Figures 33a and 33b). In the HF group, female GHAs and female WTs had lower percent liver weight than genotype- and diet-controlled males. In

the LF group, female WT mice were lower than male controls in percent kidney weight. HF fed GHA mice were lower in percent organ weights than LF fed gender- and genotype-controlled mice, most likely due to the fat mass gains (see Figures 32a and 32b). Again, no statistically significant differences were observed for gender in liver, kidney, and lung.

Table 7

Factorial ANOVA Results of Percent Tissue/Organ Weight in GHA and WT Mice

Percent Wt	Gender	Genotype	Diet	Gender x Genotype	Gender x Diet	Genotype x Diet	Gender x Genotype x Diet
Depots							
SubQ	$F(1,76) = 5.44$, **$p < .05$**	$F(1,76) = 46.78$, **$p < .001$**	$F(1,76) = 48.42$, **$p < .001$**	$F(1,76) = .00$, $p > .05$	$F(1,76) = 2.72$, $p > .05$	$F(1,76) = .01$, $p > .05$	$F(1,76) = .19$, $p > .05$
Peri	$F(1,76) = 37.68$, **$p < .001$**	$F(1,76) = 1.72$, $p > .05$	$F(1,76) = 62.23$, **$p < .001$**	$F(1,76) = .00$, $p > .05$	$F(1,76) = 4.77$, **$p < .05$**	$F(1,76) = 2.34$, $p > .05$	$F(1,76) = .00$, $p > .05$
Retro	$F(1,75) = 71.21$, **$p < .001$**	$F(1,75) = 4.51$, **$p < .05$**	$F(1,75) = 61.08$, **$p < .001$**	$F(1,75) = .00$, $p > .05$	$F(1,75) = 8.16$, **$p < .05$**	$F(1,75) = .71$, $p > .05$	$F(1,75) = .16$, $p > .05$
Mes	$F(1,76) = 35.66$, **$p < .001$**	$F(1,76) = 2.87$, $p > .05$	$F(1,76) = 47.45$ **$p < .001$**	$F(1,76) = .18$, $p > .05$	$F(1,76) = 11.07$, **$p < .05$**	$F(1,76) = .50$, $p > .05$	$F(1,76) = .39$, $p > .05$
BAT	$F(1,76) = 81.45$, **$p < 0.001$**	$F(1,76) = 35.65$, **$p < .001$**	$F(1,76) = 39.73$, **$p < .001$**	$F(1,76) = 8.62$, **$p < .05$**	$F(1,76) = 13.79$, **$p < .001$**	$F(1,76) = .03$, $p > .05$	$F(1,76) = .02$, $p > .05$

Table 7 (continued)

Percent Wt	Gender	Genotype	Diet	Gender x Genotype	Gender x Diet	Genotype x Diet	Gender x Genotype x Diet
Organs							
Liver	$F(1,76) = 1.80$, $p > .05$	$F(1,76) = 2.61$, $p > .05$	$F(1,76) = 6.01$, **$p < .05$**	$F(1,76) = 1.61$, $p > .05$	$F(1,76) = 6.01$, **$p < .05$**	$F(1,76) = 1.75$, $p > .05$	$F(1,76) = .05$, $p > .05$
Kidney	$F(1,76) = .45$, $p > .05$	$F(1,76) = 22.09$, **$p < .001$**	$F(1,76) = 16.19$, **$p < .001$**	$F(1,76) = .89$, $p > .05$	$F(1,76) = 2.40$, $p > .05$	$F(1,76) = .00$, $p > .05$	$F(1,76) = .41$, $p > .05$
Spleen	$F(1,76) = 30.83$, **$p < .001$**	$F(1,76) = 4.96$, **$p < .05$**	$F(1,76) = .10$, $p > .05$	$F(1,76) = 16.96$, **$p < .001$**	$F(1,76) = 1.46$, $p > .05$	$F(1,76) = .22$, $p > .05$	$F(1,76) = .92$, $p > .05$
Heart	$F(1,76) = 16.18$, **$p < .001$**	$F(1,76) = 13.33$, **$p < .001$**	$F(1,76) = .88$, $p > .05$	$F(1,76) = .27$, $p > .05$	$F(1,76) = 6.70$, **$p < .05$**	$F(1,76) = .39$, $p > .05$	$F(1,76) = .00$, $p > .05$
Muscle	$F(1,76) = 8.88$, **$p < .05$**	$F(1,76) = 28.37$, **$p < .001$**	$F(1,76) = 25.76$, **$p < .001$**	$F(1,76) = .27$, $p > .05$	$F(1,76) = 7.21$, **$p < .05$**	$F(1,76) = .02$, $p > .05$	$F(1,76) = .16$, $p > .05$
Lung	$F(1,76) = 3.29$, $p > .05$	$F(1,76) = .18$, $p > .05$	$F(1,76) = 9.56$, **$p < .05$**	$F(1,76) = .82$, $p > .05$	$F(1,76) = .58$, $p > .05$	$F(1,76) = 1.85$, $p > .05$	$F(1,76) = .21$, $p > .05$

Note. Bold values represent significant difference of percent tissue/organ weights. SubQ: subcutaneous; Peri: epididymal in males and parametrial in females; Retro: retroperitoneal; Mes: mesenteric; and BAT: brown adipose tissue.

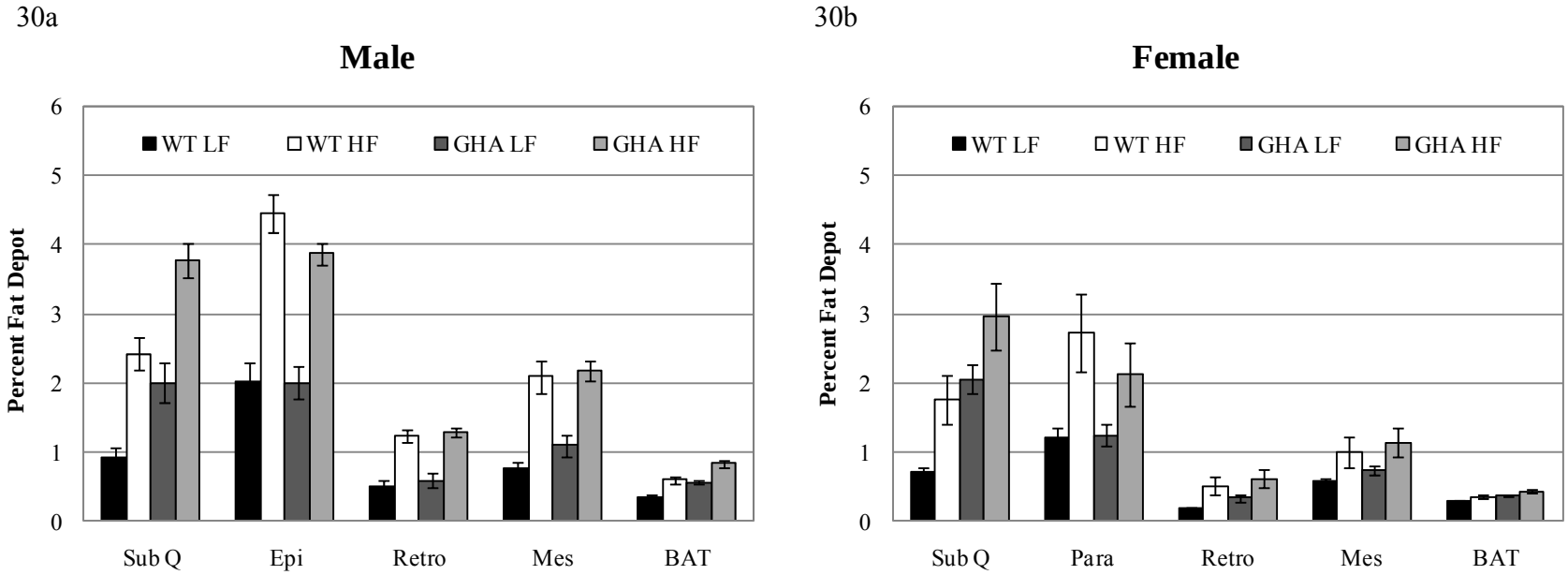


Figure 30. Normalized fat depot weights of male and female mice over the 10- to 21-week feeding study. Data are expressed as mean ± SEM. WT male on LF (n = 12), WT male on HF (n = 12), GHA male on LF (n = 9), GHA male on HF (n = 11), WT female on LF (n = 11), WT female on HF (n = 9), GHA female on LF (n = 11), GHA female on HF (n = 9).

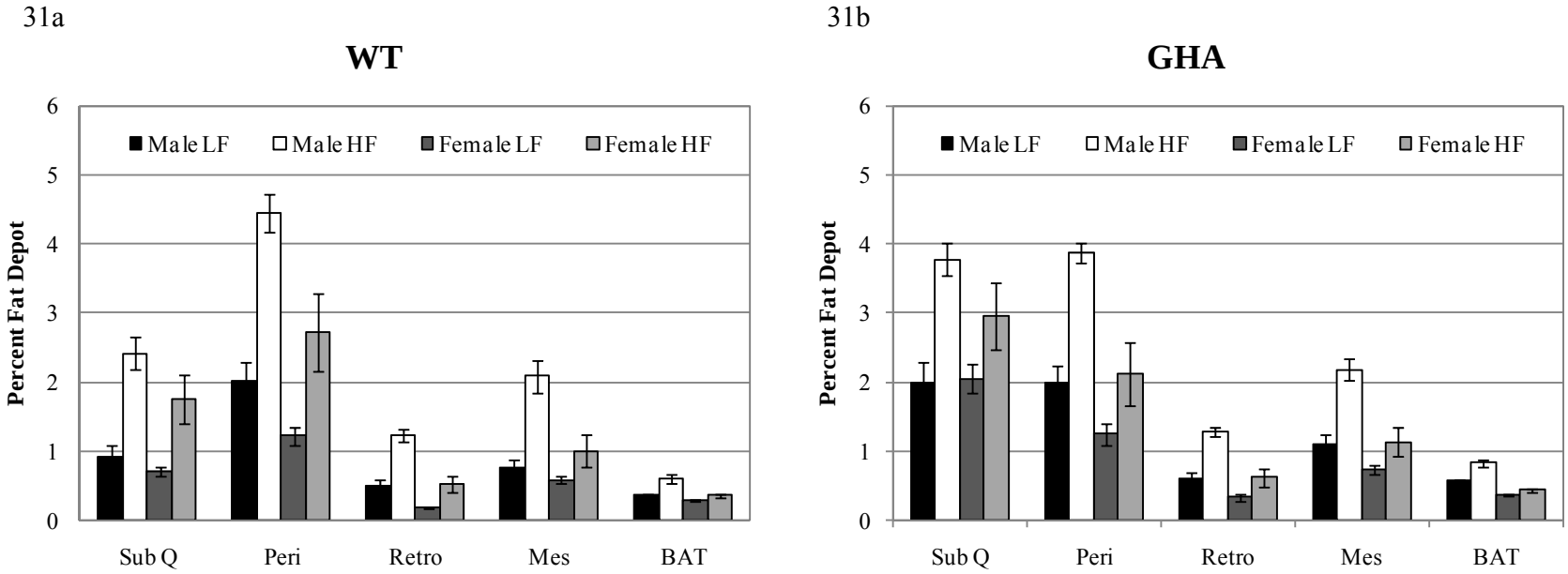
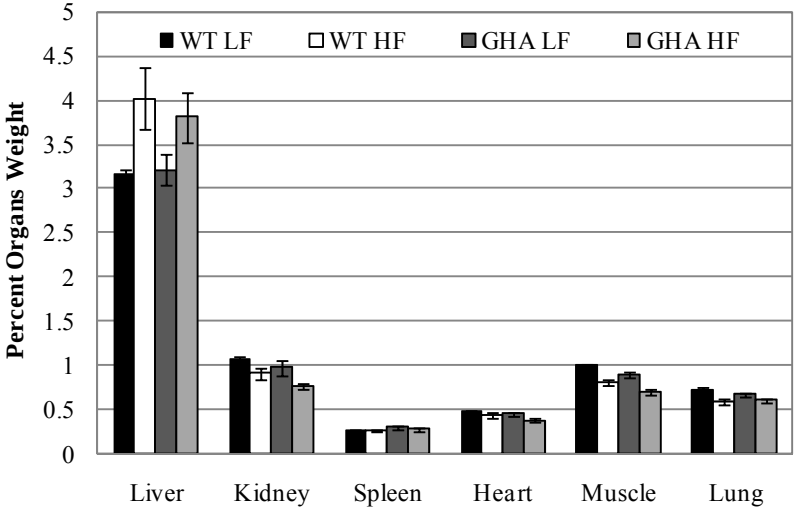


Figure 31. Normalized fat depot weights of GHA and WT mice over the 10- to 21-week feeding study. Data are expressed as mean ± SEM. WT male on LF (n = 12), WT male on HF (n = 12), GHA male on LF (n = 9), GHA male on HF (n = 11), WT female on LF (n = 11), WT female on HF (n = 9), GHA female on LF (n = 11), GHA female on HF (n = 9).

32a



32b

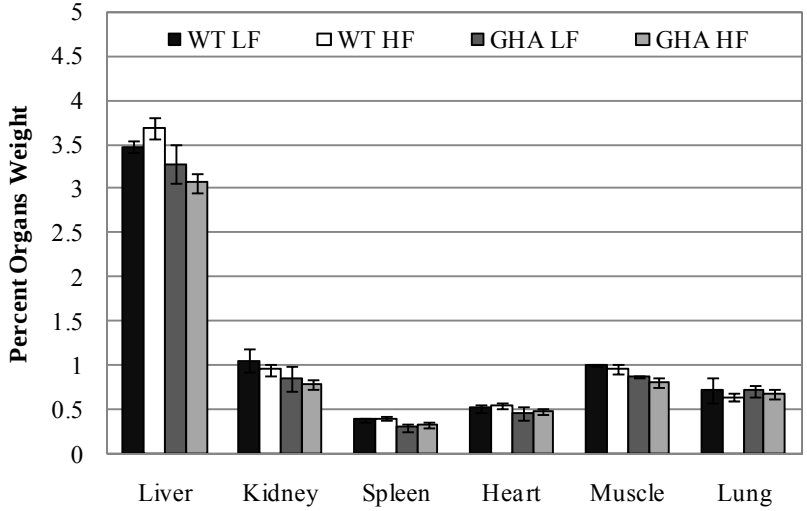


Figure 32. Normalized organ weights of male and female mice over the 10- to 21-week feeding study. Data are expressed as mean ± SEM. WT male on LF (n = 12), WT male on HF (n = 12), GHA male on LF (n = 9), GHA male on HF (n = 11), WT female on LF (n = 11), WT female on HF (n = 9), GHA female on LF (n = 11), GHA female on HF (n = 9).

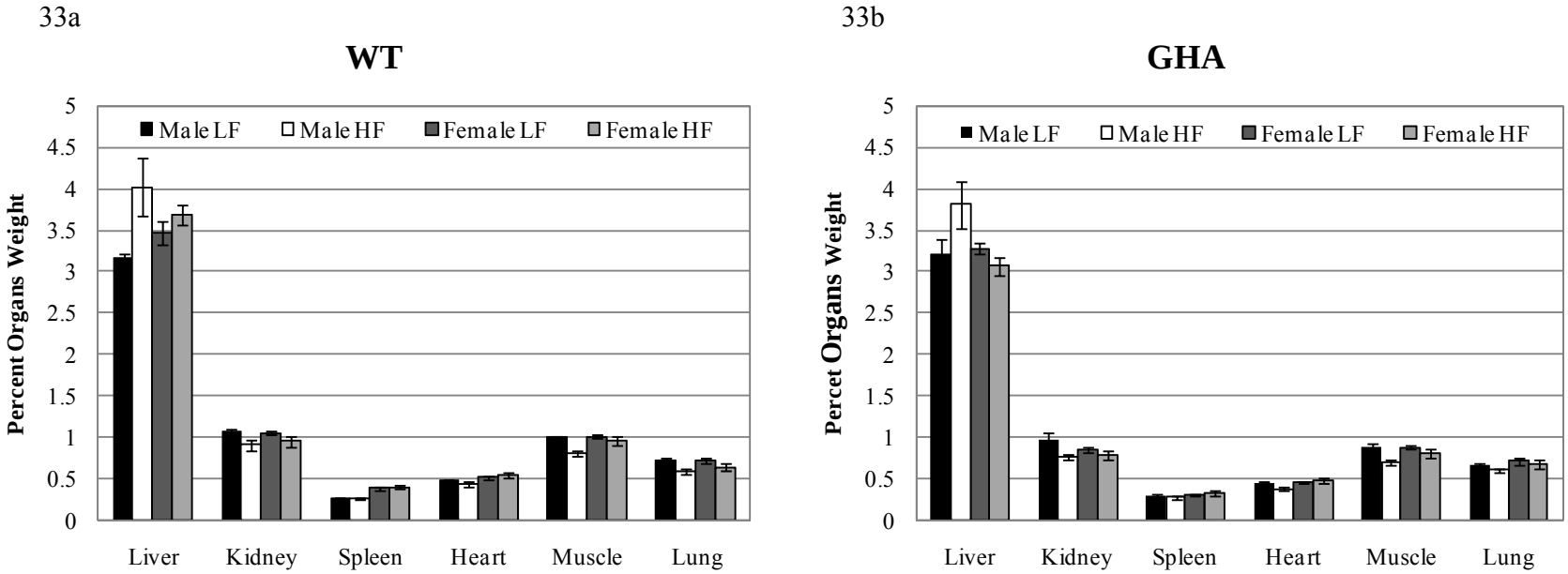


Figure 33. Normalized organ weights of GHA and WT mice over the 10- to 21-week feeding study. Data are expressed as mean ± SEM. WT male on LF (n = 12), WT male on HF (n = 12), GHA male on LF (n = 9), GHA male on HF (n = 11), WT female on LF (n = 11), WT female on HF (n = 9), GHA female on LF (n = 11), GHA female on HF (n = 9).

CHAPTER 5: DISCUSSION

The 11-week feeding study was designed to investigate diet-induced obesity in GHA mice. As compared to previous studies in which we assessed diet-induced obesity in other GH-modified mouse models (bGH and $GHR^{-/-}$ mice), females were included in this study and we observed significant gender differences with HF exposure. Namely, the females were relatively resistant to diet-induced obesity as compared to males.

Because this is the only study to date that has manipulated diet in GHA mice, much of this discussion will compare how the results from the LF fed GHA mice in this study compare to LF fed GHA mice in other studies. However, this discussion will also evaluate the genotype differences in this study as compared with other studies in which mouse models with altered GH signaling have been fed similar HF diets (bGH mice with excess GH signaling and $GHR^{-/-}$ mice with no growth hormone signaling as compared to GHA with reduced GH signaling). This discussion also focuses on the sexual dimorphic response to HF feeding for both GHA and WT mice.

Growth Hormone Receptor Antagonist Mice Comparison on the Low Fat Diet

In this section, all comparisons are made between GHA and their gender-matched WT controls unless otherwise indicated. When fed a LF diet, GHA mice had lower body weights than WT siblings. Interestingly, several studies report that the dwarf male GHA mice have lower body weight than WT controls at early ages, while they catch up in body weight with WTs by older ages (at 44-46 weeks of age in Coschigano et al; by 52 weeks of age in Li et al; and by 60 weeks of age in Magon) (Coschigano et al., 2003; Li et al., 2003; Magon, 2009). These growth patterns do not occur for female GHA mice, which

remain dwarf relative to WT females throughout lifespan (Magon, 2009). The mice in this study were only five months old by the end of the feeding study; thus, our data are consistent with these other studies, because our mice were too young to experience the "catch up" growth reported in other studies.

When assessing body composition, the GHA mice were relatively obese with lower percent lean mass. This is consistent with several previous studies, where GHA male mice have a much greater percent fat mass than WTs (Berryman et al., 2004), and female GHA mice are also relatively obese (Magon, 2009). In this study, most fat mass was localized to the subcutaneous depot, which was disproportionally enlarged, while the perigonadal fat pad was relatively small. Although there is some discrepancy in fat depots sizes in these mice, previous studies have shown specific enlargement of the subcutaneous fat depot in GHA male mice (Berryman et al., 2004; Magon, 2009). In addition, BAT increased in GHA mice in our study. In this regard, BAT is known to be a GH-responsive tissue (Li et al., 2003).

In this study, male GHA mice are hyperphagic on a LF diet as compared to male WT mice, consistent with previous studies assessing mice of a similar age as in this study (Berryman et al., 2004; Coschigano et al., 2003). Our report is the first to suggest that GHA females are also hyperphagic on a LF diet. Without energy expenditure measurement data, we can only assume that the body weight and fat mass gain in obese GHA mice is due, at least in part, to excess energy consumption.

Previous studies show that GHA mice have a normal lifespan even though they are obese (Coschigano et al., 2003; Magon, 2009). A normal lifespan despite obesity

could be due to lower or normal glucose and insulin levels in GHA mice compared to WT mice (Coschigano et al., 2003; Magon, 2009). Consistent with the previous studies, male GHA mice in this study exhibited lower blood glucose levels on the LF diet than male WT mice, suggesting reduced GH signaling protects from diabetes, despite obesity.

Diet-Induced Obesity in Growth Hormone Receptor Antagonist Mice

This feeding study is the first to address the HF diet-induced obesity of GHA mice. When compared to the LF diet, the HF diet drastically increased body weight and fat mass as well as slightly increased lean mass of both male GHA and WT mice. Nevertheless, male GHA were more obese than WT males on the HF diet.

Obesity is a result of a positive energy balance with energy intake exceeding energy expenditure. As stated before, energy expenditure was not assessed in this study. The increased obesity in HF GHA mice was associated with an even greater energy intake than LF male GHA mice, again suggesting hyperphagia contributed to the greater fat mass gains in HF fed GHA mice. The HF diet accelerated the gain in fat mass in GHA mice of both genders. The hyperphagia exists despite elevated leptin levels in these mice (Berryman et al., 2004). It is well documented that leptin reduces food intake and increases energy expenditure through specific target neurons in the brain (Schwartz, Woods, Porte, Seeley, & Baskin, 2000). Leptin is secreted from adipose tissue and is secreted in proportion to adipose tissue mass (Frederich et al., 1995; Kearns, McKeever, Roegner, Brady, & Malinowski, 2006), making it a good signal to indicate adiposity. Previous studies find that leptin is increased by months of age in male obese GHA mice as compared to littermates (Berryman et al., 2004; Magon, 2009), and male GHA mice

consume more food when normalized to body weight (Berryman et al., 2004), suggesting leptin resistance in male GHA mice. Further measurement of leptin levels or leptin receptor responsiveness would help resolve how this hormone regulates food intake and energy expenditure in GHA mice.

Unlike male GHA mice, female GHA mice were less responsive to the HF diet. Female GHA mice did not increase body weight as significantly as male GHA mice when challenged with the HF diet, with a longer lag period in the fat mass gain and no dramatic changes in lean mass. WT females show a similar trend with GHA females having only a slightly higher fat mass gain than WT females, suggesting female GHA mice are no more sensitive to the HF diet than WT female mice. Female GHA mice consumed less total energy than female WT mice, while they tended to be hyperphagic when food intake was normalized to their dwarf body size.

Altered GH signaling in bGH, $GHR^{-/-}$, and GHA mice results in different outcomes on a HF diet. Male GHA mice gained more body weight (55% gain) than male $GHR^{-/-}$ (31% gain), bGH (15% gain) or WT (18% gain) mice (Berryman et al., 2006). Male bGH mice exhibit a resistance to gaining fat mass when fed a HF diet (Berryman et al., 2006; Olsson et al., 2005). That is, in one report, half of the body weight gain in bGH is due to gains in lean mass while the other half of the body weight gain is due to fat mass gain on a HF diet, suggesting a level of resistance to diet-induced obesity (Berryman et al., 2006). However, another study using another strain of 6 months old bGH mice showed that male bGH mice did not gain any body weight or fat mass when fed a HF diet, showing a full resistance to diet-induced obesity (Olsson et al., 2005). Overall,

overexpression of GH apparently influences energy partitioning, redirecting some of the excess energy to lean tissue and away from fat tissue (Berryman et al., 2006). In contrast, previous studies have shown that male $GHR^{-/-}$ mice are more vulnerable to diet-induced obesity and their entire weight gain induced by HF feeding is attributed to fat mass gain with no gains lean mass (Berryman et al., 2006; Robertson et al., 2006). It is interesting that GHA male mice are even more susceptible to fat gain with HF feeding than $GHR^{-/-}$ mice; thus, a decrease in GH function promotes more fat mass gain than absence of GH function.

GH has ability to affect food intake to some degree (Malmlof, Din, Johansen, & Pedersen, 2002) although the impact of GH on food intake is varies with experimental manipulation. For example, in bGH mice, overexpressed GH in the central nervous system stimulates food consumption (Bohlooly et al., 2001), while local injection of GH suppresses food intake (Malmlof et al., 2002). $GHR^{-/-}$ mice also appear to have either increased food intake (Coschigano et al., 2003; Furuhata, Hirabayashi, Yonezawa, Takahashi, & Nishihara, 2002; Furuhata et al., 2000) or no change in food consumption (Berryman et al., 2004; Berryman et al., 2006). Similarly, when bGH and $GHR^{-/-}$ mice are challenged with a HF diet, they also increase food intake (Berryman et al., 2006; Olsson et al., 2005). Previous studies show male GHA mice dramatically consumed more food on a LF diet (Berryman et al., 2004). Not surprisingly, in our feeding study, male GHA mice also exhibited hyperphagia on the HF diet. Because no energy expenditure data are available for GHA mice on the HF diet, how antagonizing GH influences energy balance with HF feeding is unknown.

Fat Depots and Organ Weight Differences

Several reports show that GH impacts adipose distribution in a site-specific manner (Berryman et al., 2004; Berryman et al., 2006; Donahue & Beamer, 1993; Flint & Gardner, 1993; Kadim, McCutcheon, Purchas, & Wickham, 1996; Knapp, Chen, Turner, Byers, & Kopchick, 1994). Likewise, specific fat depots in both genders of GHA mice on HF diet are also disproportionally enlarged similar to the trend with LF feeding. That is, the subcutaneous fat depot is the largest whereas intraabdominal fat mass (perigonadal, retroperitoneal, and mesenteric) are relatively smaller as compared to WT littermates in either LF or HF diet. Consistent with the studies by Berryman et al. in 2004 and Magon et al. in 2009, the subcutaneous depot is also notably large when normalized to the dwarf body size in this study, regardless of diet. This further suggests that reduced GH action has the ability to redirect the fat pad distribution. A previous study suggests that GH influences the differentiation of adipocytes in vivo in a depot dependent manner (Flint & Gardner, 1993). In their study, the subcutaneous depot doubled in size with short-term treatment with anti-rat GH whereas the number of differentiated fat cells were drastically reduced in intraabdominal depots with the long-term anti-rat GH treatment (Flint & Gardner, 1993). It is therefore possible that reduced GH has the capability to influence differentiation and lipolysis differently depending on the depot studied. In addition, Okada et al. showed that increased fat mass in GHA mice was due to decreased differentiation and proliferation from preadipocytes to mature adipocytes as well as reduced lipolysis resulting in fewer but larger fat cells (Okada et al., 1992). Likewise, in humans, GH decreases the abdominal adipocyte size while

increasing the responsiveness of gluteal subcutaneous fat depots to the lipogenic actions of insulin (Rosenbaum et al., 1992). Further studies addressing other depot differences, such as measurement of adipocyte size and number, may help explain the depot differences observed in this study.

Interestingly, consistent with a previous study on interscapular BAT (Li et al., 2003), GHA mice in this feeding study also had a greater percent BAT mass than WT counterparts. BAT is a GH-responsive tissue (Li et al., 2003) and negatively correlates to GH levels; moreover, the HF diet increased the percent BAT mass in our study. Few studies have explored the relationship between HF diet and BAT. Only one paper reported that the BAT fat depot did not increase in lambs when fed a HF diet (Encinias, Lardy, Encinias, & Bauer, 2004). The different results may due to the species difference. However, it is worthy noted that HF feeding increases uncoupling protein 1 (UCP1) mRNA expression in BAT of rats (Li, Zhang, Wilsey, & Scarpace, 2004) or mice (Fink et al., 2007). UCP1 is the thermogenic protein that is highly expressed in BAT (Kozak, Britton, Kozak, & Wells, 1988). Increased UCP1 levels in BAT may suggest that BAT fat pad is sensitive to the HF diet. Measuring UCP1 levels in BAT in samples collected in this study may be of interest.

Overall, absolute organ weights in both genders of GHA mice were smaller while normalized weights of kidney, spleen, heart, and muscle were lower than WT littermates on either diet. Absolute and percent liver weights in male GHA and WT mice were greater on a HF diet than on a LF diet, suggesting the HF influence on lipid metabolism in liver.

Glucose Homeostasis

Both genders of GHA mice on the HF diet had lower fasting glucose levels and no change in glucose intolerance compared to WT mice on the HF diet, suggesting some level of protection in glucose homeostasis with reduced GH signaling when challenged with a HF diet. A positive correlation between glucose or glucose intolerance and fat mass was found in this study. Others have shown that reduced fat mass can improve blood glucose levels as well as insulin levels in C57BL/6J mice (Hemmeryckx et al., 2010). This relationship is also apparent in humans, where weight loss and percent fat mass loss decrease the fasting glucose levels (Matsuo et al., 2010). It is noteworthy that GHA mice had better glucose homeostasis despite having greater adiposity, suggesting that reduced GH signaling has a positive impact on glucose sensitivity which supersedes the deleterious effects of the excess fat mass. However, these data differ from GHD in humans. Patients with GHD have impaired glucose metabolism presumably due to obesity (Johansson et al., 1995). The difference among data may be attributed to species differences or the distinct causes of the lower GH function in GHA mice versus GHD.

The unique distribution of fat mass also likely plays a role in regulating glucose metabolism. As previously mentioned, many chronic disorders associated with obesity are attributed to high levels of visceral fat (Carey, Jenkins, Campbell, Freund, & Chisholm, 1996; O'Shaughnessy et al., 1995). However, subcutaneous fat can improve insulin sensitivity (Tran, Yamamoto, Gesta, & Kahn, 2008). Here, both genders of GHA mice had more subcutaneous fat mass but less intraabdominal fat mass on either diet, which may account for improved glucose metabolism. Other studies also suggest the

protective influence of subcutaneous fat mass on homeostasis. In a study by Tran and colleagues, transplanted subcutaneous fat mass in either subcutaneous or intraabdominal regions improved insulin sensitivity and decreases glucose and insulin levels (Tran et al., 2008). Thus, subcutaneous fat may have inherent properties protective for glucose metabolism. In addition, over-expressed adiponectin in the subcutaneous depot may improve metabolic disorders (Kim et al., 2007) due to the insulin sensitizing effect of adiponectin (Tschritter et al., 2003; Yamauchi et al., 2001). In contrast, excess epididymal fat pad mass in bGH mice may attribute to their hyperinsulinemia (Coschigano et al., 2003). Glucose clamping studies or insulin tolerance tests may provide more information to explain how glucose metabolism varies when challenged with the HF diet in these mice.

Adipocyte cell size and number are also related to insulin sensitivity. Although one study revealed that fat distribution was a better indicator of metabolic disorders than adipocyte size (Mundi et al.), another study showed that adipocyte size was positively correlated with insulin levels and glucose tolerance in middle-aged men whereas fat cell numbers were negatively related with insulin levels (Bjorntorp et al., 1971). Adipocyte size has been measured in mouse models with altered GH signaling fed a LF diet (Kelder et al., 2007). Kelder et al. (2007) reported that the change in adipocyte size was site-specific with the epididymal fat depot showing the least change in adipocyte size with altered GH levels and with greatest changes in the subcutaneous fat depots. So even though cell size decreased for all depots in bGH mice and increased in most depots for GHA and $GHR^{-/-}$ mice, the subcutaneous depot was most significantly altered by the

change in GH signaling at least on a LF diet. This change again suggests that the significantly increased fat cell size in subcutaneous fat depot of GHA mice may play a role in a better regulation of glucose metabolism. Measuring adipocyte cell size and number may help the interpretation of the insulin sensitivity of GHA mice.

Leptin and adiponectin help regulate glucose metabolism (Pelleymounter et al., 1995; Yamauchi et al., 2002). Normal levels of leptin are able to acutely reduce gluconeogenesis in liver (Ceddia et al., 1999). However, a feature of leptin resistance is a loss of regulation in glucose metabolism (Seufert et al., 1999). Leptin levels are positively related to total fat mass (Berryman et al., 2004; Magon, 2009). GHA mice with a similar age, as reported previously, have greater amount of leptin levels and are probablely leptin resistant (Berryman et al., 2004; Magon, 2009). However, GHA mice had a lower glucose concentration and without glucose intolerance even though challenged on the HF diet. In addition, adiponectin improves insulin sensitivity and negatively correlates with fat mass (Tschritter et al., 2003; Yamauchi et al., 2001) and male GHA mice have been shown to have elevated adiponectin levels (Berryman et al., 2004). Measuring leptin and adiponectin levels from the samples collected in this study will provide insight into how both hormones regulate fat mass and glucose homeostasis. Indeed, maintaining glucose homeostasis is a complex mechanism and much crosstalk exists.

Gender Differences

Gender differences are well known regarding obesity and insulin sensitivity. It has been shown that female WT mice on a HF diet are protected from hyperglycemia,

hyperinsulinemia, hypercholesterolemia, hyperleptinemia, hypertriglyceridemia (Hwang et al., 2009) and glucose intolerance (Gallou-Kabani et al., 2007) as compared to obese male mice. In this study, both GHA and WT female mice exhibited a lower body weight and fat mass gain than male mice on either diet, suggesting female mice, regardless of genotype, were relatively more resistant to diet-induced obesity. Less energy consumption in females when challenged with a HF diet may be a part of the reason why they gain less weight and fat mass. Fat depots weights and absolute organ weights of female mice were proportional to body size on either diet. No gender differences in fasting glucose levels were seen on the LF diet in the feeding study which was inconsistent with the GHA longitudinal study in which female GHA mice had lower blood glucose levels than males at 13 and 26 weeks of age (Magon, 2009).

The sexual dimorphism for much of the data collected may be a result of several factors. GH itself acts in a gender-specific way. That is, male rats and mice have similar GH levels with females but a higher amplitude of pulsatile GH secretion, whereas females have more constant levels of GH in circulation (Jansson, Albertsson-Wikland, Eden, Thorngren, & Isaksson, 1982; Tannenbaum & Martin, 1976).

Estrogen also plays a crucial role. First, estrogen prevents weight gain in female mice (Hong, Stubbins, Smith, Harvey, & Nunez, 2009). Female mice gain less body weight than male mice till 9-12 months old (Hwang et al., 2009). Ovariectomy in female mice abolishes the protection of estrogen, causing ovariectomized female mice to exhibit a similar trend with male mice in body weight gain (Hong et al., 2009). Second, several previous studies have shown that estradiol inhibits energy intake in different species

(Czaja & Goy, 1975; Eckel, Houpt, & Geary, 2000; Friend, 1971; Gong, Garrel, & Calloway, 1989; Houpt, Coren, Hintz, & Hilderbrant, 1979). Ovarectomized female rats increase food intake but this overeating behavior is decreased by estrogen replacement treatment (Asarian, 2006). Sex hormones affect feeding behavior in part via leptin. Leptin levels are inversely related to testosterone but positively related to estrogen in humans (Isidori et al., 1999; Luukkaa et al., 1998; Vettor et al., 1997) as well as in rats (Kristensen, Pedersen, & Richelsen, 1999). Dihydrotestosterone decreases leptin mRNA expression in adipose tissue but improves action in women (Kristensen et al., 1999). Injection of leptin in the brain reveals that leptin reduces food intake in female but not in male rats (Clegg, Riedy, Smith, Benoit, & Woods, 2003), suggesting female rats are more sensitive to catabolic action of leptin in brain than males. Third, estrogen inhibits GH action mediated through JAK-STAT pathway and suppressors of cytokine signaling-2 (Leung et al., 2003). For example, estrogen replacement therapy in postmenopausal women reduces IGF-1 levels (Cano, Castelo-Branco, & Tarin, 1999; Vestergaard, Hermann, Orskov, & Mosekilde, 1999; Weissberger, Ho, & Lazarus, 1991). Fourth, estrogen increases sensitivity to insulin and lowers insulin resistance through stimulation of Akt (an extracellular signal related kinase), increases expression of glucose and lipid metabolism genes (Macotela, Boucher, Tran, & Kahn, 2009), and increases resistance to fatty acid-induced insulin resistance when exposure a HF diet (Hevener, Reichart, Janez, & Olefsky, 2002; Macotela et al., 2009). An estrogen receptor-α knockout mouse model developed insulin resistance and glucose tolerance (Heine, Taylor, Iwamoto, Lubahn, & Cooke, 2000). Lastly, male mice express more inflammatory genes with macrophage

infiltration increased in perigonadal fat depots as compared to females when exposure to a HF diet (Grove, Fried, Greenberg, Xiao, & Clegg, 2010). This study also suggested that the perigonadal fat is considered the most sensitive fat depot with exposure to the HF diet (Grove et al., 2010). This is consistent with what we found in male WT mice on the HF diet in which male WT mice dramatically increased perigonadal fat weights with an impairment in glucose tolerance. GHA mice in this study differ from WT mice due to a greater subcutaneous fat deposition. We suggest that the normal glucose tolerance in female mice on the HF diet is due to the less inflammatory gene expression or macrophage infiltration. Therefore, measuring proinflammatory markers in fat tissues and revealing the relationship between fat distribution in both genders of GHA mice and lipid or glucose metabolism will shed more light on the mechanism of the adipose-related gender difference.

Catecholamines, sympathomimetic hormones released from adrenal glands, are able to trigger a lipolytic cascade via membrane-bound α- and β-adrenoceptors (Carpene, Bousquet-Melou, Galitzky, Berlan, & Lafontan, 1998). The lipolysis is stimulated by inhibiting α_2-adrenoceptors and stimulating β_3-adrenoceptors (Carpene et al., 1998). Since females have a lower ratio, females have more lipolytic capacity to decrease fat mass than males (Llado et al., 2002). HF diet changes the ratio of α_2/β_3-adrenoceptor in both genders in which males are greater than females, thus accumulating fat in males rather than in females (Llado et al., 2002).

Conclusion

1. A HF diet increased the body weight of all mice and male HF fed GHA mice had the greatest percent body weight change. However, HF diet did not strikingly increase weight in female GHA mice compared to LF diet. Both GHA and WT male mice had greater body weights than the females on the same diet.
2. HF diet caused male GHA and WT mice to gain more fat mass than the LF diet. Both female GHA and WT mice had lower fat mass than male mice.
3. HF diet had no apparent effect on lean mass gain. All body weight gain was attributed to fat mass gain.
4. Both genders of GHA and WT mice increased energy intake when fed a HF diet versus a LF diet. GHA mice consume less energy than WT mice; however, when normalized to body weight, GHA mice consume more energy, suggesting hyperphagia in GHA mice. Again, male mice consumed more energy than female mice.
5. Both genders of GHA mouse had lower fasting glucose levels than WT mice especially when exposed to a HF diet. Both female GHA and WT mice had similar fasting glucose levels as their male counterparts.
6. Both genders of GHA mice did not show impaired glucose tolerance when fed a HF diet. Male WT mice tended to be glucose intolerant on HF diet.
7. The largest fat pad was the subcutaneous fat pad in GHA mice both in absolute and relative weight. Males had greater fat depot weights than female mice.

8. Both genders of GHA had lower organ weights (absolute: all organs; percent: kidney, spleen, heart, and muscle) than WT mice on either diet. Females had lower absolute organ weights but greater percent organ weights.

This feeding study—the first study to address the impact of a HF diet on GH antagonist mice—demonstrated that male GHA mice were more vulnerable to diet-induced obesity than WT mice. Male GHA mice also exhibited a higher sensitivity to HF diet than bGH and $GHR^{-/-}$ mice. Their increased susceptibility to diet-induced obesity is in part due to the hyperphagia seen in male GHA mice. Importantly, both GHA and WT female mice were relatively resistant to diet-induced obesity when challenged with a HF diet, demonstrating an interesting gender difference that should be further explored. Although LF and HF fed GHA mice were relatively obese, both genders of GHA mice had lower fasting glucose levels and did not show decreased glucose tolerance with HF feeding as compared to WT mice. This feeding study provides valuable insight into how GH deficiency in mice regulates body composition, fat distribution, and metabolic homeostasis when they are exposed to HF diets. Overall, GHA mice were more susceptible to diet-induced obesity but without a significant impairment in glucose homeostasis. These results may provide insight regarding how diet affects GH deficient patients.

Future Studies

In this feeding study, the male GHA mice were more sensitive to HF diets whereas female mice were not. Additional issues raised by the present study should be addressed in the future.

Assessing energy expenditure, such as through indirect calorimetry (VO_2 or basic metabolic temperature), and more accurately measuring energy intake, such as through the use of metabolic cages, would elucidate how GHA and WT mice regulate energy balance. Examining BAT in GHA mice would be of interest as this tissue was increased in GHA mice, increased in all mice with HF feeding, and plays a significant role in determine the energy expenditure. Since UCP1 gene has previously been reported to be highly expressed in GH deficient mice, such as $GHR^{-/-}$ and GHA (Li et al., 2003), closer evaluation of this adipose depot would be of interest.

Determining adiponectin and leptin levels would help in the interpretation of the data, because these hormones are oppositely related to fat mass and glucose homeostasis.

Determining insulin levels and insulin sensitivity with a HF diet would provide a better understanding of how the HF diet influences glucose homeostasis. Although we have data for glucose levels and glucose tolerance, insulin data would offer insight into how GH deficiency alters glucose metabolism when challenged with a HF diet.

Assessing liver TAGs in GHA mice who are fed a HF diet may be of interest. Previous studies found that the GH deficiency causes severe dyslipidemia (Makimura et al., 2009; Verhelst & Abs, 2009) and liver TAG have been shown to be elevated in both male and female GHA mice (unpublished data). In the present feeding study, no genotype differences were found in percent liver weight, but there still may be differences in liver TAGs, which would influence the overall health of the mice.

Determining adipocyte size and number in all the fat depots would help establish whether altered increased adipocyte size or increased adipocyte number contributed to the greater adiposity with HF feeding.

Evaluating estrogen levels may help the interpretation of the gender differences in fat distribution, body composition, and metabolic homeostasis. An ovariectomized GHA female mouse challenged with HF diet may help to resolve these issues.

REFERENCES

Abate, N., Garg, A., Peshock, R. M., Stray-Gundersen, J., & Grundy, S. M. (1995). Relationships of generalized and regional adiposity to insulin sensitivity in men. *Journal of Clinical Investigation, 96*(1), 88-98.

Abdel-Meguid, S. S., Shieh, H. S., Smith, W. W., Dayringer, H. E., Violand, B. N., & Bentle, L. A. (1987). Three-dimensional structure of a genetically engineered variant of porcine growth hormone. *Proceedings of the National Academy of Sciences of the United States of America, 84*(18), 6434-6437.

Adamafio, N. A., & Ng, F. M. (1984). Effects of growth hormone on lipogenesis and glucose oxidation in genetically GH-deficient mice. *Molecular and Cellular Endocrinology, 37*(2), 241-244.

Agha, A., Rogers, B., Mylotte, D., Taleb, F., Tormey, W., Phillips, J., et al. (2004). Neuroendocrine dysfunction in the acute phase of traumatic brain injury. *Clinical Endocrinology, 60*(5), 584-591.

al-Shoumer, K. A., Cox, K. H., Hughes, C. L., Richmond, W., & Johnston, D. G. (1997). Fasting and postprandial lipid abnormalities in hypopituitary women receiving conventional replacement therapy. *Journal of Clinical Endocrinology and Metabolism, 82*(8), 2653-2659.

Alessi, M. C., Peiretti, F., Morange, P., Henry, M., Nalbone, G., & Juhan-Vague, I. (1997). Production of plasminogen activator inhibitor 1 by human adipose tissue: Possible link between visceral fat accumulation and vascular disease. *Diabetes, 46*(5), 860-867.

Alford, F. P., Hew, F. L., Christopher, M. C., & Rantzau, C. (1999). Insulin sensitivity in growth hormone (GH)-deficient adults and effect of GH replacement therapy. *Journal of Endocrinological Investigation, 22*(5 Suppl), 28-32.

Arden, K. C., Boutin, J. M., Djiane, J., Kelly, P. A., & Cavenee, W. K. (1990). The receptors for prolactin and growth hormone are localized in the same region of human chromosome 5. *Cytogenetics and Cell Genetics, 53*(2-3), 161-165.

Asarian, L. (2006). Membrane estrogen receptors and energy homeostasis. *Journal of Neuroscience, 26*(44), 11255-11256.

Bak, J. F., Moller, N., & Schmitz, O. (1991). Effects of growth hormone on fuel utilization and muscle glycogen synthase activity in normal humans. *American Journal of Physiology, 260*(5 Pt 1), E736-742.

Balbis, A., Dellacha, J. M., Calandra, R. S., Bartke, A., & Turyn, D. (1992). Down regulation of masked and unmasked insulin receptors in the liver of transgenic mice expressing bovine growth hormone gene. *Life Sciences, 51*(10), 771-778.

Barzilai, N., She, L., Liu, B. Q., Vuguin, P., Cohen, P., Wang, J., et al. (1999). Surgical removal of visceral fat reverses hepatic insulin resistance. *Diabetes, 48*(1), 94-98.

Bates, A. S., Van't Hoff, W., Jones, J. M., & Clayton, R. N. (1993). An audit of outcome of treatment in acromegaly. *Quarterly Journal of Medicine, 86*(5), 293-299.

Bates, A. S., Van't Hoff, W., Jones, P. J., & Clayton, R. N. (1996). The effect of hypopituitarism on life expectancy. *Journal of Clinical Endocrinology and Metabolism, 81*(3), 1169-1172.

Baumann, G., Amburn, K., & Shaw, M. A. (1988). The circulating growth hormone (GH)-binding protein complex: A major constituent of plasma GH in man. *Endocrinology, 122*(3), 976-984.

Bays, H. E. (2009). "Sick fat," metabolic disease, and atherosclerosis. *American Journal of Medicine, 122*(1 Suppl), S26-37.

Beales, P. L., Farooqi, I. S., & O'Rahilly, S. (2009). *Genetics of obesity syndromes*. New York: Oxford University Press.

Bengtsson, B. A., Brummer, R. J., Eden, S., Bosaeus, I., & Lindstedt, G. (1989). Body composition in acromegaly: The effect of treatment. *Clinical Endocrinology, 31*(4), 481-490.

Bengtsson, B. A., Eden, S., Ernest, I., Oden, A., & Sjogren, B. (1988). Epidemiology and long-term survival in acromegaly. A study of 166 cases diagnosed between 1955 and 1984. *Acta Medica Scandinavica, 223*(4), 327-335.

Bengtsson, B. A., Eden, S., Lonn, L., Kvist, H., Stokland, A., Lindstedt, G., et al. (1993). Treatment of adults with growth hormone (GH) deficiency with recombinant human GH. *Journal of Clinical Endocrinology and Metabolism, 76*(2), 309-317.

Bengtsson, B. A., & Johannsson, G. (1998). The use of growth hormone in adults: A review of the last 10 years, the present and a perspective for the future. *Growth Hormone and IGF Research, 8* (Suppl B), 27-35.

Berryman, D. E., List, E. O., Coschigano, K. T., Behar, K., Kim, J. K., & Kopchick, J. J. (2004). Comparing adiposity profiles in three mouse models with altered GH signaling. *Growth Hormone and IGF Research, 14*(4), 309-318.

Berryman, D. E., List, E. O., Kohn, D. T., Coschigano, K. T., Seeley, R. J., & Kopchick, J. J. (2006). Effect of growth hormone on susceptibility to diet-induced obesity. *Endocrinology, 147*(6), 2801-2808.

Berryman, D. E., List, E. O., Palmer, A. J., Chung, M. Y., Wright-Piekarski, J., Lubbers, E., et al. (2010). Two-year body composition analyses of long-lived GHR null mice. *Journals of Gerontology. Series A, Biological Sciences and Medical Sciences, 65*(1), 31-40.

Berti, L., Kellerer, M., Capp, E., & Haring, H. U. (1997). Leptin stimulates glucose transport and glycogen synthesis in C2C12 myotubes: Evidence for a P13-kinase mediated effect. *Diabetologia, 40*(5), 606-609.

Beshyah, S. A., Freemantle, C., Thomas, E., Rutherford, O., Page, B., Murphy, M., et al. (1995). Abnormal body composition and reduced bone mass in growth hormone deficient hypopituitary adults. *Clinical Endocrinology, 42*(2), 179-189.

Binnerts, A., Swart, G. R., Wilson, J. H., Hoogerbrugge, N., Pols, H. A., Birkenhager, J. C., et al. (1992). The effect of growth hormone administration in growth hormone deficient adults on bone, protein, carbohydrate and lipid homeostasis, as well as on body composition. *Clinical Endocrinology, 37*(1), 79-87.

Birk, R. Z., Abramovitch-Gottlib, L., Margalit, I., Aviv, M., Forti, E., Geresh, S., et al. (2006). Conversion of adipogenic to osteogenic phenotype using crystalline porous biomatrices of marine origin. *Tissue Engineering, 12*(1), 21-31.

Bjorntorp, P., Bengtsson, C., Blohme, G., Jonsson, A., Sjostrom, L., Tibblin, E., et al. (1971). Adipose tissue fat cell size and number in relation to metabolism in

randomly selected middle-aged men and women. *Metabolism: Clinical and Experimental, 20*(10), 927-935.

Black, M. M., Shuster, S., & Bottoms, E. (1972). Skin collagen and thickness in acromegaly and hypopituitarism. *Clinical Endocrinology, 1*(3), 259-263.

Bluher, S., Shah, S., & Mantzoros, C. S. (2009). Leptin deficiency: Clinical implications and opportunities for therapeutic interventions. *Journal of Investigative Medicine, 57*(7), 784-788.

Bohlooly, Y. M., Carlson, L., Olsson, B., Gustafsson, H., Andersson, I. J., Tornell, J., et al. (2001). Vascular function and blood pressure in GH transgenic mice. *Endocrinology, 142*(8), 3317-3323.

Bonnet, F., Vanderschueren-Lodeweyckx, M., Eeckels, R., & Malvaux, P. (1974). Subcutaneous adipose tissue and lipids in blood in growth hormone deficiency before and after treatment with human growth hormone. *Pediatric Research, 8*(9), 800-805.

Borkman, M., Storlien, L. H., Pan, D. A., Jenkins, A. B., Chisholm, D. J., & Campbell, L. V. (1993). The relation between insulin sensitivity and the fatty-acid composition of skeletal-muscle phospholipids. *New England Journal of Medicine, 328*(4), 238-244.

Boulware, S. D., Tamborlane, W. V., Rennert, N. J., Gesundheit, N., & Sherwin, R. S. (1994). Comparison of the metabolic effects of recombinant human insulin-like growth factor-I and insulin: Dose-response relationships in healthy young and middle-aged adults. *Journal of Clinical Investigation, 93*(3), 1131-1139.

Brameld, J. M., Atkinson, J. L., Saunders, J. C., Pell, J. M., Buttery, P. J., & Gilmour, R. S. (1996). Effects of growth hormone administration and dietary protein intake on insulin-like growth factor 1 and growth hormone receptor mRNA expression in porcine liver, skeletal muscle, and adipose tissue. *Journal of Animal Science, 74*(8), 1832-1841.

Brazeau, P., Vale, W., Burgus, R., Ling, N., Butcher, M., Rivier, J., et al. (1973). Hypothalamic polypeptide that inhibits the secretion of immunoreactive pituitary growth hormone. *Science, 179*(68), 77-79.

Brown, R. J., Adams, J. J., Pelekanos, R. A., Wan, Y., McKinstry, W. J., Palethorpe, K., et al. (2005). Model for growth hormone receptor activation based on subunit rotation within a receptor dimer. *Nature Structure & Molecular Biology, 12*(9), 814-821.

Cahill, G. F., Jr. (1976). Starvation in man. *Clinics in Endocrinology and Metabolism, 5*(2), 397-415.

Campbell, J., & Rastogi, K. S. (1969). Actions of growth hormone: Enhancement of insulin utilization with inhibition of insulin effect on blood glucose in dogs. *Metabolism: Clinical and Experimental, 18*(11), 930-944.

Campfield, L. A., Smith, F. J., Guisez, Y., Devos, R., & Burn, P. (1995). Recombinant mouse OB protein: Evidence for a peripheral signal linking adiposity and central neural networks. *Science, 269*(5223), 546-549.

Cano, A., Castelo-Branco, C., & Tarin, J. J. (1999). Effect of menopause and different combined estradiol-progestin regimens on basal and growth hormone-releasing

hormone-stimulated serum growth hormone, insulin-like growth factor-1, insulin-like growth factor binding protein (IGFBP)-1, and IGFBP-3 levels. *Fertility and Sterility, 71*(2), 261-267.

Capes, S. E., Hunt, D., Malmberg, K., Pathak, P., & Gerstein, H. C. (2001). Stress hyperglycemia and prognosis of stroke in nondiabetic and diabetic patients: A systematic overview. *Stroke, 32*(10), 2426-2432.

Carey, D. G., Jenkins, A. B., Campbell, L. V., Freund, J., & Chisholm, D. J. (1996). Abdominal fat and insulin resistance in normal and overweight women: Direct measurements reveal a strong relationship in subjects at both low and high risk of NIDDM. *Diabetes, 45*(5), 633-638.

Carey, V. J., Walters, E. E., Colditz, G. A., Solomon, C. G., Willett, W. C., Rosner, B. A., et al. (1997). Body fat distribution and risk of non-insulin-dependent diabetes mellitus in women: The Nurses' Health Study. *American Journal of Epidemiology, 145*(7), 614-619.

Carpene, C., Bousquet-Melou, A., Galitzky, J., Berlan, M., & Lafontan, M. (1998). Lipolytic effects of beta 1-, beta 2-, and beta 3-adrenergic agonists in white adipose tissue of mammals. *Annals of the New York Academy of Sciences, 839*, 186-189.

Carpinteri, R., Patelli, I., Casanueva, F. F., & Giustina, A. (2009). Pituitary tumours: Inflammatory and granulomatous expansive lesions of the pituitary. *Best Practice & Research Clinical Endocrinology & Metabolism, 23*(5), 639-650.

Carter-Su, C., Rozsa, F. W., Wang, X., & Stubbart, J. R. (1988). Rapid and transitory stimulation of 3-O-methylglucose transport by growth hormone. *American Journal of Physiology, 255*(5 Pt 1), E723-729.

Casanueva, F. F. (1992). Physiology of growth hormone secretion and action. *Endocrinology and Metabolism Clinics of North America, 21*(3), 483-517.

Ceddia, R. B., Lopes, G., Souza, H. M., Borba-Murad, G. R., William, W. N., Jr., Bazotte, R. B., et al. (1999). Acute effects of leptin on glucose metabolism of in situ rat perfused livers and isolated hepatocytes. *International Journal of Obesity and Related Metabolic Disorders, 23*(11), 1207-1212.

Chanson, P., & Salenave, S. (2008). Acromegaly. *Orphanet Journal of Rare Diseases, 3*, 17.

Charriere, G., Cousin, B., Arnaud, E., Andre, M., Bacou, F., Penicaud, L., et al. (2003). Preadipocyte conversion to macrophage: Evidence of plasticity. *Journal of Biological Chemistry, 278*(11), 9850-9855.

Chen, H. T., Schuler, L. A., & Schultz, R. D. (1998). Growth hormone receptor and regulation of gene expression in fetal lymphoid cells. *Molecular and Cellular Endocrinology, 137*(1), 21-29.

Chen, N. Y., Chen, W. Y., Striker, L. J., Striker, G. E., & Kopchick, J. J. (1997). Co-expression of bovine growth hormone (GH) and human GH antagonist genes in transgenic mice. *Endocrinology, 138*(2), 851-854.

Chen, W. Y., Chen, N., Yun, J., Wagner, T. E., & Kopchick, J. J. (1994). In vitro and in vivo studies of the antagonistic effects of human growth hormone analogs. *Journal of Biological Chemistry, 269*(32), 15892-15897.

Chen, W. Y., White, M. E., Wagner, T. E., & Kopchick, J. J. (1991). Functional antagonism between endogenous mouse growth hormone (GH) and a GH analog results in dwarf transgenic mice. *Endocrinology, 129*(3), 1402-1408.

Chen, W. Y., Wight, D. C., Mehta, B. V., Wagner, T. E., & Kopchick, J. J. (1991). Glycine 119 of bovine growth hormone is critical for growth-promoting activity. *Molecular Endocrinology, 5*(12), 1845-1852.

Chen, W. Y., Wight, D. C., Wagner, T. E., & Kopchick, J. J. (1990). Expression of a mutated bovine growth hormone gene suppresses growth of transgenic mice. *Proceedings of the National Academy of Sciences of the United States of America, 87*(13), 5061-5065.

Christoffersen, C. T., Tornqvist, H., Vlahos, C. J., Bucchini, D., Jami, J., De Meyts, P., et al. (1998). Insulin and insulin-like growth factor-I receptor mediated differentiation of 3T3-F442A cells into adipocytes: Effect of PI 3-kinase inhibition. *Biochemical and Biophysical Research Communications, 246*(2), 426-430.

Cinti, S. (2001). The adipose organ: Morphological perspectives of adipose tissues. *Proceedings of the Nutrition Society, 60*(3), 319-328.

Cinti, S., Mitchell, G., Barbatelli, G., Murano, I., Ceresi, E., Faloia, E., et al. (2005). Adipocyte death defines macrophage localization and function in adipose tissue of obese mice and humans. *Journal of Lipid Research, 46*(11), 2347-2355.

Cittadini, A., Stromer, H., Katz, S. E., Clark, R., Moses, A. C., Morgan, J. P., et al. (1996). Differential cardiac effects of growth hormone and insulin-like growth factor-1 in the rat: A combined in vivo and in vitro evaluation. *Circulation, 93*(4), 800-809.

Clark, R., Olson, K., Fuh, G., Marian, M., Mortensen, D., Teshima, G., et al. (1996). Long-acting growth hormones produced by conjugation with polyethylene glycol. *Journal of Biological Chemistry, 271*(36), 21969-21977.

Clark, R. P., Schuenke, M., Keeton, S. M., Staron, R. S., & Kopchick, J. J. (2006). Effects of growth hormone and insulin-like growth factor 1 on muscle in mouse models of human growth disorders. *Hormone Research, 66* (Suppl 1), 26-34.

Clegg, D. J., Riedy, C. A., Smith, K. A., Benoit, S. C., & Woods, S. C. (2003). Differential sensitivity to central leptin and insulin in male and female rats. *Diabetes, 52*(3), 682-687.

Clement, K., Vaisse, C., Lahlou, N., Cabrol, S., Pelloux, V., Cassuto, D., et al. (1998). A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. *Nature, 392*(6674), 398-401.

Cnop, M., Landchild, M. J., Vidal, J., Havel, P. J., Knowles, N. G., Carr, D. R., et al. (2002). The concurrent accumulation of intra-abdominal and subcutaneous fat explains the association between insulin resistance and plasma leptin

concentrations: Distinct metabolic effects of two fat compartments. *Diabetes, 51*(4), 1005-1015.

Cohen, B., Novick, D., & Rubinstein, M. (1996). Modulation of insulin activities by leptin. *Science, 274*(5290), 1185-1188.

Colao, A., Baldelli, R., Marzullo, P., Ferretti, E., Ferone, D., Gargiulo, P., et al. (2000). Systemic hypertension and impaired glucose tolerance are independently correlated to the severity of the acromegalic cardiomyopathy. *Journal of Clinical Endocrinology and Metabolism, 85*(1), 193-199.

Colao, A., Cerbone, G., Pivonello, R., Aimaretti, G., Loche, S., Di Somma, C., et al. (1999). The growth hormone (GH) response to the arginine plus GH-releasing hormone test is correlated to the severity of lipid profile abnormalities in adult patients with GH deficiency. *Journal of Clinical Endocrinology and Metabolism, 84*(4), 1277-1282.

Considine, R. V., Sinha, M. K., Heiman, M. L., Kriauciunas, A., Stephens, T. W., Nyce, M. R., et al. (1996). Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *New England Journal of Medicine, 334*(5), 292-295.

Corre, J., Barreau, C., Cousin, B., Chavoin, J. P., Caton, D., Fournial, G., et al. (2006). Human subcutaneous adipose cells support complete differentiation but not self-renewal of hematopoietic progenitors. *Journal of Cellular Physiology, 208*(2), 282-288.

Coschigano, K. T., Holland, A. N., Riders, M. E., List, E. O., Flyvbjerg, A., & Kopchick, J. J. (2003). Deletion, but not antagonism, of the mouse growth hormone receptor

results in severely decreased body weights, insulin, and insulin-like growth factor 1 levels and increased life span. *Endocrinology, 144*(9), 3799-3810.

Cousin, B., Casteilla, L., Lafontan, M., Ambid, L., Langin, D., Berthault, M. F., et al. (1993). Local sympathetic denervation of white adipose tissue in rats induces preadipocyte proliferation without noticeable changes in metabolism. *Endocrinology, 133*(5), 2255-2262.

Cousin, B., Cinti, S., Morroni, M., Raimbault, S., Ricquier, D., Penicaud, L., et al. (1992). Occurrence of brown adipocytes in rat white adipose tissue: Molecular and morphological characterization. *Journal of Cell Science, 103* (Pt 4), 931-942.

Crowe, S., Cushing, H., & Homans, J. (1910). Experimental hypohysectomy. *Bull Johns Hopkins Hospital, 21*, 127.

Crushing, H. (1909). The hypophysis cerebri. *JAMA: Journal of the American Medical Association, 53*, 250-255.

Cuneo, R. C., Salomon, F., Wiles, C. M., & Sonksen, P. H. (1990). Skeletal muscle performance in adults with growth hormone deficiency. *Hormone Research, 33* (Suppl 4), 55-60.

Cunningham, B. C., & Wells, J. A. (1991). Rational design of receptor-specific variants of human growth hormone. *Proceedings of the National Academy of Sciences of the United States of America, 88*(8), 3407-3411.

Czaja, J. A., & Goy, R. W. (1975). Ovarian hormones and food intake in female guinea pigs and rhesus monkeys. *Hormones and Behavior, 6*(4), 329-349.

Davidson, M. B. (1987). Effect of growth hormone on carbohydrate and lipid metabolism. *Endocrine Reviews, 8*(2), 115-131.

De Boer, H., Blok, G. J., Voerman, H. J., De Vries, P. M., & van der Veen, E. A. (1992). Body composition in adult growth hormone-deficient men, assessed by anthropometry and bioimpedance analysis. *Journal of Clinical Endocrinology and Metabolism, 75*(3), 833-837.

de Boer, H., Blok, G. J., Voerman, H. J., Phillips, M., & Schouten, J. A. (1994). Serum lipid levels in growth hormone-deficient men. *Metabolism: Clinical and Experimental, 43*(2), 199-203.

de Vos, A. M., Ultsch, M., & Kossiakoff, A. A. (1992). Human growth hormone and extracellular domain of its receptor: Crystal structure of the complex. *Science, 255*(5042), 306-312.

del Rincon, J. P., Iida, K., Gaylinn, B. D., McCurdy, C. E., Leitner, J. W., Barbour, L. A., et al. (2007). Growth hormone regulation of p85alpha expression and phosphoinositide 3-kinase activity in adipose tissue: Mechanism for growth hormone-mediated insulin resistance. *Diabetes, 56*(6), 1638-1646.

Dietz, J., & Schwartz, J. (1991). Growth hormone alters lipolysis and hormone-sensitive lipase activity in 3T3-F442A adipocytes. *Metabolism: Clinical and Experimental, 40*(8), 800-806.

Doglio, A., Dani, C., Grimaldi, P., & Ailhaud, G. (1986). Growth hormone regulation of the expression of differentiation-dependent genes in preadipocyte Ob1771 cells. *Biochemical Journal, 238*(1), 123-129.

Dominici, F. P., Arostegui Diaz, G., Bartke, A., Kopchick, J. J., & Turyn, D. (2000). Compensatory alterations of insulin signal transduction in liver of growth hormone receptor knockout mice. *Journal of Endocrinology, 166*(3), 579-590.

Donahue, L. R., & Beamer, W. G. (1993). Growth hormone deficiency in "little" mice results in aberrant body composition, reduced insulin-like growth factor-I and insulin-like growth factor-binding protein-3 (IGFBP-3), but does not affect IGFBP-2, -1 or -4. *Journal of Endocrinology, 136*(1), 91-104.

Ducy, P., Amling, M., Takeda, S., Priemel, M., Schilling, A. F., Beil, F. T., et al. (2000). Leptin inhibits bone formation through a hypothalamic relay: A central control of bone mass. *Cell, 100*(2), 197-207.

Dudley, G. A., & Portanova, R. (1987). Histochemical characteristics of soleus muscle in hGH transgenic mice. *Proceedings of the Society for Experimental Biology and Medicine, 185*(4), 403-408.

Eckel, L. A., Houpt, T. A., & Geary, N. (2000). Spontaneous meal patterns in female rats with and without access to running wheels. *Physiology and Behavior, 70*(3-4), 397-405.

Eden, S. (1979). Age- and sex-related differences in episodic growth hormone secretion in the rat. *Endocrinology, 105*(2), 555-560.

Egecioglu, E., Bjursell, M., Ljungberg, A., Dickson, S. L., Kopchick, J. J., Bergstrom, G., et al. (2005). Growth hormone receptor deficiency results in blunted ghrelin feeding response, obesity and hypolipidemia in mice. *American Journal of Physiology- Endocrinology and Metabolism, 290*(2), E317-325.

Encinias, H. B., Lardy, G. P., Encinias, A. M., & Bauer, M. L. (2004). High linoleic acid safflower seed supplementation for gestating ewes: Effects on ewe performance, lamb survival, and brown fat stores. *Journal of Animal Science, 82*(12), 3654-3661.

Entingh-Pearsall, A., & Kahn, C. R. (2004). Differential roles of the insulin and insulin-like growth factor-I (IGF-I) receptors in response to insulin and IGF-I. *Journal of Biological Chemistry, 279*(36), 38016-38024.

Esposito, C., Liu, Z. H., Striker, G. E., Phillips, C., Chen, N. Y., Chen, W. Y., et al. (1996). Inhibition of diabetic nephropathy by a GH antagonist: A molecular analysis. *Kidney International, 50*(2), 506-514.

Esposito, N., Paterlini, P., Kelly, P. A., Postel-Vinay, M. C., & Finidori, J. (1994). Expression of two isoforms of the human growth hormone receptor in normal liver and hepatocarcinoma. *Molecular and Cellular Endocrinology, 103*(1-2), 13-20.

Evans, H. M., & Long, J. A. (1921). The effect of the anterior lobe administered intraperitoneally upon growth, maturity and oestrus cycles of the rat. *Anatomical Record, 21*, 62-63.

Ezzat, S., Forster, M. J., Berchtold, P., Redelmeier, D. A., Boerlin, V., & Harris, A. G. (1994). Acromegaly. Clinical and biochemical features in 500 patients. *Medicine, 73*(5), 233-240.

Farmer, S. R. (2008). Molecular determinants of brown adipocyte formation and function. *Genes and Development, 22*(10), 1269-1275.

Farooqi, I. S., Matarese, G., Lord, G. M., Keogh, J. M., Lawrence, E., Agwu, C., et al. (2002). Beneficial effects of leptin on obesity, T cell hyporesponsiveness, and neuroendocrine/metabolic dysfunction of human congenital leptin deficiency. *Journal of Clinical Investigation, 110*(8), 1093-1103.

Fei, H., Okano, H. J., Li, C., Lee, G. H., Zhao, C., Darnell, R., et al. (1997). Anatomic localization of alternatively spliced leptin receptors (Ob-R) in mouse brain and other tissues. *Proceedings of the National Academy of Sciences of the United States of America, 94*(13), 7001-7005.

Felig, P., Marliss, E. B., & Cahill, G. F., Jr. (1971). Metabolic response to human growth hormone during prolonged starvation. *Journal of Clinical Investigation, 50*(2), 411-421.

Ferrannini, E., Barrett, E. J., Bevilacqua, S., & DeFronzo, R. A. (1983). Effect of fatty acids on glucose production and utilization in man. *Journal of Clinical Investigation, 72*(5), 1737-1747.

Festa, A., D'Agostino, R., Jr., Howard, G., Mykkanen, L., Tracy, R. P., & Haffner, S. M. (2000). Chronic subclinical inflammation as part of the insulin resistance syndrome: the Insulin Resistance Atherosclerosis Study (IRAS). *Circulation, 102*(1), 42-47.

Fink, B. D., Herlein, J. A., Almind, K., Cinti, S., Kahn, C. R., & Sivitz, W. I. (2007). Mitochondrial proton leak in obesity-resistant and obesity-prone mice. *American Journal of Physiology–Regulatory, Integrative and Comparative Physiology, 293*(5), R1773-1780.

Fisker, S., Hansen, B., Fuglsang, J., Kristensen, K., Ovesen, P., Orskov, H., et al. (2004). Gene expression of the GH receptor in subcutaneous and intraabdominal fat in healthy females: Relationship to GH-binding protein. *European Journal of Endocrinology / European Federation of Endocrine Societies, 150*(6), 773-777.

Fisker, S., Kristensen, K., Rosenfalck, A. M., Pedersen, S. B., Ebdrup, L., Richelsen, B., et al. (2001). Gene expression of a truncated and the full-length growth hormone (GH) receptor in subcutaneous fat and skeletal muscle in GH-deficient adults: Impact of GH treatment. *Journal of Clinical Endocrinology and Metabolism, 86*(2), 792-796.

Fleenor, D., Arumugam, R., & Freemark, M. (2006). Growth hormone and prolactin receptors in adipogenesis: STAT-5 activation, suppressors of cytokine signaling, and regulation of insulin-like growth factor 1. *Hormone Research, 66*(3), 101-110.

Flint, D. J., & Gardner, M. J. (1993). Influence of growth hormone deficiency on growth and body composition in rats: Site-specific effects upon adipose tissue development. *Journal of Endocrinology, 137*(2), 203-211.

Florini, J. R., Ewton, D. Z., & Coolican, S. A. (1996). Growth hormone and the insulin-like growth factor system in myogenesis. *Endocrine Reviews, 17*(5), 481-517.

Flyvbjerg, A., Bennett, W. F., Rasch, R., Kopchick, J. J., & Scarlett, J. A. (1999). Inhibitory effect of a growth hormone receptor antagonist (G120K-PEG) on renal enlargement, glomerular hypertrophy, and urinary albumin excretion in experimental diabetes in mice. *Diabetes, 48*(2), 377-382.

Fontana, L., Eagon, J. C., Trujillo, M. E., Scherer, P. E., & Klein, S. (2007). Visceral fat adipokine secretion is associated with systemic inflammation in obese humans. *Diabetes, 56*(4), 1010-1013.

Foss, M. C., Saad, M. J., Paccola, G. M., Paula, F. J., Piccinato, C. E., & Moreira, A. C. (1991). Peripheral glucose metabolism in acromegaly. *Journal of Clinical Endocrinology and Metabolism, 72*(5), 1048-1053.

Fowelin, J., Attvall, S., von Schenck, H., Smith, U., & Lager, I. (1991). Characterization of the insulin-antagonistic effect of growth hormone in man. *Diabetologia, 34*(7), 500-506.

Frederich, R. C., Hamann, A., Anderson, S., Lollmann, B., Lowell, B. B., & Flier, J. S. (1995). Leptin levels reflect body lipid content in mice: Evidence for diet-induced resistance to leptin action. *Nature Medicine, 1*(12), 1311-1314.

Frick, F., Bohlooly, Y. M., Linden, D., Olsson, B., Tornell, J., Eden, S., et al. (2001). Long-term growth hormone excess induces marked alterations in lipoprotein metabolism in mice. *American Journal of Physiology–Endocrinology and Metabolism, 281*(6), E1230-1239.

Fried, S. K., Bunkin, D. A., & Greenberg, A. S. (1998). Omental and subcutaneous adipose tissues of obese subjects release interleukin-6: Depot difference and regulation by glucocorticoid. *Journal of Clinical Endocrinology and Metabolism, 83*(3), 847-850.

Friedman, J. M. (2002). The function of leptin in nutrition, weight, and physiology. *Nutrition Reviews, 60*(10 Pt 2), S1-14; discussion S68-84, 85-17.

Friend, D. W. (1971). Self-selection of feeds and water by swine during pregnancy and lactation. *Journal of Animal Science, 32*(4), 658-666.

Fruhbeck, G. (2008). Overview of adipose tissue and its role in obesity and metabolic disorders. In K. Yang (Ed.), *Adipose Tissue Protocols* (pp. 1-22). Totowa, NJ: Humana Press.

Fruhbeck, G., Aguado, M., & Martinez, J. A. (1997). In vitro lipolytic effect of leptin on mouse adipocytes: Evidence for a possible autocrine/paracrine role of leptin. *Biochemical and Biophysical Research Communications, 240*(3), 590-594.

Fuh, G., Cunningham, B. C., Fukunaga, R., Nagata, S., Goeddel, D. V., & Wells, J. A. (1992). Rational design of potent antagonists to the human growth hormone receptor. *Science, 256*(5064), 1677-1680.

Furuhata, Y., Hirabayashi, K., Yonezawa, T., Takahashi, M., & Nishihara, M. (2002). Effects of pair-feeding and growth hormone treatment on obese transgenic rats. *European Journal of Endocrinology / European Federation of Endocrine Societies, 146*(2), 245-249.

Furuhata, Y., Kagaya, R., Hirabayashi, K., Ikeda, A., Chang, K. T., Nishihara, M., et al. (2000). Development of obesity in transgenic rats with low circulating growth hormone levels: Involvement of leptin resistance. *European Journal of Endocrinology / European Federation of Endocrine Societies, 143*(4), 535-541.

Gallou-Kabani, C., Vige, A., Gross, M. S., Rabes, J. P., Boileau, C., Larue-Achagiotis, C., et al. (2007). C57BL/6J and A/J mice fed a high-fat diet delineate components of metabolic syndrome. *Obesity (Silver Spring), 15*(8), 1996-2005.

Geloen, A., Roy, P. E., & Bukowiecki, L. J. (1989). Regression of white adipose tissue in diabetic rats. *American Journal of Physiology, 257*(4 Pt 1), E547-553.

Gemmell, R. T., Bell, A. W., & Alexander, G. (1972). Morphology of adipose cells in lambs at birth and during subsequent transition of brown to white adipose tissue in cold and in warm conditons. *American Journal of Anatomy, 133*(2), 143-164.

Ginsberg, H., Kimmerling, G., Olefsky, J. M., & Reaven, G. M. (1975). Demonstration of insulin resistance in untreated adult onset diabetic subjects with fasting hyperglycemia. *Journal of Clinical Investigation, 55*(3), 454-461.

Goffin, V., Bernichtein, S., Carriere, O., Bennett, W. F., Kopchick, J. J., & Kelly, P. A. (1999). The human growth hormone antagonist B2036 does not interact with the prolactin receptor. *Endocrinology, 140*(8), 3853-3856.

Gomez, J. M. (2006). The role of insulin-like growth factor 1 components in the regulation of vitamin D. *Current Pharmaceutical Biotechnology, 7*(2), 125-132.

Gong, E. J., Garrel, D., & Calloway, D. H. (1989). Menstrual cycle and voluntary food intake. *American Journal of Clinical Nutrition, 49*(2), 252-258.

Goodman, H. M. (1965a). Early and late effects of growth hormone on the metabolism of glucose in adipose tissue. *Endocrinology, 76*, 1134-1140.

Goodman, H. M. (1965b). In vitro actions of growth hormone on glucose metabolism in adipose tissue. *Endocrinology, 76*, 216-225.

Goodman, H. M. (1978). The effects of growth hormone on the utilization of L-leucine in adipose tissue. *Endocrinology, 102*(1), 210-217.

Goodman, H. M. (2009). *Basic medical endocrinology* (4th ed.): Amsterdam: Elsevier/Academic Press.

Goodyear, L. J., Giorgino, F., Sherman, L. A., Carey, J., Smith, R. J., & Dohm, G. L. (1995). Insulin receptor phosphorylation, insulin receptor substrate-1 phosphorylation, and phosphatidylinositol 3-kinase activity are decreased in intact skeletal muscle strips from obese subjects. *Journal of Clinical Investigation, 95*(5), 2195-2204.

Green, H., Morikawa, M., & Nixon, T. (1985). A dual effector theory of growth-hormone action. *Differentiation, 29*(3), 195-198.

Greenspan, F. S., Li, C. H., Simpson, M. E., & Evans, H. M. (1949). Bioassay of hypophyseal growth hormone: The tibia test. *Endocrinology, 45*, 455-463.

Griffin, M. E., Marcucci, M. J., Cline, G. W., Bell, K., Barucci, N., Lee, D., et al. (1999). Free fatty acid-induced insulin resistance is associated with activation of protein kinase C theta and alterations in the insulin signaling cascade. *Diabetes, 48*(6), 1270-1274.

Grove, K. L., Fried, S. K., Greenberg, A. S., Xiao, X. Q., & Clegg, D. J. (2010). A microarray analysis of sexual dimorphism of adipose tissues in high-fat-diet-induced obese mice. *International Journal of Obesity* (London), *34*(6), 989-1000.

Grunstein, R. R., Ho, K. Y., & Sullivan, C. E. (1991). Sleep apnea in acromegaly. *Annals of Internal Medicine, 115*(7), 527-532.

Guerra, C., Koza, R. A., Yamashita, H., Walsh, K., & Kozak, L. P. (1998). Emergence of brown adipocytes in white fat in mice is under genetic control: Effects on body weight and adiposity. *Journal of Clinical Investigation, 102*(2), 412-420.

Guilak, F., Lott, K. E., Awad, H. A., Cao, Q., Hicok, K. C., Fermor, B., et al. (2006). Clonal analysis of the differentiation potential of human adipose-derived adult stem cells. *Journal of Cellular Physiology, 206*(1), 229-237.

Guler, H. P., Zapf, J., & Froesch, E. R. (1987). Short-term metabolic effects of recombinant human insulin-like growth factor 1 in healthy adults. *New England Journal of Medicine, 317*(3), 137-140.

Hadden, D. R., & Prout, T. E. (1964). A growth hormone binding protein in normal human serum. *Nature, 202*, 1342-1343.

Hansen, L. H., Madsen, B., Teisner, B., Nielsen, J. H., & Billestrup, N. (1998). Characterization of the inhibitory effect of growth hormone on primary preadipocyte differentiation. *Molecular Endocrinology, 12*(8), 1140-1149.

Hansen, T. B., Vahl, N., Jorgensen, J. O., Christiansen, J. S., & Hagen, C. (1995). Whole body and regional soft tissue changes in growth hormone deficient adults after one year of growth hormone treatment: A double-blind, randomized, placebo-controlled study. *Clinical Endocrinology, 43*(6), 689-696.

Haque, M. S., Minokoshi, Y., Hamai, M., Iwai, M., Horiuchi, M., & Shimazu, T. (1999). Role of the sympathetic nervous system and insulin in enhancing glucose uptake in peripheral tissues after intrahypothalamic injection of leptin in rats. *Diabetes, 48*(9), 1706-1712.

Harding, P. A., Wang, X., Okada, S., Chen, W. Y., Wan, W., & Kopchick, J. J. (1996). Growth hormone (GH) and a GH antagonist promote GH receptor dimerization and internalization. *Journal of Biological Chemistry, 271*(12), 6708-6712.

Hauck, S. J., Hunter, W. S., Danilovich, N., Kopchick, J. J., & Bartke, A. (2001). Reduced levels of thyroid hormones, insulin, and glucose, and lower body core temperature in the growth hormone receptor/binding protein knockout mouse. *Experimental Biology and Medicine* (Maywood), *226*(6), 552-558.

Hauner, H., Wabitsch, M., & Pfeiffer, E. F. (1988). Differentiation of adipocyte precursor cells from obese and nonobese adult women and from different adipose tissue sites. *Hormone and Metabolic Research* (Suppl), *19*, 35-39.

Heine, P. A., Taylor, J. A., Iwamoto, G. A., Lubahn, D. B., & Cooke, P. S. (2000). Increased adipose tissue in male and female estrogen receptor-alpha knockout mice. *Proceedings of the National Academy of Sciences of the United States of America, 97*(23), 12729-12734.

Hemmeryckx, B., Loeckx, D., Dresselaers, T., Himmelreich, U., Hoylaerts, M. F., & Lijnen, H. R. (2010). Age-associated adaptations in murine adipose tissues. *Endocrine Journal*. Advance online publication. Retrieved October 26, 2010. PMID: 20686275.

Henderson, M. J., Morgan, H. E., & Park, C. R. (1961). Regulation of glucose uptake in muscle. V. The effect of growth hormone on glucose transport in the isolated, perfused rat heart. *Journal of Biological Chemistry, 236*, 2157-2161.

Henneman, D. H., & Henneman, P. H. (1960). Effects of human growth hormone on levels of blood urinary carbohydrate and fat metabolites in man. *Journal of Clinical Investigation, 39*, 1239-1245.

Hennes, M. M., Dua, A., & Kissebah, A. H. (1997). Effects of free fatty acids and glucose on splanchnic insulin dynamics. *Diabetes, 46*(1), 57-62.

Hevener, A., Reichart, D., Janez, A., & Olefsky, J. (2002). Female rats do not exhibit free fatty acid-induced insulin resistance. *Diabetes, 51*(6), 1907-1912.

Hew, F. L., Koschmann, M., Christopher, M., Rantzau, C., Vaag, A., Ward, G., et al. (1996). Insulin resistance in growth hormone-deficient adults: Defects in glucose utilization and glycogen synthase activity. *Journal of Clinical Endocrinology and Metabolism, 81*(2), 555-564.

Higham, C. E., & Trainer, P. J. (2008). Growth hormone excess and the development of growth hormone receptor antagonists. *Experimental Physiology, 93*(11), 1157-1169.

Himms-Hagen, J. (1990). Brown adipose tissue thermogenesis: Interdisciplinary studies. *Federation of American Societies for Experimental Biology Journal, 4*(11), 2890-2898.

Hindmarsh, P., Smith, P. J., Brook, C. G., & Matthews, D. R. (1987). The relationship between height velocity and growth hormone secretion in short prepubertal children. *Clinical Endocrinology, 27*(5), 581-591.

Hirt, H., Kimelman, J., Birnbaum, M. J., Chen, E. Y., Seeburg, P. H., Eberhardt, N. L., et al. (1987). The human growth hormone gene locus: Structure, evolution, and allelic variations. *DNA, 6*(1), 59-70.

Holly, J. M., Cotterill, A. M., Jemmott, R. C., Shears, D., al-Othman, S., Chard, T., et al. (1991). Inter-relations between growth hormone, insulin, insulin-like growth factor-1 (IGF-1), IGF-binding protein-1 (IGFBP-1) and sex hormone-binding globulin in acromegaly. *Clinical Endocrinology, 34*(4), 275-280.

Hommel, J. D., Trinko, R., Sears, R. M., Georgescu, D., Liu, Z. W., Gao, X. B., et al. (2006). Leptin receptor signaling in midbrain dopamine neurons regulates feeding. *Neuron, 51*(6), 801-810.

Honeyman, T. W., & Goodman, H. M. (1980). Effects of growth hormone on glycogen metabolism in adipose tissue of hypophysectomized rats. *American Journal of Physiology, 238*(4), E389-394.

Hong, J., Stubbins, R. E., Smith, R. R., Harvey, A. E., & Nunez, N. P. (2009). Differential susceptibility to obesity between male, female and ovariectomized female mice. *Nutrition Journal, 8*, 11. doi:10.1186/1475-2891-8-11

Hotamisligil, G. S., Murray, D. L., Choy, L. N., & Spiegelman, B. M. (1994). Tumor necrosis factor alpha inhibits signaling from the insulin receptor. *Proceedings of the National Academy of Sciences of the United States of America, 91*(11), 4854-4858.

Hotamisligil, G. S., Shargill, N. S., & Spiegelman, B. M. (1993). Adipose expression of tumor necrosis factor-alpha: Direct role in obesity-linked insulin resistance. *Science, 259*(5091), 87-91.

Houpt, K. A., Coren, B., Hintz, H. F., & Hilderbrant, J. E. (1979). Effect of sex and reproductive status on sucrose preference, food intake, and body weight of dogs. *Journal of the American Veterinary Medical Association, 174*(10), 1083-1085.

Houssay, B. A., & Biasotti, A. (1930). Hipofisectomia y diabetes pancreatica en el sapo. *Revista de la Sociedad Argentina de Biologia, 6*, 8-24.

Hube, F., Birgel, M., Lee, Y. M., & Hauner, H. (1999). Expression pattern of tumour necrosis factor receptors in subcutaneous and omental human adipose tissue: Role of obesity and non-insulin-dependent diabetes mellitus. *European Journal of Clinical Investigation, 29*(8), 672-678.

Hull, D. (1966). The structure and function of brown adipose tissue. *British Medical Bulletin, 22*(1), 92-96.

Hwang, L. L., Wang, C. H., Li, T. L., Chang, S. D., Lin, L. C., Chen, C. P., et al. (2009). Sex differences in high-fat diet-induced obesity, metabolic alterations and learning, and synaptic plasticity deficits in mice. *Obesity (Silver Spring), 18*(3), 463-469.

Ikeda, A., Chang, K. T., Matsumoto, Y., Furuhata, Y., Nishihara, M., Sasaki, F., et al. (1998). Obesity and insulin resistance in human growth hormone transgenic rats. *Endocrinology, 139*(7), 3057-3063.

Isgaard, J., Nilsson, A., Vikman, K., & Isaksson, O. G. (1989). Growth hormone regulates the level of insulin-like growth factor-1 mRNA in rat skeletal muscle. *Journal of Endocrinology, 120*(1), 107-112.

Isidori, A. M., Caprio, M., Strollo, F., Moretti, C., Frajese, G., Isidori, A., et al. (1999). Leptin and androgens in male obesity: Evidence for leptin contribution to reduced androgen levels. *Journal of Clinical Endocrinology and Metabolism, 84*(10), 3673-3680.

Jansson, J. O., Albertsson-Wikland, K., Eden, S., Thorngren, K. G., & Isaksson, O. (1982). Effect of frequency of growth hormone administration on longitudinal bone growth and body weight in hypophysectomized rats. *Acta Physiologica Scandinavica, 114*(2), 261-265.

Jensen, M. D., Haymond, M. W., Rizza, R. A., Cryer, P. E., & Miles, J. M. (1989). Influence of body fat distribution on free fatty acid metabolism in obesity. *Journal of Clinical Investigation, 83*(4), 1168-1173.

Jensen, M. D., & Johnson, C. M. (1996). Contribution of leg and splanchnic free fatty acid (FFA) kinetics to postabsorptive FFA flux in men and women. *Metabolism: Clinical and Experimental, 45*(5), 662-666.

Johansson, J. O., Fowelin, J., Landin, K., Lager, I., & Bengtsson, B. A. (1995). Growth hormone-deficient adults are insulin-resistant. *Metabolism: Clinical and Experimental, 44*(9), 1126-1129.

Jones, J. I., & Clemmons, D. R. (1995). Insulin-like growth factors and their binding proteins: Biological actions. *Endocrine Reviews, 16*(1), 3-34.

Kadim, I. T., McCutcheon, S. N., Purchas, R. W., & Wickham, G. A. (1996). Manipulation of adult body composition by treatment of the neonatal rat with growth hormone and prolactin. *Growth Regulation, 6*(4), 201-205.

Kahn, B. B., Alquier, T., Carling, D., & Hardie, D. G. (2005). AMP-activated protein kinase: Ancient energy gauge provides clues to modern understanding of metabolism. *Cell Metababolism, 1*(1), 15-25.

Kahn, B. B., & Flier, J. S. (2000). Obesity and insulin resistance. *Journal of Clinical Investigation, 106*(4), 473-481.

Kamel, A., Norgren, S., Elimam, A., Danielsson, P., & Marcus, C. (2000). Effects of growth hormone treatment in obese prepubertal boys. *Journal of Clinical Endocrinology and Metabolism, 85*(4), 1412-1419.

Kamohara, S., Burcelin, R., Halaas, J. L., Friedman, J. M., & Charron, M. J. (1997). Acute stimulation of glucose metabolism in mice by leptin treatment. *Nature, 389*(6649), 374-377.

Kato, H., Faria, T. N., Stannard, B., Roberts, C. T., Jr., & LeRoith, D. (1993). Role of tyrosine kinase activity in signal transduction by the insulin-like growth factor-1 (IGF-1) receptor: Characterization of kinase-deficient IGF-1 receptors and the action of an IGF-1-mimetic antibody (alpha IR-3). *Journal of Biological Chemistry, 268*(4), 2655-2661.

Katznelson, L. (2009). Alterations in body composition in acromegaly. *Pituitary, 12*(2), 136-142.

Kearns, C. F., McKeever, K. H., Roegner, V., Brady, S. M., & Malinowski, K. (2006). Adiponectin and leptin are related to fat mass in horses. *Veterinary Journal, 172*(3), 460-465.

Kelder, B., Berryman, D. E., Clark, R., Li, A., List, E. O., & Kopchick, J. J. (2007). CIDE-A gene expression is decreased in white adipose tissue of growth hormone receptor/binding protein gene disrupted mice and with high-fat feeding of normal mice. *Growth Hormone and IGF Research, 17*(4), 346-351.

Kellerer, M., Koch, M., Metzinger, E., Mushack, J., Capp, E., & Haring, H. U. (1997). Leptin activates PI-3 kinase in C2C12 myotubes via janus kinase-2 (JAK-2) and insulin receptor substrate-2 (IRS-2) dependent pathways. *Diabetologia, 40*(11), 1358-1362.

Kelley, D. E., Thaete, F. L., Troost, F., Huwe, T., & Goodpaster, B. H. (2000). Subdivisions of subcutaneous abdominal adipose tissue and insulin resistance. *American Journal of Physiology–Endocrinology and Metabolism, 278*(5), E941-948.

Khaleeli, A. A., Levy, R. D., Edwards, R. H., McPhail, G., Mills, K. R., Round, J. M., et al. (1984). The neuromuscular features of acromegaly: A clinical and pathological study. *Journal of Neurology, Neurosurgery and Psychiatry, 47*(9), 1009-1015.

Kim, J. Y., van de Wall, E., Laplante, M., Azzara, A., Trujillo, M. E., Hofmann, S. M., et al. (2007). Obesity-associated improvements in metabolic profile through expansion of adipose tissue. *Journal of Clinical Investigation, 117*(9), 2621-2637.

Kintscher, U., Hartge, M., Hess, K., Foryst-Ludwig, A., Clemenz, M., Wabitsch, M., et al. (2008). T-lymphocyte infiltration in visceral adipose tissue: a primary event in adipose tissue inflammation and the development of obesity-mediated insulin resistance. *Arteriosclerosis, Thrombosis, and Vascular Biology, 28*(7), 1304-1310.

Klaus, S., Cassard-Doulcier, A. M., & Ricquier, D. (1991). Development of Phodopus sungorus brown preadipocytes in primary cell culture: Effect of an atypical beta-adrenergic agonist, insulin, and triiodothyronine on differentiation, mitochondrial development, and expression of the uncoupling protein UCP. *Journal of Cell Biology, 115*(6), 1783-1790.

Knapp, J. R., Chen, W. Y., Turner, N. D., Byers, F. M., & Kopchick, J. J. (1994). Growth patterns and body composition of transgenic mice expressing mutated bovine somatotropin genes. *Journal of Animal Science, 72*(11), 2812-2819.

Kopchick, J. J., & Andry, J. M. (2000). Growth hormone (GH), GH receptor, and signal transduction. *Molecular Genetics and Metabolism, 71*(1-2), 293-314.

Kozak, L. P., Britton, J. H., Kozak, U. C., & Wells, J. M. (1988). The mitochondrial uncoupling protein gene: Correlation of exon structure to transmembrane domains. *Journal of Biological Chemistry, 263*(25), 12274-12277.

Kristensen, K., Pedersen, S. B., & Richelsen, B. (1999). Regulation of leptin by steroid hormones in rat adipose tissue. *Biochemical and Biophysical Research Communications, 259*(3), 624-630.

Kubota, N., Terauchi, Y., Yamauchi, T., Kubota, T., Moroi, M., Matsui, J., et al. (2002). Disruption of adiponectin causes insulin resistance and neointimal formation. *Journal of Biological Chemistry, 277*(29), 25863-25866.

LaFranchi, S., Hanna, C. E., Torresani, T., Schoenle, E., & Illig, R. (1985). Comparison of growth hormone binding and metabolic response in rat adipocytes of epididymal, subcutaneous, and retroperitoneal origin. *Acta Endocrinologica, 110*(1), 50-55.

Lee, G. H., Proenca, R., Montez, J. M., Carroll, K. M., Darvishzadeh, J. G., Lee, J. I., et al. (1996). Abnormal splicing of the leptin receptor in diabetic mice. *Nature, 379*(6566), 632-635.

Leung, D. W., Spencer, S. A., Cachianes, G., Hammonds, R. G., Collins, C., Henzel, W. J., et al. (1987). Growth hormone receptor and serum binding protein: Purification, cloning and expression. *Nature, 330*(6148), 537-543.

Leung, K. C., Doyle, N., Ballesteros, M., Sjogren, K., Watts, C. K., Low, T. H., et al. (2003). Estrogen inhibits GH signaling by suppressing GH-induced JAK2 phosphorylation, an effect mediated by SOCS-2. *Proceedings of the National Academy of Sciences of the United States of America, 100*(3), 1016-1021.

Lewis, U. J., Singh, R. N., Tutwiler, G. F., Sigel, M. B., VanderLaan, E. F., & VanderLaan, W. P. (1980). Human growth hormone: A complex of proteins. *Recent Progress in Hormone Research, 36*, 477-508.

Li, C. H., & Evans, H. M. (1944). The isolation of pituitary growth hormone. *Science, 99*, 183-184.

Li, G., Zhang, Y., Wilsey, J. T., & Scarpace, P. J. (2004). Unabated anorexic and enhanced thermogenic responses to melanotan II in diet-induced obese rats despite reduced melanocortin 3 and 4 receptor expression. *Journal of Endocrinology, 182*(1), 123-132.

Li, S., Crenshaw, E. B., 3rd, Rawson, E. J., Simmons, D. M., Swanson, L. W., & Rosenfeld, M. G. (1990). Dwarf locus mutants lacking three pituitary cell types result from mutations in the POU-domain gene pit-1. *Nature, 347*(6293), 528-533.

Li, Y., Knapp, J. R., & Kopchick, J. J. (2003). Enlargement of interscapular brown adipose tissue in growth hormone antagonist transgenic and in growth hormone receptor gene-disrupted dwarf mice. *Experimental Biology and Medicine (Maywood), 228*(2), 207-215.

Lindberg, O. (1970). *Brown adipose tissue*. New York: Elsevier.

Llado, I., Rodriguez-Cuenca, S., Pujol, E., Monjo, M., Estrany, M. E., Roca, P., et al. (2002). Gender effects on adrenergic receptor expression and lipolysis in white adipose tissue of rats. *Obesity Research, 10*(4), 296-305.

Lord, G. M., Matarese, G., Howard, J. K., Baker, R. J., Bloom, S. R., & Lechler, R. I. (1998). Leptin modulates the T-cell immune response and reverses starvation-induced immunosuppression. *Nature, 394*(6696), 897-901.

Lottati, M., Kolka, C. M., Stefanovski, D., Kirkman, E. L., & Bergman, R. N. (2009). Greater omentectomy improves insulin sensitivity in nonobese dogs. *Obesity (Silver Spring), 17*(4), 674-680.

Louis Casteilla, L. P., Beatrice Cousin, Denis Calise. (2008). Choosing an Adipose Tissue Depot for Sampling. In K. Yang (Ed.), *Adipose tissue protocols* (2nd ed., pp. 23-38). Totowa, NJ: Humana Press.

Lowe, W. L., Jr., Lasky, S. R., LeRoith, D., & Roberts, C. T., Jr. (1988). Distribution and regulation of rat insulin-like growth factor 1 messenger ribonucleic acids encoding alternative carboxyterminal E-peptides: Evidence for differential processing and regulation in liver. *Molecular Endocrinology, 2*(6), 528-535.

Luukkaa, V., Pesonen, U., Huhtaniemi, I., Lehtonen, A., Tilvis, R., Tuomilehto, J., et al. (1998). Inverse correlation between serum testosterone and leptin in men. *Journal of Clinical Endocrinology and Metabolism, 83*(9), 3243-3246.

MacLeod, J. N., Pampori, N. A., & Shapiro, B. H. (1991). Sex differences in the ultradian pattern of plasma growth hormone concentrations in mice. *Journal of Endocrinology, 131*(3), 395-399.

Macotela, Y., Boucher, J., Tran, T. T., & Kahn, C. R. (2009). Sex and depot differences in adipocyte insulin sensitivity and glucose metabolism. *Diabetes, 58*(4), 803-812.

Madsen, K., Friberg, U., Roos, P., Eden, S., & Isaksson, O. (1983). Growth hormone stimulates the proliferation of cultured chondrocytes from rabbit ear and rat rib growth cartilage. *Nature, 304*(5926), 545-547.

Maffei, M., Halaas, J., Ravussin, E., Pratley, R. E., Lee, G. H., Zhang, Y., et al. (1995). Leptin levels in human and rodent: Measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. *Nature Medicine, 1*(11), 1155-1161.

Magon, V. (2009). *Body Composition and Adipokine Levels in Growth Hormone Antagonist Mice.* Unpublished master's thesis, Ohio University, Athens.

Makimura, H., Stanley, T., Mun, D., Chen, C., Wei, J., Connelly, J. M., et al. (2009). Reduced growth hormone secretion is associated with increased carotid intima-media thickness in obesity. *Journal of Clinical Endocrinology and Metabolism, 94*(12), 5131-5138.

Malina, R., Bouchard, C., & Bar-Or, O. (2004). *Growth, maturation, and physical activity* (2nd ed.). Champaign, IL: Human Kinetics.

Malmlof, K., Din, N., Johansen, T., & Pedersen, S. B. (2002). Growth hormone affects both adiposity and voluntary food intake in old and obese female rats. *European Journal of Endocrinology / European Federation of Endocrine Societies, 146*(1), 121-128.

Martial, J. A., Hallewell, R. A., Baxter, J. D., & Goodman, H. M. (1979). Human growth hormone: Complementary DNA cloning and expression in bacteria. *Science, 205*(4406), 602-607.

Martin, M. L., & Jensen, M. D. (1991). Effects of body fat distribution on regional lipolysis in obesity. *Journal of Clinical Investigation, 88*(2), 609-613.

Martini, J. F., Villares, S. M., Nagano, M., Delehaye-Zervas, M. C., Eymard, B., Kelly, P. A., et al. (1995). Quantitative analysis by polymerase chain reaction of growth hormone receptor gene expression in human liver and muscle. *Endocrinology, 136*(4), 1355-1360.

Mathews, L. S., Enberg, B., & Norstedt, G. (1989). Regulation of rat growth hormone receptor gene expression. *Journal of Biological Chemistry, 264*(17), 9905-9910.

Matsuo, T., Murotake, Y., Nakata, Y., Seino, S., Okura, T., & Tanaka, K. (2010). [Effects of a community-based weight loss program, jointly established by local government and university faculty, on weight loss and metabolic syndrome components: The Sodegaura Weight Management Study]. *Nippon Koshu Eisei Zasshi, 57*(5), 390-402.

Matsuzawa, Y. (2005). White adipose tissue and cardiovascular disease. *Best Practice & Research Clinical Endocrinology & Metabolism, 19*(4), 637-647.

Matthias, A., Ohlson, K. B., Fredriksson, J. M., Jacobsson, A., Nedergaard, J., & Cannon, B. (2000). Thermogenic responses in brown fat cells are fully UCP1-dependent. UCP2 or UCP3 do not substitute for UCP1 in adrenergically or fatty acid-induced thermogenesis. *Journal of Biological Chemistry, 275*(33), 25073-25081.

McGauley, G. A. (1989). Quality of life assessment before and after growth hormone treatment in adults with growth hormone deficiency. *Acta Paediatrica Scandinavica* (Suppl), *356*, 70-72; discussion 73-74.

McGrane, M. M., Yun, J. S., Moorman, A. F., Lamers, W. H., Hendrick, G. K., Arafah, B. M., et al. (1990). Metabolic effects of developmental, tissue-, and cell-specific expression of a chimeric phosphoenolpyruvate carboxykinase (GTP)/bovine growth hormone gene in transgenic mice. *Journal of Biological Chemistry, 265*(36), 22371-22379.

McTernan, C. L., McTernan, P. G., Harte, A. L., Levick, P. L., Barnett, A. H., & Kumar, S. (2002). Resistin, central obesity, and type 2 diabetes. *Lancet, 359*(9300), 46-47.

Mercer, J. G., Hoggard, N., Williams, L. M., Lawrence, C. B., Hannah, L. T., & Trayhurn, P. (1996). Localization of leptin receptor mRNA and the long form splice variant (Ob-Rb) in mouse hypothalamus and adjacent brain regions by in situ hybridization. *Federation of European Biochemical Societies Letters, 387*(2-3), 113-116.

Mohamed-Ali, V., Goodrick, S., Rawesh, A., Katz, D. R., Miles, J. M., Yudkin, J. S., et al. (1997). Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis factor-alpha, in vivo. *Journal of Clinical Endocrinology and Metabolism, 82*(12), 4196-4200.

Mohamed-Ali, V., Pinkney, J. H., & Coppack, S. W. (1998). Adipose tissue as an endocrine and paracrine organ. *International Journal of Obesity and Related Metabolic Disorders, 22*(12), 1145-1158.

Montague, C. T., Farooqi, I. S., Whitehead, J. P., Soos, M. A., Rau, H., Wareham, N. J., et al. (1997). Congenital leptin deficiency is associated with severe early-onset obesity in humans. *Nature, 387*(6636), 903-908.

Morikawa, M., Nixon, T., & Green, H. (1982). Growth hormone and the adipose conversion of 3T3 cells. *Cell, 29*(3), 783-789.

Morton, N. M., Emilsson, V., de Groot, P., Pallett, A. L., & Cawthorne, M. A. (1999). Leptin signalling in pancreatic islets and clonal insulin-secreting cells. *Journal of Molecular Endocrinology, 22*(2), 173-184.

Mouroux, I., Bertin, R., & Portet, R. (1990). Thermogenic capacity of the brown adipose tissue of developing rats: Effects of rearing temperature. *Journal of Developmental Physiology, 14*(6), 337-342.

Mundi, M. S., Karpyak, M. V., Koutsari, C., Votruba, S. B., O'Brien, P. C., & Jensen, M. D. Body fat distribution, adipocyte size, and metabolic characteristics of nondiabetic adults. *Journal of Clinical Endocrinology and Metabolism, 95*(1), 67-73.

Murphy, L. J., & Friesen, H. G. (1988). Differential effects of estrogen and growth hormone on uterine and hepatic insulin-like growth factor 1 gene expression in the ovariectomized hypophysectomized rat. *Endocrinology, 122*(1), 325-332.

Murphy, W. J., Durum, S. K., Anver, M., Frazier, M., & Longo, D. L. (1992). Recombinant human growth hormone promotes human lymphocyte engraftment in immunodeficient mice and results in an increased incidence of human Epstein Barr virus-induced B-cell lymphoma. *Brain, Behavior, and Immunity, 6*(4), 355-364.

Murray, R. D., Adams, J. E., & Shalet, S. M. (2004). Adults with partial growth hormone deficiency have an adverse body composition. *Journal of Clinical Endocrinology and Metabolism, 89*(4), 1586-1591.

Nabarro, J. D. (1987). Acromegaly. *Clinical Endocrinology, 26*(4), 481-512.

Napolitano, L. (1963). The differentiation of white adipose cells: An electron microscope study. *Journal of Cell Biology, 18*, 663-679.

Nedergaard, J., Bengtsson, T., & Cannon, B. (2007). Unexpected evidence for active brown adipose tissue in adult humans. *American Journal of Physiology–Endocrinology and Metababolism, 293*(2), E444-452.

Nedergaard, J., Connolly, E., & Cannon, B. (1986). Brown adipose tissue in mammalian neonate. In P. N. D. Trayhurn (Ed.), *Brown adipose tissue* (pp. 269-298). London: Edward Arnold.

Newman, C. B., Melmed, S., Snyder, P. J., Young, W. F., Boyajy, L. D., Levy, R., et al. (1995). Safety and efficacy of long-term octreotide therapy of acromegaly: Results of a multicenter trial in 103 patients–A clinical research center study. *Journal of Clinical Endocrinology and Metabolism, 80*(9), 2768-2775.

Nicoll, C. S., Mayer, G. L., & Russell, S. M. (1986). Structural features of prolactins and growth hormones that can be related to their biological properties [published erratum appears in Endocr Rev 1987 Feb;8(1):43]. *Endocrine Reviews, 7*(2), 169-203.

Nikkila, E. A., & Pelkonen, R. (1975). Serum lipids in acromegaly. *Metabolism: Clinical and Experimental, 24*(7), 829-838.

O'Shaughnessy, I. M., Myers, T. J., Stepniakowski, K., Nazzaro, P., Kelly, T. M., Hoffmann, R. G., et al. (1995). Glucose metabolism in abdominally obese hypertensive and normotensive subjects. *Hypertension, 26*(1), 186-192.

Ohlsson, C., Lovstedt, K., Holmes, P. V., Nilsson, A., Carlsson, L., & Tornell, J. (1993). Embryonic stem cells express growth hormone receptors: Regulation by retinoic acid. *Endocrinology, 133*(6), 2897-2903.

Okada, S., Chen, W. Y., Wiehl, P., Kelder, B., Goodman, H. M., Guller, S., et al. (1992). A growth hormone (GH) analog can antagonize the ability of native GH to promote differentiation of 3T3-F442A preadipocytes and stimulate insulin-like and lipolytic activities in primary rat adipocytes. *Endocrinology, 130*(4), 2284-2290.

Olsson, B., Bohlooly, Y. M., Fitzgerald, S. M., Frick, F., Ljungberg, A., Ahren, B., et al. (2005). Bovine growth hormone transgenic mice are resistant to diet-induced obesity but develop hyperphagia, dyslipidemia, and diabetes on a high-fat diet. *Endocrinology, 146*(2), 920-930.

Orme, S. M., McNally, R. J., Cartwright, R. A., & Belchetz, P. E. (1998). Mortality and cancer incidence in acromegaly: A retrospective cohort study. United Kingdom Acromegaly Study Group. *Journal of Clinical Endocrinology and Metabolism, 83*(8), 2730-2734.

Oscarsson, J., Wiklund, O., Jakobsson, K. E., Petruson, B., & Bengtsson, B. A. (1994). Serum lipoproteins in acromegaly before and 6-15 months after transsphenoidal adenomectomy. *Clinical Endocrinology, 41*(5), 603-608.

Owerbach, D., Rutter, W. J., Martial, J. A., Baxter, J. D., & Shows, T. B. (1980). Genes for growth hormone, chorionic somatommammotropin, and growth hormones-like gene on chromosome 17 in humans. *Science, 209*(4453), 289-292.

Palmer, A. J., Chung, M. Y., List, E. O., Walker, J., Okada, S., Kopchick, J. J., et al. (2009). Age-related changes in body composition of bovine growth hormone transgenic mice. *Endocrinology, 150*(3), 1353-1360.

Palmiter, R. D., Brinster, R. L., Hammer, R. E., Trumbauer, M. E., Rosenfeld, M. G., Birnberg, N. C., et al. (1982). Dramatic growth of mice that develop from eggs microinjected with metallothionein-growth hormone fusion genes. *Nature, 300*(5893), 611-615.

Paolisso, G., Barbieri, M., Bonafe, M., & Franceschi, C. (2000). Metabolic age modelling: The lesson from centenarians. *European Journal of Clinical Investigation, 30*(10), 888-894.

Parkinson, C., Drake, W. M., Wieringa, G., Yates, A. P., Besser, G. M., & Trainer, P. J. (2002). Serum lipoprotein changes following IGF-1 normalization using a growth hormone receptor antagonist in acromegaly. *Clinical Endocrinology, 56*(3), 303-311.

Pelleymounter, M. A., Cullen, M. J., Baker, M. B., Hecht, R., Winters, D., Boone, T., et al. (1995). Effects of the obese gene product on body weight regulation in ob/ob mice. *Science, 269*(5223), 540-543.

Peraldi, P., Hotamisligil, G. S., Buurman, W. A., White, M. F., & Spiegelman, B. M. (1996). Tumor necrosis factor (TNF)-alpha inhibits insulin signaling through stimulation of the p55 TNF receptor and activation of sphingomyelinase. *Journal of Biological Chemistry, 271*(22), 13018-13022.

Peter, M. A., Winterhalter, K. H., Boni-Schnetzler, M., Froesch, E. R., & Zapf, J. (1993). Regulation of insulin-like growth factor-1 (IGF-1) and IGF-binding proteins by growth hormone in rat white adipose tissue. *Endocrinology, 133*(6), 2624-2631.

Planat-Benard, V., Menard, C., Andre, M., Puceat, M., Perez, A., Garcia-Verdugo, J. M., et al. (2004). Spontaneous cardiomyocyte differentiation from adipose tissue stroma cells. *Circulation Research, 94*(2), 223-229.

Planat-Benard, V., Silvestre, J. S., Cousin, B., Andre, M., Nibbelink, M., Tamarat, R., et al. (2004). Plasticity of human adipose lineage cells toward endothelial cells: Physiological and therapeutic perspectives. *Circulation, 109*(5), 656-663.

Pomp, D., Oberbauer, A. M., & Murray, J. D. (1996). Development of obesity following inactivation of a growth hormone transgene in mice. *Transgenic Research, 5*(1), 13-23.

Potteiger, J. A., Jacobsen, D. J., & Donnelly, J. E. (2002). A comparison of methods for analyzing glucose and insulin areas under the curve following nine months of exercise in overweight adults. *International Journal of Obesity and Related Metabolic Disorders, 26*(1), 87-89.

Pradhananga, S., Wilkinson, I., & Ross, R. J. (2002). Pegvisomant: Structure and function. *Journal of Molecular Endocrinology, 29*(1), 11-14.

Press, M., Tamborlane, W. V., & Sherwin, R. S. (1984). Importance of raised growth hormone levels in mediating the metabolic derangements of diabetes. *New England Journal of Medicine, 310*(13), 810-815.

Prins, J. B., Walker, N. I., Winterford, C. M., & Cameron, D. P. (1994). Apoptosis of human adipocytes in vitro. *Biochemical and Biophysical Research Communications, 201*(2), 500-507.

Prunet-Marcassus, B., Cousin, B., Caton, D., Andre, M., Penicaud, L., & Casteilla, L. (2006). From heterogeneity to plasticity in adipose tissues: Site-specific differences. *Experimental Cell Research, 312*(6), 727-736.

Quabbe, H. J., Schilling, E., & Helge, H. (1966). Pattern of growth hormone secretion during a 24-hour fast in normal adults. *Journal of Clinical Endocrinology and Metabolism, 26*(10), 1173-1177.

Raben, M. S., & Hollenberg, C. H. (1959). Effect of growth hormone on plasma fatty acids. *Journal of Clinical Investigation, 38*(3), 484-488.

Rafael, J., & Heldt, H. W. (1976). Binding of guanine nucleotides to the outer surface of the inner membrane of guinea pig brown fat mitochondria in correlation with the thermogenic activity of the tissue. *Federation of European Biochemical Societies Letters, 63*(2), 304-308.

Rajala, M. W., & Scherer, P. E. (2003). Minireview: The adipocyte–At the crossroads of energy homeostasis, inflammation, and atherosclerosis. *Endocrinology, 144*(9), 3765-3773.

Rajasoorya, C., Holdaway, I. M., Wrightson, P., Scott, D. J., & Ibbertson, H. K. (1994). Determinants of clinical outcome and survival in acromegaly. *Clinical Endocrinology, 41*(1), 95-102.

Ravussin, E., Pratley, R. E., Maffei, M., Wang, H., Friedman, J. M., Bennett, P. H., et al. (1997). Relatively low plasma leptin concentrations precede weight gain in Pima Indians. *Nature Medicine, 3*(2), 238-240.

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.

Rennie, M. J. (2003). Claims for the anabolic effects of growth hormone: A case of the emperor's new clothes? *British Journal of Sports Medicine, 37*(2), 100-105.

Rinderknecht, E., & Humbel, R. E. (1978). The amino acid sequence of human insulin-like growth factor 1 and its structural homology with proinsulin. *Journal of Biological Chemistry, 253*(8), 2769-2776.

Rizza, R. A., Mandarino, L. J., & Gerich, J. E. (1982). Effects of growth hormone on insulin action in man. Mechanisms of insulin resistance, impaired suppression of glucose production, and impaired stimulation of glucose utilization. *Diabetes, 31*(8 Pt 1), 663-669.

Robertson, K., Kopchick, J. J., & Liu, J. L. (2006). Growth hormone receptor gene deficiency causes delayed insulin responsiveness in skeletal muscles without affecting compensatory islet cell overgrowth in obese mice. *American Journal of Physiology–Endocrinology and Metabolism, 291*(3), E491-498.

Roby, K. F., Deb, S., Gibori, G., Szpirer, C., Levan, G., Kwok, S. C., et al. (1993). Decidual prolactin-related protein: Identification, molecular cloning, and characterization. *Journal of Biological Chemistry, 268*(5), 3136-3142.

Ronchi, C. L., Giavoli, C., Ferrante, E., Verrua, E., Bergamaschi, S., Ferrari, D. I., et al. (2009). Prevalence of GH deficiency in cured acromegalic patients: Impact of

different previous treatments. *European Journal of Endocrinology / European Federation of Endocrine Societies, 161*(1), 37-42.

Rondinone, C. M. (2006). Adipocyte-derived hormones, cytokines, and mediators. *Endocrine, 29*(1), 81-90.

Rosen, T., & Bengtsson, B. A. (1990). Premature mortality due to cardiovascular disease in hypopituitarism. *Lancet, 336*(8710), 285-288.

Rosen, T., Bosaeus, I., Tolli, J., Lindstedt, G., & Bengtsson, B. A. (1993). Increased body fat mass and decreased extracellular fluid volume in adults with growth hormone deficiency. *Clinical Endocrinology, 38*(1), 63-71.

Rosen, T., Wiren, L., Wilhelmsen, L., Wiklund, I., & Bengtsson, B. A. (1994). Decreased psychological well-being in adult patients with growth hormone deficiency. *Clinical Endocrinology, 40*(1), 111-116.

Rosenbaum, M., Gertner, J. M., Gidfar, N., Hirsch, J., & Leibel, R. L. (1992). Effects of systemic growth hormone (GH) administration on regional adipose tissue in children with non-GH-deficient short stature. *Journal of Clinical Endocrinology and Metabolism, 75*(1), 151-156.

Rosenfeld, R. G., & Hwa, V. (2009). The growth hormone cascade and its role in mammalian growth. *Hormone Research, 71* (Suppl 2), 36-40.

Ross, R. J., Leung, K. C., Maamra, M., Bennett, W., Doyle, N., Waters, M. J., et al. (2001). Binding and functional studies with the growth hormone receptor antagonist, B2036-PEG (pegvisomant), reveal effects of pegylation and evidence

that it binds to a receptor dimer. *Journal of Clinical Endocrinology and Metabolism, 86*(4), 1716-1723.

Roust, L. R., & Jensen, M. D. (1993). Postprandial free fatty acid kinetics are abnormal in upper body obesity. *Diabetes, 42*(11), 1567-1573.

Safford, K. M., Hicok, K. C., Safford, S. D., Halvorsen, Y. D., Wilkison, W. O., Gimble, J. M., et al. (2002). Neurogenic differentiation of murine and human adipose-derived stromal cells. *Biochemical and Biophysical Research Communications, 294*(2), 371-379.

Salomon, F., Cuneo, R. C., Hesp, R., & Sonksen, P. H. (1989). The effects of treatment with recombinant human growth hormone on body composition and metabolism in adults with growth hormone deficiency. *New England Journal of Medicine, 321*(26), 1797-1803.

Sato, T., Nagafuku, M., Shimizu, K., Taira, T., Igarashi, Y., & Inokuchi, J. (2008). Physiological levels of insulin and IGF-1 synergistically enhance the differentiation of mesenteric adipocytes. *Cell Biology International, 32*(11), 1397-1404.

Sawdey, M. S., & Loskutoff, D. J. (1991). Regulation of murine type 1 plasminogen activator inhibitor gene expression in vivo: Tissue specificity and induction by lipopolysaccharide, tumor necrosis factor-alpha, and transforming growth factor-beta. *Journal of Clinical Investigation, 88*(4), 1346-1353.

Schmitz, F., Hartmann, H., Stumpel, F., & Creutzfeldt, W. (1991). In vivo metabolic action of insulin-like growth factor 1 in adult rats. *Diabetologia, 34*(3), 144-149.

Schwartz, M. W., Woods, S. C., Porte, D., Jr., Seeley, R. J., & Baskin, D. G. (2000). Central nervous system control of food intake. *Nature, 404*(6778), 661-671.

Seeburg, P. H. (1982). The human growth hormone gene family: Nucleotide sequences show recent divergence and predict a new polypeptide hormone. *DNA, 1*(3), 239-249.

Seidell, J. C., Bjorntorp, P., Sjostrom, L., Kvist, H., & Sannerstedt, R. (1990). Visceral fat accumulation in men is positively associated with insulin, glucose, and C-peptide levels, but negatively with testosterone levels. *Metabolism: Clinical and Experimental, 39*(9), 897-901.

Sekimoto, H., & Boney, C. M. (2003). C-terminal Src kinase (CSK) modulates insulin-like growth factor-I signaling through Src in 3T3-L1 differentiation. *Endocrinology, 144*(6), 2546-2552.

Seldin, M. F., Mott, D., Bhat, D., Petro, A., Kuhn, C. M., Kingsmore, S. F., et al. (1994). Glycogen synthase: A putative locus for diet-induced hyperglycemia. *Journal of Clinical Investigation, 94*(1), 269-276.

Senn, J. J., Klover, P. J., Nowak, I. A., Zimmers, T. A., Koniaris, L. G., Furlanetto, R. W., et al. (2003). Suppressor of cytokine signaling-3 (SOCS-3), a potential mediator of interleukin-6-dependent insulin resistance in hepatocytes. *Journal of Biological Chemistry, 278*(16), 13740-13746.

Seufert, J., Kieffer, T. J., Leech, C. A., Holz, G. G., Moritz, W., Ricordi, C., et al. (1999). Leptin suppression of insulin secretion and gene expression in human pancreatic

islets: Implications for the development of adipogenic diabetes mellitus. *Journal of Clinical Endocrinology and Metabolism, 84*(2), 670-676.

Sierra-Honigmann, M. R., Nath, A. K., Murakami, C., Garcia-Cardena, G., Papapetropoulos, A., Sessa, W. C., et al. (1998). Biological action of leptin as an angiogenic factor. *Science, 281*(5383), 1683-1686.

Sjogren, K., Wallenius, K., Liu, J. L., Bohlooly, Y. M., Pacini, G., Svensson, L., et al. (2001). Liver-derived IGF-I is of importance for normal carbohydrate and lipid metabolism. *Diabetes, 50*(7), 1539-1545.

Smith, S. R., Lovejoy, J. C., Greenway, F., Ryan, D., deJonge, L., de la Bretonne, J., et al. (2001). Contributions of total body fat, abdominal subcutaneous adipose tissue compartments, and visceral adipose tissue to the metabolic complications of obesity. *Metabolism: Clinical and Experimental, 50*(4), 425-435.

Smith, T. M., Cong, Z., Gilliland, K. L., Clawson, G. A., & Thiboutot, D. M. (2006). Insulin-like growth factor-1 induces lipid production in human SEB-1 sebocytes via sterol response element-binding protein-1. *Journal of Investigative Dermatology, 126*(6), 1226-1232.

Smith, T. M., Gilliland, K., Clawson, G. A., & Thiboutot, D. (2008). IGF-1 induces SREBP-1 expression and lipogenesis in SEB-1 sebocytes via activation of the phosphoinositide 3-kinase/Akt pathway. *Journal of Investigative Dermatology, 128*(5), 1286-1293.

Sobrier, M. L., Duquesnoy, P., Duriez, B., Amselem, S., & Goossens, M. (1993). Expression and binding properties of two isoforms of the human growth hormone receptor. *Federation of European Biochemical Societies Letters, 319*(1-2), 16-20.

Sonksen, P. H., Salomon, F., & Cuneo, R. (1991). Metabolic effects of hypopituitarism and acromegaly. *Hormone Research, 36*(Suppl 1), 27-31.

Sotiropoulos, A., Ohanna, M., Kedzia, C., Menon, R. K., Kopchick, J. J., Kelly, P. A., et al. (2006). Growth hormone promotes skeletal muscle cell fusion independent of insulin-like growth factor 1 up-regulation. *Proceedings of the National Academy of Sciences of the United States of America, 103*(19), 7315-7320.

Spalding, K. L., Arner, E., Westermark, P. O., Bernard, S., Buchholz, B. A., Bergmann, O., et al. (2008). Dynamics of fat cell turnover in humans. *Nature, 453*(7196), 783-787.

Stephens, T. W., Basinski, M., Bristow, P. K., Bue-Valleskey, J. M., Burgett, S. G., Craft, L., et al. (1995). The role of neuropeptide Y in the antiobesity action of the obese gene product. *Nature, 377*(6549), 530-532.

Steppan, C. M., Bailey, S. T., Bhat, S., Brown, E. J., Banerjee, R. R., Wright, C. M., et al. (2001). The hormone resistin links obesity to diabetes. *Nature, 409*(6818), 307-312.

Stewart, C. E., & Rotwein, P. (1996). Growth, differentiation, and survival: Multiple physiological functions for insulin-like growth factors. *Physiological Reviews, 76*(4), 1005-1026.

Sucunza, N., Barahona, M. J., Resmini, E., Fernandez-Real, J. M., Farrerons, J., Lluch, P., et al. (2008). Gender dimorphism in body composition abnormalities in acromegaly: Males are more affected than females. *European Journal of Endocrinology / European Federation of Endocrine Societies, 159*(6), 773-779.

Suh, Y., Atzmon, G., Cho, M. O., Hwang, D., Liu, B., Leahy, D. J., et al. (2008). Functionally significant insulin-like growth factor 1 receptor mutations in centenarians. *Proceedings of the National Academy of Sciences of the United States of America, 105*(9), 3438-3442.

Surwit, R. S., Feinglos, M. N., Rodin, J., Sutherland, A., Petro, A. E., Opara, E. C., et al. (1995). Differential effects of fat and sucrose on the development of obesity and diabetes in C57BL/6J and A/J mice. *Metabolism: Clinical and Experimental, 44*(5), 645-651.

Surwit, R. S., Kuhn, C. M., Cochrane, C., McCubbin, J. A., & Feinglos, M. N. (1988). Diet-induced type II diabetes in C57BL/6J mice. *Diabetes, 37*(9), 1163-1167.

Tai, E. S., Lau, T. N., Ho, S. C., Fok, A. C., & Tan, C. E. (2000). Body fat distribution and cardiovascular risk in normal weight women. Associations with insulin resistance, lipids and plasma leptin. *International Journal of Obesity and Related Metabolic Disorders, 24*(6), 751-757.

Takano, A., Haruta, T., Iwata, M., Usui, I., Uno, T., Kawahara, J., et al. (2001). Growth hormone induces cellular insulin resistance by uncoupling phosphatidylinositol 3-kinase and its downstream signals in 3T3-L1 adipocytes. *Diabetes, 50*(8), 1891-1900.

Tan, G. D., Goossens, G. H., Humphreys, S. M., Vidal, H., & Karpe, F. (2004). Upper and lower body adipose tissue function: A direct comparison of fat mobilization in humans. *Obesity Research, 12*(1), 114-118.

Tang, B., Jeoung, D. I., & Sonenberg, M. (1995). Effect of human growth hormone and insulin on [3H]thymidine incorporation, cell cycle progression, and cyclin D expression in 3T3-F442A preadipose cells. *Endocrinology, 136*(7), 3062-3069.

Tannenbaum, G. S. (1991). Neuroendocrine control of growth hormone secretion. *Acta Paediatrica Scandinavica* (Suppl), *372*, 5-16.

Tannenbaum, G. S., & Ling, N. (1984). The interrelationship of growth hormone (GH)-releasing factor and somatostatin in generation of the ultradian rhythm of GH secretion. *Endocrinology, 115*(5), 1952-1957.

Tannenbaum, G. S., & Martin, J. B. (1976). Evidence for an endogenous ultradian rhythm governing growth hormone secretion in the rat. *Endocrinology, 98*(3), 562-570.

Tartaglia, L. A., Dembski, M., Weng, X., Deng, N., Culpepper, J., Devos, R., et al. (1995). Identification and expression cloning of a leptin receptor, OB-R. *Cell, 83*(7), 1263-1271.

Thompson, R. G., Rodriguez, A., Kowarski, A., & Blizzard, R. M. (1972). Growth hormone: Metabolic clearance rates, integrated concentrations, and production rates in normal adults and the effect of prednisone. *Journal of Clinical Investigation, 51*(12), 3193-3199.

Tiong, T. S., & Herington, A. C. (1991). Tissue distribution, characterization, and regulation of messenger ribonucleic acid for growth hormone receptor and serum binding protein in the rat. *Endocrinology, 129*(3), 1628-1634.

Tomii, R., Kurome, M., Ochiai, T., Wako, N., Ueda, H., Hirakawa, K., et al. (2005). Production of cloned pigs by nuclear transfer of preadipocytes established from adult mature adipocytes. *Cloning Stem Cells, 7*(4), 279-288.

Tominaga, S., Morikawa, M., & Osumi, T. (2002). Growth hormone has dual stage-specific effects on the differentiation of 3T3-L1 preadipocytes. *Journal of Biochemistry, 132*(6), 881-889.

Tordjman, J., Guerre-Millo, M., & Clement, K. (2008). Adipose tissue inflammation and liver pathology in human obesity. *Diabetes and Metabolism, 34*(6 Pt 2), 658-663.

Trainer, P. J., Drake, W. M., Katznelson, L., Freda, P. U., Herman-Bonert, V., van der Lely, A. J., et al. (2000). Treatment of acromegaly with the growth hormone-receptor antagonist pegvisomant. *New England Journal of Medicine, 342*(16), 1171-1177.

Tran, T. T., Yamamoto, Y., Gesta, S., & Kahn, C. R. (2008). Beneficial effects of subcutaneous fat transplantation on metabolism. *Cell Metabolism, 7*(5), 410-420.

Truong, M. T., Erasmus, J. J., Munden, R. F., Marom, E. M., Sabloff, B. S., Gladish, G. W., et al. (2004). Focal FDG uptake in mediastinal brown fat mimicking malignancy: A potential pitfall resolved on PET/CT. *American Journal of Roentgenology, 183*(4), 1127-1132.

Tschritter, O., Fritsche, A., Thamer, C., Haap, M., Shirkavand, F., Rahe, S., et al. (2003). Plasma adiponectin concentrations predict insulin sensitivity of both glucose and lipid metabolism. *Diabetes, 52*(2), 239-243.

Ueki, K., Algenstaedt, P., Mauvais-Jarvis, F., & Kahn, C. R. (2000). Positive and negative regulation of phosphoinositide 3-kinase-dependent signaling pathways by three different gene products of the p85alpha regulatory subunit. *Molecular and Cellular Biology, 20*(21), 8035-8046.

Ullrich, A., Gray, A., Tam, A. W., Yang-Feng, T., Tsubokawa, M., Collins, C., et al. (1986). Insulin-like growth factor 1 receptor primary structure: Comparison with insulin receptor suggests structural determinants that define functional specificity. *European Molecular Biology Organization Journal, 5*(10), 2503-2512.

Unger, R. H., & Orci, L. (2002). Lipoapoptosis: Its mechanism and its diseases. *Biochimica et Biophysica Acta, 1585*(2-3), 202-212.

Vahl, N., Jorgensen, J. O., Jurik, A. G., & Christiansen, J. S. (1996). Abdominal adiposity and physical fitness are major determinants of the age associated decline in stimulated GH secretion in healthy adults. *Journal of Clinical Endocrinology and Metabolism, 81*(6), 2209-2215.

Valera, A., Rodriguez-Gil, J. E., Yun, J. S., McGrane, M. M., Hanson, R. W., & Bosch, F. (1993). Glucose metabolism in transgenic mice containing a chimeric P-enolpyruvate carboxykinase/bovine growth hormone gene. *Federation of American Societies for Experimental Biology Journal, 7*(9), 791-800.

van der Lely, A. J., Muller, A., Janssen, J. A., Davis, R. J., Zib, K. A., Scarlett, J. A., et al. (2001). Control of tumor size and disease activity during cotreatment with octreotide and the growth hormone receptor antagonist pegvisomant in an acromegalic patient. *Journal of Clinical Endocrinology and Metabolism, 86*(2), 478-481.

Van Harmelen, V., Reynisdottir, S., Eriksson, P., Thorne, A., Hoffstedt, J., Lonnqvist, F., et al. (1998). Leptin secretion from subcutaneous and visceral adipose tissue in women. *Diabetes, 47*(6), 913-917.

Van Heek, M., Compton, D. S., France, C. F., Tedesco, R. P., Fawzi, A. B., Graziano, M. P., et al. (1997). Diet-induced obese mice develop peripheral, but not central, resistance to leptin. *Journal of Clinical Investigation, 99*(3), 385-390.

Van Vliet, G., Bosson, D., Craen, M., Du Caju, M. V., Malvaux, P., & Vanderschueren-Lodeweyckx, M. (1987). Comparative study of the lipolytic potencies of pituitary-derived and biosynthetic human growth hormone in hypopituitary children. *Journal of Clinical Endocrinology and Metabolism, 65*(5), 876-879.

Vance, M. L., & Harris, A. G. (1991). Long-term treatment of 189 acromegalic patients with the somatostatin analog octreotide: Results of the International Multicenter Acromegaly Study Group. *Archives of Internal Medicine, 151*(8), 1573-1578.

Vassaux, G., Negrel, R., Ailhaud, G., & Gaillard, D. (1994). Proliferation and differentiation of rat adipose precursor cells in chemically defined medium: Differential action of anti-adipogenic agents. *Journal of Cellular Physiology, 161*(2), 249-256.

Vazquez-Vela, M. E., Torres, N., & Tovar, A. R. (2008). White adipose tissue as endocrine organ and its role in obesity. *Archives of Medical Research, 39*(8), 715-728.

Verhelst, J., & Abs, R. (2009). Cardiovascular risk factors in hypopituitary GH-deficient adults. *European Journal of Endocrinology / European Federation of Endocrine Societies, 161* (Suppl 1), S41-49.

Vestergaard, P., Hermann, A. P., Orskov, H., & Mosekilde, L. (1999). Effect of sex hormone replacement on the insulin-like growth factor system and bone mineral: A cross-sectional and longitudinal study in 595 perimenopausal women participating in the Danish Osteoporosis Prevention Study. *Journal of Clinical Endocrinology and Metabolism, 84*(7), 2286-2290.

Vettor, R., De Pergola, G., Pagano, C., Englaro, P., Laudadio, E., Giorgino, F., et al. (1997). Gender differences in serum leptin in obese people: Relationships with testosterone, body fat distribution and insulin sensitivity. *European Journal of Clinical Investigation, 27*(12), 1016-1024.

Villafuerte, B. C., Fine, J. B., Bai, Y., Zhao, W., Fleming, S., & DiGirolamo, M. (2000). Expressions of leptin and insulin-like growth factor-1 are highly correlated and region-specific in adipose tissue of growing rats. *Obesity Research, 8*(9), 646-655.

Wabitsch, M., Hauner, H., Heinze, E., & Teller, W. M. (1995). The role of growth hormone/insulin-like growth factors in adipocyte differentiation. *Metabolism: Clinical and Experimental, 44*(10 Suppl 4), 45-49.

Wabitsch, M., Heinze, E., Hauner, H., Shymko, R. M., Teller, W. M., De Meyts, P., et al. (1996). Biological effects of human growth hormone in rat adipocyte precursor cells and newly differentiated adipocytes in primary culture. *Metabolism: Clinical and Experimental, 45*(1), 34-42.

Wahlander, H., Isgaard, J., Jennische, E., & Friberg, P. (1992). Left ventricular insulin-like growth factor 1 increases in early renal hypertension. *Hypertension, 19*(1), 25-32.

Waine, H., Bennet, G.A., & Bauer, W. (1945). Joint disease associated with acromegaly. *The American Journal of the Medical Sciences, 209*, 671-678.

Wang, P., Mariman, E., Renes, J., & Keijer, J. (2008). The secretory function of adipocytes in the physiology of white adipose tissue. *Journal of Cellular Physiology, 216*(1), 3-13.

Weisberg, S. P., McCann, D., Desai, M., Rosenbaum, M., Leibel, R. L., & Ferrante, A. W., Jr. (2003). Obesity is associated with macrophage accumulation in adipose tissue. *Journal of Clinical Investigation, 112*(12), 1796-1808.

Weissberger, A. J., Ho, K. K., & Lazarus, L. (1991). Contrasting effects of oral and transdermal routes of estrogen replacement therapy on 24-hour growth hormone (GH) secretion, insulin-like growth factor 1, and GH-binding protein in postmenopausal women. *Journal of Clinical Endocrinology and Metabolism, 72*(2), 374-381.

Werther, G. A., Haynes, K., & Waters, M. J. (1993). Growth hormone (GH) receptors are expressed on human fetal mesenchymal tissues–Identification of messenger

ribonucleic acid and GH-binding protein. *Journal of Clinical Endocrinology and Metabolism, 76*(6), 1638-1646.

Weyer, C., Foley, J. E., Bogardus, C., Tataranni, P. A., & Pratley, R. E. (2000). Enlarged subcutaneous abdominal adipocyte size, but not obesity itself, predicts type II diabetes independent of insulin resistance. *Diabetologia, 43*(12), 1498-1506.

Wiesenthal, S. R., Sandhu, H., McCall, R. H., Tchipashvili, V., Yoshii, H., Polonsky, K., et al. (1999). Free fatty acids impair hepatic insulin extraction in vivo. *Diabetes, 48*(4), 766-774.

Wolf, E., Kahnt, E., Ehrlein, J., Hermanns, W., Brem, G., & Wanke, R. (1993). Effects of long-term elevated serum levels of growth hormone on life expectancy of mice: Lessons from transgenic animal models. *Mechanisms of Ageing and Development, 68*(1-3), 71-87.

Wolverton, C. K., Azain, M. J., Duffy, J. Y., White, M. E., & Ramsay, T. G. (1992). Influence of somatotropin on lipid metabolism and IGF gene expression in porcine adipose tissue. *American Journal of Physiology, 263*(4 Pt 1), E637-645.

World Health Organization. (2006). Obesity and overweight. Retrieved October 20, 2010, from http://www.who.int/mediacentre/factsheets/fs311/en/

Yakar, S., Liu, J. L., & Le Roith, D. (2000). The growth hormone/insulin-like growth factor-I system: implications for organ growth and development. *Pediatric Nephrology, 14*(7), 544-549.

Yakar, S., Liu, J. L., Stannard, B., Butler, A., Accili, D., Sauer, B., et al. (1999). Normal growth and development in the absence of hepatic insulin-like growth factor 1.

Proceedings of the National Academy of Sciences of the United States of America, 96(13), 7324-7329.

Yakar, S., Setser, J., Zhao, H., Stannard, B., Haluzik, M., Glatt, V., et al. (2004). Inhibition of growth hormone action improves insulin sensitivity in liver IGF-1-deficient mice. *Journal of Clinical Investigation, 113*(1), 96-105.

Yamauchi, T., Kamon, J., Minokoshi, Y., Ito, Y., Waki, H., Uchida, S., et al. (2002). Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nature Medicine, 8*(11), 1288-1295.

Yamauchi, T., Kamon, J., Waki, H., Terauchi, Y., Kubota, N., Hara, K., et al. (2001). The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nature Medicine, 7*(8), 941-946.

Yarwood, S. J., Kilgour, E., & Anderson, N. G. (1998). Cyclic AMP potentiates growth hormone-dependent differentiation of 3T3-F442A preadipocytes: Possible involvement of the transcription factor CREB. *Molecular and Cellular Endocrinology, 138*(1-2), 41-50.

Yoshizato, H., Fujikawa, T., Soya, H., Tanaka, M., & Nakashima, K. (1998). The growth hormone (GH) gene is expressed in the lateral hypothalamus: Enhancement by GH-releasing hormone and repression by restraint stress. *Endocrinology, 139*(5), 2545-2551.

Zapf, J., Hauri, C., Waldvogel, M., & Froesch, E. R. (1986). Acute metabolic effects and half-lives of intravenously administered insulin like growth factors 1 and 2 in

normal and hypophysectomized rats. *Journal of Clinical Investigation, 77*(6), 1768-1775.

Zenobi, P. D., Graf, S., Ursprung, H., & Froesch, E. R. (1992). Effects of insulin-like growth factor-1 on glucose tolerance, insulin levels, and insulin secretion. *Journal of Clinical Investigation, 89*(6), 1908-1913.

Zezulak, K. M., & Green, H. (1986). The generation of insulin-like growth factor-1–Sensitive cells by growth hormone action. *Science, 233*(4763), 551-553.

Zhang, Y., Proenca, R., Maffei, M., Barone, M., Leopold, L., & Friedman, J. M. (1994). Positional cloning of the mouse obese gene and its human homologue. *Nature, 372*(6505), 425-432.

Zhou, Y., Xu, B. C., Maheshwari, H. G., He, L., Reed, M., Lozykowski, M., et al. (1997). A mammalian model for Laron syndrome produced by targeted disruption of the mouse growth hormone receptor/binding protein gene (the Laron mouse). *Proceedings of the National Academy of Sciences of the United States of America, 94*(24), 13215-13220.

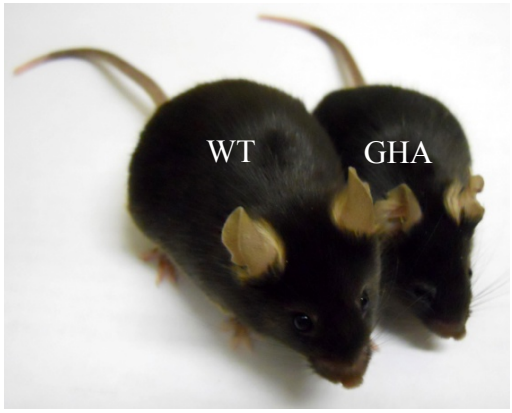
Zou, L., Menon, R. K., & Sperling, M. A. (1997). Induction of mRNAs for the growth hormone receptor gene during mouse 3T3-L1 preadipocyte differentiation. *Metabolism: Clinical and Experimental, 46*(1), 114-118.

Zuk, P. A., Zhu, M., Ashjian, P., De Ugarte, D. A., Huang, J. I., Mizuno, H., et al. (2002). Human adipose tissue is a source of multipotent stem cells. *Molecular Biology of the Cell, 13*(12), 4279-4295.

APPENDIX A: GHA AND WT MICE IN 11 WEEKS OF FEEDING STUDY

MALES

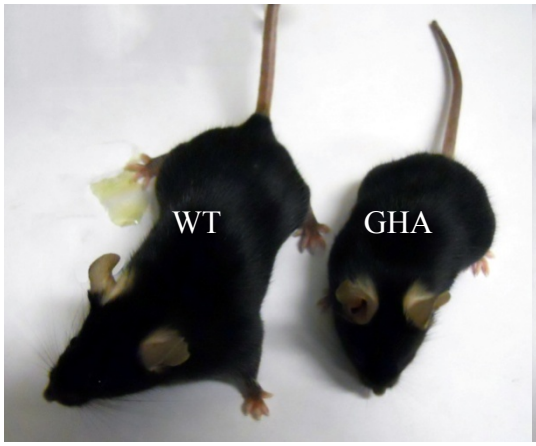
LF WT and GHA



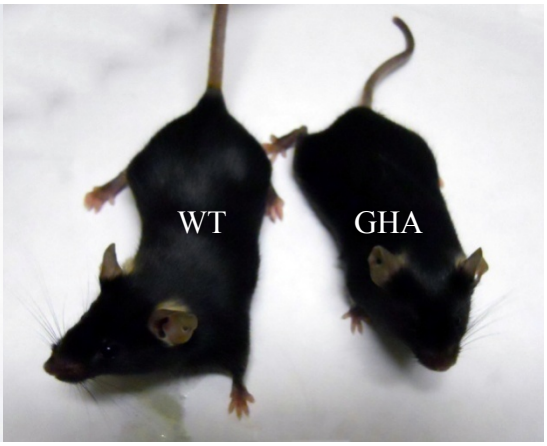
HF WT and GHA

FEMALES

LF WT and GHA



HF WT and GHA



APPENDIX B: THREE-WAY REPEATED ANOVA RESULTS OF BODY WEIGHT IN GHA AND WT MICE

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	500385.815	1	500385.815	5471.720	.000000
Gender	13945.018	1	13945.018	152.489	**.000000**
Genotype	12378.612	1	12378.612	135.360	**.000000**
Diet	4349.663	1	4349.663	47.564	**.000000**
Gender * Genotype	203.778	1	203.778	2.228	.139642
Gender * Diet	532.601	1	532.601	5.824	**.018218**
Genotype * Diet	52.509	1	52.509	.574	.450942
Gender * Genotype * Diet	59.622	1	59.622	.652	.421932
Error	6950.159	76	91.449		

Note. Bold values represent significant difference.

APENDIX C: THREE-WAY ANOVA RESULTS OF BODY WEIGHT IN GHA AND WT MICE

Weeks	Gender	Genotype	Diet	Gender x Genotype	Gender x Diet	Genotype x Diet	Gender x Genotype x Diet
10	$F(1,76) = 329.84$, **$p < .001$**	$F(1,76) = 1008.46$, **$p < .001$**	$F(1,76) = .09$, $p > .05$	$F(1,76) = 24.76$, **$p < .001$**	$F(1,76) = .22$, $p > .05$	$F(1,76) = .35$, $p > .05$	$F(1,76) = .56$, $p > .05$
11	$F(1,76) = 227.39$, **$p < .001$**	$F(1,76) = 334.83$, **$p < .001$**	$F(1,76) = 17.29$, **$p < .001$**	$F(1,76) = 13.37$, **$p < .001$**	$F(1,76) = 1.92$, $p > .05$	$F(1,76) = .06$, $p > .05$	$F(1,76) = .10$, $p > .05$
12	$F(1,76) = 184.64$, **$p < .001$**	$F(1,76) = 203.34$, **$p < .001$**	$F(1,76) = 22.99$, **$p < .001$**	$F(1,76) = 7.25$, **$p < .05$**	$F(1,76) = 5.00$, **$p < .05$**	$F(1,76) = .00$, $p > .05$	$F(1,76) = .34$, $p > .05$
13	$F(1,76) = 165.86$, **$p < .001$**	$F(1,76) = 154.70$, **$p < .001$**	$F(1,76) = 26.82$, **$p < .001$**	$F(1,76) = 2.53$, $p > .05$	$F(1,76) = 5.68$, **$p < .05$**	$F(1,76) = .08$, $p > .05$	$F(1,76) = .47$, $p > .05$
14	$F(1,76) = 151.45$, **$p < .001$**	$F(1,76) = 139.85$, **$p < .001$**	$F(1,76) = 31.78$, **$p < .001$**	$F(1,76) = 2.07$, $p > .05$	$F(1,76) = 4.78$, **$p < .05$**	$F(1,76) = .22$, $p > .05$	$F(1,76) = 1.45$, $p > .05$
15	$F(1,76) = 127.52$, **$p < .001$**	$F(1,76) = 116.84$, **$p < .001$**	$F(1,76) = 45.47$, **$p < .001$**	$F(1,76) = 1.09$, $p > .05$	$F(1,76) = 6.37$, **$p < .05$**	$F(1,76) = .88$, $p > .05$	$F(1,76) = 1.69$, $p > .05$
16	$F(1,76) = 120.93$, **$p < .001$**	$F(1,76) = 106.21$, **$p < .001$**	$F(1,76) = 45.03$, **$p < .001$**	$F(1,76) = .67$, $p > .05$	$F(1,76) = 4.81$, **$p < .05$**	$F(1,76) = .42$, $p > .05$	$F(1,76) = 1.22$, $p > .05$
17	$F(1,76) =120.83$, **$p < .001$**	$F(1,76) = 83.89$, **$p < .001$**	$F(1,76) = 50.95$, **$p < .001$**	$F(1,76) = .95$, $p > .05$	$F(1,76) = 5.45$, **$p < .05$**	$F(1,76) = .57$, $p > .05$	$F(1,76) = .71$, $p > .05$
18	$F(1,76) = 117.68$, $p < .001$	$F(1,76) = 79.96$, $p < .001$	$F(1,76) = 60.10$, $p < .001$	$F(1,76) = .74$, $p > .05$	$F(1,76) = 5.77$, $p < .05$	$F(1,76) = .96$, $p > .05$	$F(1,76) = .62$, $p > .05$
19	$F(1,76) = 120.12$, **$p < .001$**	$F(1,76) = 73.84$, **$p < .001$**	$F(1,76) = 58.16$, **$p < .001$**	$F(1,76) = 1.31$, $p > .05$	$F(1,76) = 6.51$, **$p < .05$**	$F(1,76) = 1.21$, $p > .05$	$F(1,76) = .54$, $p > .05$
20	$F(1,76) = 115.96$, **$p < .001$**	$F(1,76) = 72.11$, **$p < .001$**	$F(1,76) =63.90$, **$p < .001$**	$F(1,76) = .89$, $p > .05$	$F(1,76) = 6.39$, **$p < .05$**	$F(1,76) = 1.47$, $p > .05$	$F(1,76) = .31$, $p > .05$
21	$F(1,76) = 122.63$, **$p < .001$**	$F(1,76) = 71.43$, **$p < .001$**	$F(1,76) = 67.37$, **$p < .001$**	$F(1,76) = 1.00$, $p > .05$	$F(1,76) = 9.25$, **$p < .05$**	$F(1,76) = .73$, $p > .05$	$F(1,76) = .13$, $p > .05$

Note. Bold values represent significant difference.

APPENDIX D: GENOTYPE COMPARISON: PERCENT VALUES IN BODY WEIGHT AND BODY COMPOSITION OF GHA MICE AS COMPARED TO WT CONTROLS OF THE SAME DIET AND GENDER AT THE BEGINNING AND END OF THE FEEDING STUDY

Genotype Comparison	Body Weight		Absolute Weight						Normalized Weight					
			Fat		Lean		Fluid		Fat		Lean		Fluid	
	10 wk	21 wk	10 wk	21 wk	10 wk	21 wk	10 wk	21 wk	10 wk	21 wk	10 wk	21 wk	10 wk	21 wk
Male LF GHA vs. WT	63%	74%	210%	182%	57%	64%	80%	82%	332%	243%	91%	87%	126%	112%
Female LF GHA vs. WT	67%	75%	171%	190%	61%	65%	72%	82%	252%	244%	91%	87%	101%	109%
Male HF GHA vs. WT	64%	79%	**229%**	**102%**	58%	69%	83%	92%	**356%**	**133%**	91%	87%	130%	117%
Female HF GHA vs. WT	64%	73%	**142%**	**102%**	59%	65%	67%	68%	**217%**	**146%**	92%	88%	105%	96%

Note. Bold values highlight drastic changes between the beginning (10 weeks) and end (21 weeks) of the feeding study.

APPENDIX E: GENDER COMPARISON: PERCENT VALUES IN BODY WEIGHT AND BODY COMPOSITION OF FEMALE VERSUS MALE MICE OF THE SAME DIET AND GENOTYPE AT THE BEGINNING AND END OF THE FEEDING STUDY.

Gender Comparison	Body Weight		Absolute Weight						Percent Weight					
			Fat		Lean		Fluid		Fat		Lean		Fluid	
	10 wk	21wk	10 wk	21 wk	10 wk	21 wk	10 wk	21 wk	10 wk	21 wk	10 wk	21 wk	10 wk	21 wk
WT LF Female vs.Male	76%	73%	**95%**	**60%**	73%	75%	104%	78%	**128%**	**85%**	97%	102%	137%	108%
GHA LF Female vs.Male	**80%**	**41%**	**78%**	**63%**	78%	76%	87%	79%	**97%**	**86%**	98%	102%	109%	105%
WT HF Female vs.Male	78%	68%	**123%**	**49%**	76%	76%	104%	88%	**154%**	**66%**	97%	112%	132%	128%
GHA HF Female vs.Male	79%	63%	**76%**	**49%**	77%	71%	83%	65%	**94%**	**73%**	98%	114%	106%	105%

Note. Bold values highlight drastic changes between the beginning (10 weeks) and end (21 weeks) of the feeding study.

APPENDIX F: THREE-WAY REPEATED ANOVA RESULTS OF FAT MASS IN GHA AND WT MICE

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Intercept	15235.723	1	15235.723	291.118	.000000
Gender	1370.588	1	1370.588	26.189	**.000002**
Genotype	203.931	1	203.931	3.897	.052017
Diet	2681.836	1	2681.836	51.243	**.000000**
Gender * Genotype	26.302	1	26.302	.503	.480546
Gender * Diet	449.523	1	449.523	8.589	**.004464**
Genotype * Diet	62.174	1	62.174	1.188	.279179
Gender * Genotype * Diet	1.323	1	1.323	.025	.874104
Error	3977.480	76	52.335		

Note. Bold values represent significant difference.

APPENDIX G: PAIRED T-TEST RESULTS OF BODY WEIGHT AND BODY COMPOSITION IN GHA AND WT MICE AT WEEK 10 AND 21 OF FEEDING STUDY

	♂ WT LF	♂ GHA LF	♂ WT HF	♂ GHA HF	♀ WT LF	♀ GHA LF	♀ WT HF	♀ GHA HF
BW	***p* < .001**	***p* < .001**	***p* < .001**	***P* < .001**	***p* < .001**	***P* < .001**	***P* < .05**	***p* < .001**
Fat Mass	***p* < .05**	***p* < .05**	***p* < .001**	***p* < .001**	***p* < .001**	***p* < .001**	***p* < .05**	***p* < .05**
Percent Fat Mass	***p* < .05**	***p* < .05**	***p* < .001**	***p* < .001**	***p* < .001**	***p* < .001**	***p* < .05**	***p* < .001**
Lean Mass	***p* < .001**	***p* < .001**	***p* < .001**	***p* < .001**	***p* < .001**	***p* < .001**	***p* < .001**	***p* < .001**
Percent Lean Mass	***p* < .05**	***p* < .05**	***p* < .001**	***p* < .001**	$p > .05$	***p* < .05**	***p* < .05**	***p* < .05**
Fluid Mass	***p* < .05**	***p* < .001**	***p* < .001**	***p* < .001**	$p > .05$	***p* < .05**	$p > .05$	***p* < .001**
Percent Fluid Mass	$p > .05$	$p > .05$	***p* < .05**	***p* < .001**	***p* < .05**	$p > .05$	***p* < .001**	***p* < .001**

Note. Bold values represent significant difference.

APPENDIX H: THREE-WAY ANOVA RESULTS OF FAT MASS IN GHA AND WT MICE

Weeks	Gender	Genotype	Diet	Gender x Genotype	Gender x Diet	Genotype x Diet	Gender x Genotype x Diet
10	$F(1,76) = 2.59$, $p > .05$	$F(1,76) = 44.27$, **$p < .001$**	$F(1,76) = .02$, $p > .05$	$F(1,76) = 4.87$, **$p < .05$**	$F(1,76) = .18$, $p > .05$	$F(1,76) = .11$, $p > .05$	$F(1,76) = .27$, $p > .05$
11	$F(1,76) = 20.91$, **$p < .001$**	$F(1,76) = 29.08$, **$p < .001$**	$F(1,76) = 37.05$, **$p < .001$**	$F(1,76) = .41$, $P > .05$	$F(1,76) = 3.84$, $p > .05$	$F(1,76) = .04$, $p > .05$	$F(1,76) = .19$, $p > .05$
12	$F(1,76) = 22.62$, **$p < .001$**	$F(1,76) = 11.90$, **$p < .001$**	$F(1,76) = 27.64$, **$p < .001$**	$F(1,76) = .45$, $p > .05$	$F(1,76) = 6.78$, **$p < .05$**	$F(1,76) = .11$, $p > .05$	$F(1,76) = .01$, $p > .05$
13	$F(1,76) = 22.86$, **$p < .001$**	$F(1,76) = 5.81$, **$p < .05$**	$F(1,76) = 26.92$, **$p < .001$**	$F(1,76) = .94$, $p > .05$	$F(1,76) = 8.81$, **$p < .05$**	$F(1,76) = .46$, $p > .05$	$F(1,76) = .00$, $p > .05$
14	$F(1,76) = 21.03$, **$p < .001$**	$F(1,76) = 4.21$, **$p < .05$**	$F(1,76) = 28.15$, **$p < .001$**	$F(1,76) = .78$, $p > .05$	$F(1,76) = 7.81$, **$p < .05$**	$F(1,76) = .54$, $p > .05$	$F(1,76) = .11$, $p > .05$
15	$F(1,76) = 20.67$, **$p < .001$**	$F(1,76) = 2.61$, $p > .05$	$F(1,76) = 40.74$, **$p < .001$**	$F(1,76) = .67$, $p > .05$	$F(1,76) = 9.33$, **$p < .05$**	$F(1,76) = 1.22$, $p > .05$	$F(1,76) = .21$, $p > .05$
16	$F(1,76) = 20.38$, **$p < .001$**	$F(1,76) = 2.21$, $p > .05$	$F(1,76) = 43.21$, **$p < .001$**	$F(1,76) = .94$, $p > .05$	$F(1,76) = 7.46$, **$p < .05$**	$F(1,76) = .93$, $p > .05$	$F(1,76) = .06$, $p > .05$
17	$F(1,76) = 23.26$, **$p < .001$**	$F(1,76) = 2.31$, $p > .05$	$F(1,76) = 50.35$, **$p < .001$**	$F(1,76) = .60$, $p > .05$	$F(1,76) = 7.32$, **$p < .05$**	$F(1,76) = 1.12$, $p > .05$	$F(1,76) = .04$, $p > .05$
18	$F(1,76) = 25.26$, **$p < .001$**	$F(1,76) = 1.54$, $p > .05$	$F(1,76) = 58.08$, **$p < .001$**	$F(1,76) = .42$, $p > .05$	$F(1,76) = 7.66$, **$p < .05$**	$F(1,76) = 1.73$, $p > .05$	$F(1,76) = .06$, $p > .05$
19	$F(1,76) = 29.42$, **$p < .001$**	$F(1,76) = 1.73$, $p > .05$	$F(1,76) = 61.32$, **$p < .001$**	$F(1,76) = .12$, $p > .05$	$F(1,76) = 8.91$, **$p < .05$**	$F(1,76) = 1.98$, $p > .05$	$F(1,76) = .05$, $p > .05$
20	$F(1,76) = 30.11$, **$p < .001$**	$F(1,76) = 1.71$, $p > .05$	$F(1,76) = 68.27$, **$p < .001$**	$F(1,76) = .20$, $p > .05$	$F(1,76) = 8.43$, **$p < .05$**	$F(1,76) = 2.64$, $p > .05$	$F(1,76) = .02$, $p > .05$
21	$F(1,76) = 30.80$, **$p < .001$**	$F(1,76) = 2.02$, $p > .05$	$F(1,76) = 76.21$, **$p < .001$**	$F(1,76) = .09$, $p > .05$	$F(1,76) = 12.06$, **$p < .001$**	$F(1,76) = 1.47$, $p > .05$	$F(1,76) = .06$, $p > .05$

Note. Bold values represent significant difference.

APPENDIX I: THREE-WAY REPEATED ANOVA RESULTS OF PERCENT FAT MASS IN GHA AND WT MICE

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	257434.531	1	257434.531	554.994	.000000
Gender	4866.371	1	4866.371	10.491	**.001780**
Genotype	21273.551	1	21273.551	45.863	**.000000**
Diet	20898.821	1	20898.821	45.055	**.000000**
Gender * Genotype	369.725	1	369.725	.797	.374787
Gender * Diet	1994.087	1	1994.087	4.299	**.041524**
Genotype * Diet	223.955	1	223.955	.483	.489268
Gender * Genotype * Diet	58.307	1	58.307	.126	.723912
Error	35252.698	76	463.851		

Note. Bold values represent significant difference.

APPENDIX J: THREE-WAY ANOVA RESULTS OF PERCENT FAT MASS IN GHA AND WT MICE

Weeks	Gender	Genotype	Diet	Gender x Genotype	Gender x Diet	Genotype x Diet	Gender x Genotype x Diet
10	$F(1,76) = .47$, $p > .05$	$F(1,76) = 142.83$, **$p < .001$**	$F(1,76) = .10$, $p > .05$	$F(1,76) = 2.44$, $p > .05$	$F(1,76) = .03$, $p > .05$	$F(1,76) = .18$, $p > .05$	$F(1,76) = .21$, $p > .05$
11	$F(1,76) = 4.83$, **$p < .05$**	$F(1,76) = 130.54$, **$p < .001$**	$F(1,76) = 39.89$, **$p < .001$**	$F(1,76) = .39$, $P > .05$	$F(1,76) = 1.12$, $p > .05$	$F(1,76) = .86$, $p > .05$	$F(1,76) = .18$, $p > .05$
12	$F(1,76) = 8.70$, **$p < .05$**	$F(1,76) = 74.65$, **$p < .001$**	$F(1,76) = 27.10$, **$p < .001$**	$F(1,76) = .88$, $p > .05$	$F(1,76) = 3.94$, $p > .05$	$F(1,76) = .00$, $p > .05$	$F(1,76) = .14$, $p > .05$
13	$F(1,76) = 8.725$, **$p < .05$**	$F(1,76) = 47.408$, **$p < .001$**	$F(1,76) = 21.813$, **$p < .001$**	$F(1,76) = 1.715$, $p > .05$	$F(1,76) = 6.286$, **$p < .05$**	$F(1,76) = .275$, $p > .05$	$F(1,76) = .145$, $p > .05$
14	$F(1,76) = 8.61$, **$p < .05$**	$F(1,76) = 38.64$, **$p < .001$**	$F(1,76) = 22.72$, **$p < .001$**	$F(1,76) = 1.29$, $p > .05$	$F(1,76) = 5.55$, **$p < .05$**	$F(1,76) = .31$, $p > .05$	$F(1,76) = .33$, $p > .05$
15	$F(1,76) = 8.56$, **$p < .001$**	$F(1,76) = 32.89$, **$p < .001$**	$F(1,76) = 35.25$, **$p < .001$**	$F(1,76) = .90$, $p > .05$	$F(1,76) = 6.25$, **$p < .05$**	$F(1,76) = .69$, $p < .05$	$F(1,76) = .50$, $p > .05$
16	$F(1,76) = 9.41$, **$p < .05$**	$F(1,76) = 29.88$, **$p < .001$**	$F(1,76) = 40.24$, **$p < .001$**	$F(1,76) = 1.30$, $p > .05$	$F(1,76) = 4.51$, **$p < .05$**	$F(1,76) = .35$, $p > .05$	$F(1,76) = .10$, $p > .05$
17	$F(1,76) = 11.30$, **$p < .05$**	$F(1,76) = 30.32$, **$p < .001$**	$F(1,76) = 48.30$, **$p < .001$**	$F(1,76) = .97$, $p > .05$	$F(1,76) = 3.55$, $p > .05$	$F(1,76) = .58$, $p > .05$	$F(1,76) = .12$, $p > .05$
18	$F(1,76) = 12.45$, **$p < .001$**	$F(1,76) = 28.53$, **$p < .001$**	$F(1,76) = 56.91$, **$p < .001$**	$F(1,76) = .74$, $p > .05$	$F(1,76) = 3.43$, $p > .05$	$F(1,76) = 1.02$, $p > .05$	$F(1,76) = .23$, $p > .05$
19	$F(1,76) = 13.81$, **$p < .001$**	$F(1,76) = 32.16$, **$p < .001$**	$F(1,76) = 57.89$, **$p < .001$**	$F(1,76) = .19$, $p > .05$	$F(1,76) = 3.86$, $p > .05$	$F(1,76) = 1.12$, $p > .05$	$F(1,76) = .20$, $p > .05$
20	$F(1,76) = 13.77$, **$p < .001$**	$F(1,76) = 33.60$, **$p < .001$**	$F(1,76) = 63.15$, **$p < .001$**	$F(1,76) = .29$, $p > .05$	$F(1,76) = 3.30$, $p > .05$	$F(1,76) = 1.62$, $p > .05$	$F(1,76) = .13$, $p > .05$
21	$F(1,76) = 12.22$, **$p < .001$**	$F(1,76) = 34.69$, **$p < .001$**	$F(1,76) = 73.76$, **$p < .001$**	$F(1,76) = .11$, $p > .05$	$F(1,76) = 5.23$, **$p < .05$**	$F(1,76) = .40$, $p > .05$	$F(1,76) = .03$, $p > .05$

Note. Bold values represent significant difference.

APPENDIX K: THREE-WAY REPEATED ANOVA RESULTS OF LEAN MASS IN GHA AND WT MICE

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	267425.057	1	267425.057	24411.237	.000000
Gender	5476.042	1	5476.042	499.867	**.000000**
Genotype	12736.856	1	12736.856	1162.652	**.000000**
Diet	238.287	1	238.287	21.751	**.000013**
Gender * Genotype	271.091	1	271.091	24.746	**.000004**
Gender * Diet	5.509	1	5.509	.503	.480397
Genotype * Diet	.420	1	.420	.038	.845266
Gender * Genotype * Diet	41.626	1	41.626	3.800	.054952
Error	832.580	76	10.955		

Note. Bold values represent significant difference.

APPENDIX L: THREE-WAY ANOVA RESULTS OF LEAN MASS IN GHA AND WT MICE

Weeks	Gender	Genotype	Diet	Gender x Genotype	Gender x Diet	Genotype x Diet	Gender x Genotype x Diet
10	$F(1,76) = 503.16$, **$p < .001$**	$F(1,76) = 1770.13$, **$p < .001$**	$F(1,76) = .54$, $p > .05$	$F(1,76) = 53.23$, **$p < .05$**	$F(1,76) = .14$, $p > .05$	$F(1,76) = .31$, $p > .05$	$F(1,76) = .67$, $p > .05$
11	$F(1,76) = 391.36$, **$p < .001$**	$F(1,76) = 1090.11$, **$p < .001$**	$F(1,76) = 1.70$, $p > .05$	$F(1,76) = 36.54$, **$P < .001$**	$F(1,76) = .11$, $p > .05$	$F(1,76) = .65$, $p > .05$	$F(1,76) = 1.18$, $p > .05$
12	$F(1,76) = 403.57$, **$p < .001$**	$F(1,76) = 1052.71$, **$p < .001$**	$F(1,76) = 3.89$, $p > .05$	$F(1,76) = 32.48$, **$p < .001$**	$F(1,76) = .79$, $p > .05$	$F(1,76) = .68$, $p > .05$	$F(1,76) = 1.72$, $p > .05$
13	$F(1,76) = 465.84$, **$p < .001$**	$F(1,76) = 1094.89$, **$p < .001$**	$F(1,76) = 14.93$, **$p < .001$**	$F(1,76) = 27.61$, **$p < .001$**	$F(1,76) = .08$, $p > .05$	$F(1,76) = .71$, $p > .05$	$F(1,76) = 3.52$, $p > .05$
14	$F(1,76) = 464.66$, **$p < .001$**	$F(1,76) = 1059.56$, **$p < .001$**	$F(1,76) = 16.57$, **$p < .001$**	$F(1,76) = 23.58$, **$p < .001$**	$F(1,76) = .03$, $p > .05$	$F(1,76) = .104$, $p > .05$	$F(1,76) = 5.80$, **$p < .05$**
15	$F(1,76) = 442.14$, **$p < .001$**	$F(1,76) = 1067.42$, **$p < .001$**	$F(1,76) = 30.52$, **$p < .001$**	$F(1,76) = 17.72$, **$p < .001$**	$F(1,76) = 1.08$, $p > .05$	$F(1,76) = .01$, $p > .05$	$F(1,76) = 8.76$, $p > .05$
16	$F(1,76) = 455.18$, **$p < .001$**	$F(1,76) = 1032.15$, **$p < .001$**	$F(1,76) = 29.43$, **$p < .001$**	$F(1,76) = 17.96$, **$p < .001$**	$F(1,76) = .01$, $p > .05$	$F(1,76) = .03$, $p > .05$	$F(1,76) = 6.45$, $p > .05$
17	$F(1,76) = 416.86$, **$p < .001$**	$F(1,76) = 845.16$, **$p < .001$**	$F(1,76) = 32.97$, **$p < .001$**	$F(1,76) = 15.39$, **$p < .001$**	$F(1,76) = .83$, $p > .05$	$F(1,76) = .00$, $p > .05$	$F(1,76) = 3.70$, $p > .05$
18	$F(1,76) = 495.28$, **$p < .001$**	$F(1,76) = 997.51$, **$p < .001$**	$F(1,76) = 38.31$, **$p < .001$**	$F(1,76) = 16.73$, **$p < .001$**	$F(1,76) = .38$, $p > .05$	$F(1,76) = .05$, $p > .05$	$F(1,76) = 3.76$, $p > .05$
19	$F(1,76) = 467.29$, **$p < .001$**	$F(1,76) = 955.25$, **$p < .001$**	$F(1,76) = 38.78$, **$p < .001$**	$F(1,76) = 17.78$, **$p < .001$**	$F(1,76) = 1.61$, $p > .05$	$F(1,76) = .04$, $p > .05$	$F(1,76) = 4.46$, **$p < .05$**
20	$F(1,76) = 388.44$, **$p < .001$**	$F(1,76) = 810.25$, **$p < .001$**	$F(1,76) = 30.83$, **$p < .001$**	$F(1,76) = 12.74$, **$p < .001$**	$F(1,76) = 1.04$, $p > .05$	$F(1,76) = .00$, $p > .05$	$F(1,76) = 2.60$, $p > .05$
21	$F(1,76) = 430.75$, **$p < .001$**	$F(1,76) = 852.98$, **$p < .001$**	$F(1,76) = 24.92$, **$p < .001$**	$F(1,76) = 12.14$, **$p < .001$**	$F(1,76) = 1.95$, $p > .05$	$F(1,76) = .03$, $p > .05$	$F(1,76) = 1.75$, $p > .05$

Note. Bold values represent significant difference.

APPENDIX M: THREE-WAY REPEATED ANOVA RESULTS OF PERCENT LEAN MASS IN GHA AND WT MICE

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	5453689.900	1	5453689.900	16662.113	.000000
Gender	2543.469	1	2543.469	7.771	**.006704**
Genotype	19374.160	1	19374.160	59.192	**.000000**
Diet	13760.877	1	13760.877	42.042	**.000000**
Gender * Genotype	119.596	1	119.596	.365	.547328
Gender * Diet	1472.833	1	1472.833	4.500	**.037159**
Genotype * Diet	253.090	1	253.090	.773	.381988
Gender * Genotype * Diet	13.724	1	13.724	.042	.838304
Error	24875.623	76	327.311		

Note. Bold values represent significant difference.

APPENDIX N: THREE-WAY ANOVA RESULTS OF PERCENT LEAN MASS IN GHA AND WT MICE

Weeks	Gender	Genotype	Diet	Gender x Genotype	Gender x Diet	Genotype x Diet	Gender x Genotype x Diet
10	$F(1,76) = 18.99$, **$p < .001$**	$F(1,76) = 189.21$, **$p < .001$**	$F(1,76) = .81$, $p > .05$	$F(1,76) = .80$, $p > .05$	$F(1,76) = .00$, $p > .05$	$F(1,76) = .14$, $p > .05$	$F(1,76) = .00$, $p > .05$
11	$F(1,76) = .81$, $p > .05$	$F(1,76) = 179.17$, **$p < .001$**	$F(1,76) = 38.12$, **$p < .001$**	$F(1,76) = .02$, $p > .05$	$F(1,76) = 1.81$, $p > .05$	$F(1,76) = .01$, $p > .05$	$F(1,76) = .44$, $p > .05$
12	$F(1,76) = 8.18$, **$p < .05$**	$F(1,76) = 100.27$, **$p < .001$**	$F(1,76) = 35.46$, **$p < .001$**	$F(1,76) = .19$, $p > .05$	$F(1,76) = 4.16$, **$p < .05$**	$F(1,76) = .33$, $p > .05$	$F(1,76) = .01$, $p > .05$
13	$F(1,76) = 9.86$, **$p < .05$**	$F(1,76) = 54.01$, **$p < .001$**	$F(1,76) = 17.60$, **$p < .001$**	$F(1,76) = 1.62$, $p > .05$	$F(1,76) = 7.87$, **$p < .05$**	$F(1,76) = .77$, $p > .05$	$F(1,76) = .02$, $p > .05$
14	$F(1,76) = 5.49$, **$p < .05$**	$F(1,76) = 57.26$, **$p < .001$**	$F(1,76) = 23.68$, **$p < .001$**	$F(1,76) = .70$, $p > .05$	$F(1,76) = 6.03$, **$p < .05$**	$F(1,76) = .55$, $p > .05$	$F(1,76) = .20$, $p > .05$
15	$F(1,76) = 8.32$, **$p < .05$**	$F(1,76) = 41.46$, **$p < .001$**	$F(1,76) = 34.47$, **$p < .001$**	$F(1,76) = .54$, $p > .05$	$F(1,76) = 4.69$, **$p < .05$**	$F(1,76) = .99$, $p > .05$	$F(1,76) = .13$, $p > .05$
16	$F(1,76) = 6.67$, **$p < .05$**	$F(1,76) = 36.90$, **$p < .001$**	$F(1,76) = 33.89$, **$p < .001$**	$F(1,76) = .92$, $p > .05$	$F(1,76) = 5.85$, **$p < .05$**	$F(1,76) = .47$, $p > .05$	$F(1,76) = .11$, $p > .05$
17	$F(1,76) = 10.47$, **$p < .05$**	$F(1,76) = 39.89$, **$p < .001$**	$F(1,76) = 42.74$, **$p < .001$**	$F(1,76) = .40$, $p > .05$	$F(1,76) = 3.79$, $p > .05$	$F(1,76) = .83$, $p > .05$	$F(1,76) = .14$, $p > .05$
18	$F(1,76) = 8.90$, **$p < .05$**	$F(1,76) = 34.95$, **$p < .001$**	$F(1,76) = 52.25$, **$p < .001$**	$F(1,76) = .36$, $p > .05$	$F(1,76) = 4.00$, **$p < .05$**	$F(1,76) = .77$, $p > .05$	$F(1,76) = .15$, $p > .05$
19	$F(1,76) = 11.73$, **$p < .001$**	$F(1,76) = 39.61$, **$p < .001$**	$F(1,76) = 48.30$, **$p < .001$**	$F(1,76) = .05$, $p > .05$	$F(1,76) = 3.08$, $p > .05$	$F(1,76) = 1.20$, $p > .05$	$F(1,76) = .04$, $p > .05$
20	$F(1,76) = 12.12$, **$p < .001$**	$F(1,76) = 39.46$, **$p < .001$**	$F(1,76) = 59.69$, **$p < .001$**	$F(1,76) = .10$, $p > .05$	$F(1,76) = 3.18$, $p > .05$	$F(1,76) = 1.77$, $p > .05$	$F(1,76) = .03$, $p > .05$
21	$F(1,76) = 10.72$, **$p < .05$**	$F(1,76) = 43.77$, **$p < .001$**	$F(1,76) = 73.63$, **$p < .001$**	$F(1,76) = .00$, $p > .05$	$F(1,76) = 5.08$, $p < .05$	$F(1,76) = .55$, $p > .05$	$F(1,76) = .00$, $p > .05$

Note. Bold values represent significant difference.

APPENDIX O: THREE-WAY REPEATED ANOVA RESULTS OF FLUID MASS IN GHA AND WT MICE

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	1722.730	1	1722.730	3222.395	.000000
Gender	16.474	1	16.474	30.815	**.000000**
Genotype	21.925	1	21.925	41.012	**.000000**
Diet	4.515	1	4.515	8.446	**.004791**
Gender * Genotype	.040	1	.040	.075	.785509
Gender * Diet	.265	1	.265	.496	.483523
Genotype * Diet	.013	1	.013	.024	.876397
Gender * Genotype * Diet	.484	1	.484	.906	.344214
Error	40.630	76	.535		

Note. Bold values represent significant difference.

APPENDIX P: THREE-WAY ANOVA RESULTS OF FLUID MASS IN GHA AND WT MICE

Weeks	Gender	Genotype	Diet	Gender x Genotype	Gender x Diet	Genotype x Diet	Gender x Genotype x Diet
10	$F(1,76) = 1.78$, $p > .05$	$F(1,76) = 73.87$, **$p < .001$**	$F(1,76) = .00$, $p > .05$	$F(1,76) = 6.88$, **$p < .05$**	$F(1,76) = .10$, $p > .05$	$F(1,76) = .11$, $p > .05$	$F(1,76) = .04$, $p > .05$
11	$F(1,76) = 2.36$, $p > .05$	$F(1,76) = 26.29$, **$p < .001$**	$F(1,76) = 1.14$, $p > .05$	$F(1,76) = .07$, $p > .05$	$F(1,76) = 1.05$, $p > .05$	$F(1,76) = .40$, $p > .05$	$F(1,76) = .09$, $p > .05$
12	$F(1,76) = 10.86$, **$p < .05$**	$F(1,76) = 19.13$, **$p < .001$**	$F(1,76) = 1.58$, $p > .05$	$F(1,76) = .00$, $p > .05$	$F(1,76) = .11$, $p > .05$	$F(1,76) = .06$, $p > .05$	$F(1,76) = .00$, $p > .05$
13	$F(1,76) = 40.46$, **$p < .001$**	$F(1,76) = 26.81$, **$p < .001$**	$F(1,76) = 4.29$, **$p < .05$**	$F(1,76) = .09$, $p > .05$	$F(1,76) = 3.55$, $p > .05$	$F(1,76) = .00$, $p > .05$	$F(1,76) = .10$, $p > .05$
14	$F(1,76) = 15.74$, **$p < .001$**	$F(1,76) = 29.05$, **$p < .001$**	$F(1,76) = 6.44$, **$p < .05$**	$F(1,76) = .06$, $p > .05$	$F(1,76) = .16$, $p > .05$	$F(1,76) = .00$, $p > .05$	$F(1,76) = .73$, $p > .05$
15	$F(1,76) = 26.45$, **$p < .001$**	$F(1,76) = 26.62$, **$p < .001$**	$F(1,76) = 8.79$, **$p < .05$**	$F(1,76) = .01$, $p > .05$	$F(1,76) = .01$, $p > .05$	$F(1,76) = .66$, $p > .05$	$F(1,76) = .43$, $p > .05$
16	$F(1,76) = 20.97$, **$p < .001$**	$F(1,76) = 20.40$, **$p < .001$**	$F(1,76) = 7.00$, **$p < .05$**	$F(1,76) = .23$, $p > .05$	$F(1,76) = .50$, $p > .05$	$F(1,76) = .21$, $p > .05$	$F(1,76) = 1.29$, $p > .05$
17	$F(1,76) = 29.29$, **$p < .001$**	$F(1,76) = 18.83$, **$p < .001$**	$F(1,76) = 8.36$, **$p < .001$**	$F(1,76) = .34$, $p > .05$	$F(1,76) = .43$, $p > .05$	$F(1,76) = .28$, $p > .05$	$F(1,76) = 3.28$, $p > .05$
18	$F(1,76) = 31.31$, **$p < .001$**	$F(1,76) = 22.07$, **$p < .001$**	$F(1,76) = 13.82$, **$p < .001$**	$F(1,76) = .16$, $p > .05$	$F(1,76) = .38$, $p > .05$	$F(1,76) = .02$, $p > .05$	$F(1,76) = .69$, $p > .05$
19	$F(1,76) = 34.80$, **$p < .001$**	$F(1,76) = 39.47$, **$p < .001$**	$F(1,76) = 17.15$, **$p < .001$**	$F(1,76) = .57$, $p > .05$	$F(1,76) = .33$, $p > .05$	$F(1,76) = .17$, $p > .05$	$F(1,76) = 2.26$, $p > .05$
20	$F(1,76) = 24.99$, **$p < .001$**	$F(1,76) = 31.25$, **$p < .001$**	$F(1,76) = 7.50$, **$p < .05$**	$F(1,76) = .03$, $p > .05$	$F(1,76) = .04$, $p > .05$	$F(1,76) = .04$, $p > .05$	$F(1,76) = .39$, $p > .05$
21	$F(1,76) = 35.23$, **$p < .001$**	$F(1,76) = 22.36$, **$p < .001$**	$F(1,76) = 5.21$, **$p < .05$**	$F(1,76) = 1.30$, $p > .05$	$F(1,76) = .34$, $p > .05$	$F(1,76) = .17$, $p > .05$	$F(1,76) = 3.22$, $p > .05$

Note. Bold values represent significant difference.

APPENDIX Q: THREE-WAY REPEATED ANOVA RESULTS OF PERCENT FLUID MASS IN GHA AND WT MICE

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	36239.731	1	36239.731	8951.811	.000000
Gender	164.851	1	164.851	40.721	**.000000**
Genotype	79.463	1	79.463	19.629	**.000031**
Diet	41.041	1	41.041	10.138	**.002106**
Gender * Genotype	2.089	1	2.089	.516	.474713
Gender * Diet	3.297	1	3.297	.814	.369698
Genotype * Diet	.273	1	.273	.068	.795651
Gender * Genotype * Diet	.011	1	.011	.003	.957881
Error	307.672	76	4.048		

Note. Bold values represent significant difference.

APPENDIX S: THREE-WAY ANOVA RESULTS OF PERCENT FLUID MASS IN GHA AND WT MICE

Weeks	Gender	Genotype	Diet	Gender x Genotype	Gender x Diet	Genotype x Diet	Gender x Genotype x Diet
10	$F(1,76) = 37.31$, **$p < .001$**	$F(1,76) = 19.72$, **$p < .001$**	$F(1,76) = .00$, $p > .05$	$F(1,76) = 11.97$, **$p < .001$**	$F(1,76) = .33$, $p > .05$	$F(1,76) = .42$, $p > .05$	$F(1,76) = .01$, $p > .05$
11	$F(1,76) = 30.14$, **$p < .001$**	$F(1,76) = 6.23$, **$p < .05$**	$F(1,76) = .91$, $p > .05$	$F(1,76) = .66$, $p > .05$	$F(1,76) = 1.05$, $p > .05$	$F(1,76) = 1.67$, $p > .05$	$F(1,76) = .23$, $p > .05$
12	$F(1,76) = 12.00$, **$p < .001$**	$F(1,76) = 7.08$, **$p < .05$**	$F(1,76) = 1.00$, $p > .05$	$F(1,76) = .32$, $p > .05$	$F(1,76) = .40$, $p > .05$	$F(1,76) = .22$, $p > .05$	$F(1,76) = .38$, $p > .05$
13	$F(1,76) = 2.27$, $p > .05$	$F(1,76) = 7.34$, **$p < .05$**	$F(1,76) = 1.32$, $p > .05$	$F(1,76) = .03$, $p > .05$	$F(1,76) = .49$, $p > .05$	$F(1,76) = .00$, $p > .05$	$F(1,76) = 1.12$, $p > .05$
14	$F(1,76) = 19.34$, **$p < .001$**	$F(1,76) = 5.37$, **$p < .05$**	$F(1,76) = .32$, $p > .05$	$F(1,76) = .14$, $p > .05$	$F(1,76) = .97$, $p > .05$	$F(1,76) = .01$, $p > .05$	$F(1,76) = .06$, $p > .05$
15	$F(1,76) = 10.20$, **$p < .05$**	$F(1,76) = 9.30$, **$p < .05$**	$F(1,76) = 2.62$, $p > .05$	$F(1,76) = .00$, $p > .05$	$F(1,76) = 5.28$, $p < .05$	$F(1,76) = .49$, $p > .05$	$F(1,76) = .88$, $p > .05$
16	$F(1,76) = 18.12$, **$p < .001$**	$F(1,76) = 14.38$, **$p < .001$**	$F(1,76) = 5.94$, **$p < .05$**	$F(1,76) = .03$, $p > .05$	$F(1,76) = .23$, $p > .05$	$F(1,76) = .32$, $p > .05$	$F(1,76) = .01$, $p > .05$
17	$F(1,76) = 22.03$, **$p < .001$**	$F(1,76) = 19.20$, **$p < .001$**	$F(1,76) = 11.75$, **$p < .001$**	$F(1,76) = 1.40$, $p > .05$	$F(1,76) = .22$, $p > .05$	$F(1,76) = .15$, $p > .05$	$F(1,76) = 1.91$, $p > .05$
18	$F(1,76) = 37.32$, **$p < .001$**	$F(1,76) = 25.95$, **$p < .001$**	$F(1,76) = 18.65$, **$p < .001$**	$F(1,76) = .08$, $p > .05$	$F(1,76) = 2.03$, $p > .05$	$F(1,76) = .11$, $p > .05$	$F(1,76) = .47$, $p > .05$
19	$F(1,76) = 28.57$, **$p < .001$**	$F(1,76) = 2.33$, $p > .05$	$F(1,76) = 9.37$, **$p < .05$**	$F(1,76) = .35$, $p > .05$	$F(1,76) = 1.98$, $p > .05$	$F(1,76) = .01$, $p > .05$	$F(1,76) = 1.18$, $p > .05$
20	$F(1,76) = 26.13$, **$p < .001$**	$F(1,76) = 4.25$, **$p < .05$**	$F(1,76) = 21.93$, **$p < .001$**	$F(1,76) = .14$, $p > .05$	$F(1,76) = 4.53$, $p < .05$	$F(1,76) = .01$, $p > .05$	$F(1,76) = .36$, $p > .05$
21	$F(1,76) = 13.44$, **$p < .001$**	$F(1,76) = 8.01$, **$p < .05$**	$F(1,76) = 33.82$, **$p < .001$**	$F(1,76) = 3.21$, $p > .05$	$F(1,76) = 1.41$, $p > .05$	$F(1,76) = 1.28$, $p > .05$	$F(1,76) = 1.97$, $p > .05$

Note. Bold values represent significant difference.

APPENDIX T: THREE-WAY ANOVA RESULTS OF ENERGY EXPENDITURE IN GHA AND WT MICE

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2.326E+06	7	332312.145	73.627	.000000
Intercept	5.779E+07	1	5.779E+07	12803.041	.000000
Gender	513235.754	1	513235.754	113.712	**.000000**
Genotype	103476.244	1	103476.244	22.926	**.000008**
Diet	1.419E+06	1	1.419E+06	314.480	**.000000**
Gender * Genotype	4893.587	1	4893.587	1.084	.301056
Gender * Diet	53128.566	1	53128.566	11.771	**.000976**
Genotype * Diet	3983.917	1	3983.917	.883	.350446
Gender * Genotype * Diet	104825.937	1	104825.937	23.225	**.000007**
Error	343022.383	76	4513.452		
Total	6.190E+07	84			
Corrected Total	2.669E+06	83			

Note. Bold values represent significant difference.

APPENDIX U: THREE-WAY ANOVA RESULTS OF BLOOD GLUCOSE IN GHA AND WT MICE

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	34366.528[a]	7	4909.504	6.273	.000008
Intercept	3185656.642	1	3185656.642	4070.216	.000000
Gender	2526.007	1	2526.007	3.227	.076442
Genotype	10405.032	1	10405.032	13.294	**.000489**
Diet	17128.534	1	17128.534	21.885	**.000013**
Gender * Genotype	523.251	1	523.251	.669	.416150
Gender * Diet	1008.575	1	1008.575	1.289	.259916
Genotype * Diet	1102.914	1	1102.914	1.409	.238942
Gender * Genotype * Diet	194.325	1	194.325	.248	.619744
Error	58700.629	75	782.675		
Total	3358890.000	83			
Corrected Total	93067.157	82			

a. R Squared = .369 (Adjusted R Squared = .310)

Note. Bold values represent significant difference.

APPENDIX V: THREE-WAY ANOVA RESULTS OF AREA UNDER THE CURVE IN GHA AND WT MICE

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1.985E+09	7	2.835E+08	2.658	.016393
Intercept	1.629E+11	1	1.629E+11	1527.183	.000000
Gender	3.571E+08	1	3.571E+08	3.348	.071241
Genotype	1.674E+07	1	1.674E+07	.157	.693130
Diet	1.025E+08	1	1.025E+08	.961	.330189
Gender * Genotype	4.663E+07	1	4.663E+07	.437	.510488
Gender * Diet	9.392E+07	1	9.392E+07	.881	.351061
Genotype * Diet	7.132E+08	1	7.132E+08	6.687	**.011653**
Gender * Genotype * Diet	4.739E+08	1	4.739E+08	4.443	**.038388**
Error	7.999E+09	75	1.067E+08		
Total	1.760E+11	83			
Corrected Total	9.984E+09	82			

Note. Bold values represent significant difference.

APPENDIX W: PERMISSION TO REPRODUCE FIGURE

Figure 1

ELSEVIER LICENSE
TERMS AND CONDITIONS

Oct 21, 2010

This is a License Agreement between Tianxu Yang ("You") and Elsevier ("Elsevier") provided by Copyright Clearance Center ("CCC"). The license consists of your order details, the terms and conditions provided by Elsevier, and the payment terms and conditions.

All payments must be made in full to CCC. For payment instructions, please see information listed at the bottom of this form.

Supplier

Elsevier Limited
The Boulevard,Langford Lane
Kidlington,Oxford,OX5 1GB,UK

Registered Company Number

1982084

Customer name

Tianxu Yang

Customer address

1 Water Tower Street,

Athens, OH 45701

License number

2532651466203

License date

Oct 19, 2010

Licensed content publisher

Elsevier

Licensed content publication

Molecular Genetics and Metabolism

Licensed content title

Growth Hormone (GH), GH Receptor, and Signal Transduction

Licensed content author

John J. Kopchick, Jerald M. Andry

Licensed content date

September 2000

Licensed content volume number 71

Licensed content issue number 1-2

Number of pages 22

Type of Use

reuse in a thesis/dissertation

Portion

figures/tables/illustrations

Number of figures/tables/illustrations 1

Format

both print and electronic

Are you the author of this Elsevier article? No

Will you be translating? No

Order reference number

Title of your thesis/dissertation

Diet-induced Obesity in Growth Hormone Receptor Antagonist Mice

Expected completion date

Oct 2010

Estimated size (number of pages) 227

Elsevier VAT number

GB 494 6272 12

Terms and Conditions

INTRODUCTION

1. The publisher for this copyrighted material is Elsevier. By clicking "accept" in connection with completing this licensing transaction, you agree that the following terms and conditions apply to this transaction (along with the Billing and Payment terms and conditions established by Copyright Clearance Center, Inc. ("CCC"), at the time that you opened your Rightslink account and that are available at any time at http://myaccount.copyright.com).

GENERAL TERMS

2. Elsevier hereby grants you permission to reproduce the aforementioned material subject to the terms and conditions indicated.

3. Acknowledgement: If any part of the material to be used (for example, figures) has appeared in our publication with credit or acknowledgement to another source, permission must also be sought from that source. If such permission is not obtained then that material may not be included in your publication/copies. Suitable acknowledgement to the source must be made, either as a footnote or in a reference list at the end of your publication, as follows:

"Reprinted from Publication title, Vol /edition number, Author(s), Title of article / title of chapter, Pages No., Copyright (Year), with permission from Elsevier [OR APPLICABLE SOCIETY COPYRIGHT OWNER]." Also Lancet special credit - "Reprinted from The Lancet, Vol. number, Author(s), Title of article, Pages No., Copyright (Year), with permission from Elsevier."

4. Reproduction of this material is confined to the purpose and/or media for which permission is hereby given.

5. Altering/Modifying Material: Not Permitted. However figures and illustrations may be altered/adapted minimally to serve your work. Any other abbreviations, additions, deletions and/or any other alterations shall be made only with prior written authorization of Elsevier Ltd. (Please contact Elsevier at permissions@elsevier.com)

6. If the permission fee for the requested use of our material is waived in this instance, please be advised that your future requests for Elsevier materials may attract a fee.

7. Reservation of Rights: Publisher reserves all rights not specifically granted in the combination of (i) the license details provided by you and accepted in the course of this licensing transaction, (ii) these terms and conditions and (iii) CCC's Billing and Payment terms and conditions.

8. License Contingent Upon Payment: While you may exercise the rights licensed immediately upon issuance of the license at the end of the licensing process for the transaction, provided that you have disclosed complete and accurate details of your proposed use, no license is finally effective unless and until full payment is received from you (either by publisher or by CCC) as provided in CCC's Billing and Payment terms and conditions. If full payment is not received on a timely basis, then any license preliminarily granted shall be deemed automatically revoked and shall be void as if never granted. Further, in the event that you breach any of these terms and conditions or any of CCC's Billing and Payment terms and conditions, the license is automatically revoked and shall be void as if never granted. Use of materials as described in a revoked license, as well as any use of the materials beyond the scope of an unrevoked license, may constitute copyright infringement and publisher reserves the right to take any and all action to protect its copyright in the materials.

9. Warranties: Publisher makes no representations or warranties with respect to the licensed material.

10. Indemnity: You hereby indemnify and agree to hold harmless publisher and CCC, and their respective officers, directors, employees and agents, from and against any and all claims arising out of your use of the licensed material other than as specifically authorized pursuant to this license.

11. No Transfer of License: This license is personal to you and may not be sublicensed, assigned, or transferred by you to any other person without publisher's written permission.

12. No Amendment Except in Writing: This license may not be amended except in a writing signed by both parties (or, in the case of publisher, by CCC on publisher's behalf).

13. Objection to Contrary Terms: Publisher hereby objects to any terms contained in any purchase order, acknowledgment, check endorsement or other writing prepared by you, which terms are inconsistent with these terms and conditions or CCC's Billing and Payment terms and conditions. These terms and conditions, together with CCC's Billing and Payment terms and conditions (which are incorporated herein), comprise the entire agreement between you and publisher (and CCC) concerning this licensing transaction. In the event of any conflict between your obligations established by these terms and conditions and those established by CCC's Billing and Payment terms and conditions, these terms and conditions shall control.

14. Revocation: Elsevier or Copyright Clearance Center may deny the permissions described in this License at their sole discretion, for any reason or no reason, with a full refund payable to you. Notice of such denial will be made using the contact information provided by you. Failure to receive such notice will not alter or invalidate the denial. In no event will Elsevier or Copyright Clearance Center be responsible or liable for any costs, expenses or damage incurred by you as a result of a denial of your permission request, other than a refund of the amount(s) paid by you to Elsevier and/or Copyright Clearance Center for denied permissions.

LIMITED LICENSE

The following terms and conditions apply only to specific license types:

15. **Translation**: This permission is granted for non-exclusive world **English** rights only unless your license was granted for translation rights. If you licensed translation rights you may only translate this content into the languages you requested. A professional translator must perform all translations and reproduce the content word for word preserving the integrity of the article. If this license is to re-use 1 or 2 figures then permission is granted for non-exclusive world rights in all languages.

16. **Website**: The following terms and conditions apply to electronic reserve and author websites:
Electronic reserve: If licensed material is to be posted to website, the web site is to be password-protected and made available only to bona fide students registered on a relevant course if:
This license was made in connection with a course,

This permission is granted for 1 year only. You may obtain a license for future website posting,
All content posted to the web site must maintain the copyright information line on the bottom of each image,
A hyper-text must be included to the Homepage of the journal from which you are licensing at http://www.sciencedirect.com/science/journal/xxxxx or the Elsevier homepage for books at http://www.elsevier.com , and
Central Storage: This license does not include permission for a scanned version of the material to be stored in a central repository such as that provided by Heron/XanEdu.

17. **Author website** for journals with the following additional clauses:

All content posted to the web site must maintain the copyright information line on the bottom of each image, and
he permission granted is limited to the personal version of your paper. You are not allowed to download and post the published electronic version of your article (whether PDF or HTML, proof or final version), nor may you scan the printed edition to create an electronic version,
A hyper-text must be included to the Homepage of the journal from which you are licensing at http://www.sciencedirect.com/science/journal/xxxxx , As part of our normal production process, you will receive an e-mail notice when your article appears on Elsevier's online service ScienceDirect (www.sciencedirect.com). That e-mail will include the article's Digital Object Identifier (DOI). This number provides the electronic link to the published article and should be included in the posting of your personal version. We ask that you wait until you receive this e-mail and have the DOI to do any posting.
Central Storage: This license does not include permission for a scanned version of the material to be stored in a central repository such as that provided by Heron/XanEdu.

18. **Author website** for books with the following additional clauses:
Authors are permitted to place a brief summary of their work online only.
A hyper-text must be included to the Elsevier homepage at http://www.elsevier.com

All content posted to the web site must maintain the copyright information line on the bottom of each image
You are not allowed to download and post the published electronic version of your chapter, nor may you scan the printed edition to create an electronic version.
Central Storage: This license does not include permission for a scanned version of the material to be stored in a central repository such as that provided by Heron/XanEdu.

19. **Website** (regular and for author): A hyper-text must be included to the Homepage of the journal from which you are licensing at http://www.sciencedirect.com/science/journal/xxxxx. or for books to the Elsevier homepage at http://www.elsevier.com

20. **Thesis/Dissertation**: If your license is for use in a thesis/dissertation your thesis may be submitted to your institution in either print or electronic form. Should your thesis be published commercially, please reapply for permission. These requirements include permission for the Library and Archives of Canada to supply single copies, on demand, of the complete thesis and include permission for UMI to supply single copies, on demand, of the complete thesis. Should your thesis be published commercially, please reapply for permission.

21. **Other Conditions**:

v1.6

Gratis licenses (referencing $0 in the Total field) are free. Please retain this printable license for your reference. No payment is required.

If you would like to pay for this license now, please remit this license along with your payment made payable to "COPYRIGHT CLEARANCE CENTER" otherwise you will be invoiced within 48 hours of the license date. Payment should be in the form of a check or money order referencing your account number and this invoice number RLNK10868889.
Once you receive your invoice for this order, you may pay your invoice by credit card. Please follow instructions provided at that time.

Make Payment To:
Copyright Clearance Center
Dept 001
P.O. Box 843006
Boston, MA 02284-3006

If you find copyrighted material related to this license will not be used and wish to cancel, please contact us referencing this license number 2532651466203 and noting the reason for cancellation.

Questions? customercare@copyright.com or +1-877-622-5543 (toll free in the US) or +1-978-646-2777.