ABSTRACT

Title of Document: THE EFFECT OF DIET-INDUCED OBESITY AND SUBSEQUENT WEIGHT LOSS ON BODY COMPOSITION, GLUCOSE CLEARANCE, METABOLITE PROFILE AND LIVER AMP-ACTIVATED PROTEIN KINASE IN MICE

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Obesity, currently an epidemic, is a difficult disease to combat because it is marked by both a change in body weight and an underlying dysregulation in metabolism, making consistent weight loss challenging. We sought to elucidate this metabolic dysregulation resulting from diet-induced obesity (DIO) that persists through subsequent weight loss. We hypothesized that weight gain imparts a change in "metabolic set point" persisting through subsequent weight loss and that this modification may involve a persistent change in hepatic AMP-activated protein kinase (AMPK), a key energy-sensing enzyme in the body. To test these hypotheses, we tracked metabolic perturbations through this period, measuring changes in hepatic AMPK. To further understand the role of AMPK we used AICAR, an AMPK activator, following DIO. Our findings established a more dynamic metabolic model of DIO and subsequent weight loss. We observed hepatic AMPK elevation following weight loss, but AICAR administration without similar dieting was unsuccessful in improving metabolic dysregulation. Our findings provide an approach to modeling DIO and subsequent dieting that can be built upon in future studies and hopefully contribute to more effective long-term treatments of obesity.

THE EFFECT OF DIET-INDUCED OBESITY AND SUBSEQUENT WEIGHT LOSS ON BODY COMPOSITION, GLUCOSE CLEARANCE METABOLITE PROFILE AND LIVER AMP-ACTIVATED PROTEIN KINASE IN MICE

BY

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Chapter 1 - Introduction

Prevalence and Consequences of Obesity in America

Obesity has become an increasing problem in the United States (U.S.), affecting 35.7% of adults and 17% of children (Blackburn, 2012). The percentage of Americans who are classified as "morbidly obese" (BMI > 40) has risen six fold, up from 0.9% in 1962 to 6% in 2010 (Fryar, Carroll & Ogden, 2012). Projections estimate that by 2030, 51% of Americans will be obese and 11% will be morbidly obese (Blackburn, 2012).

The economic consequences of obesity in the U.S. can be seen in reduced productivity in the workforce and elevated healthcare costs. Finkelstein et al. (2009) estimate that obesity-related productivity loss costs employers roughly $36.4 billion per year. Obese individuals take 5.9 to 9.4 more absent days per year than their healthy weight counterparts and when they are present at work, their efficiency is reduced due to obesity-related health problems (Fryar et al., 2012). In 2008, roughly 9% of healthcare expenses in the U.S., or $147 billion, were obesity-related costs (Blackburn, 2012).

Aside from the economic implications, obesity in humans has many health-related consequences. Research has shown that once a level of obesity is reached, there are increased risks of developing such conditions as coronary heart disease, Type II diabetes, certain types of cancer, hypertension, stroke, liver disease and osteoarthritis (Pi-Sunyer, 2002). In addition, obesity has been documented to increase discrimination and social stigma, possibly impacting psychological health (Schafer & Ferraro, 2011).

Etiology of Overweight and Obesity

In a broad sense, the terms "overweight" and "obesity" describe a state of body weight that is greater than what is considered healthy and therefore individuals in these

categories have an increased chance of diseases and health complications (Centers for Disease Control, 2010). Body mass index (BMI), calculated by dividing weight by the square of the height (kg/m^2), is most commonly used to define obesity because of its ease of measurement and low cost for assessment. The range for a healthy weight is considered to be 18.5 – 24.9 BMI units. At the same time, the range for overweight individuals is 25.0 – 29.9 BMI units while the range for obese individuals is 30.0 BMI units or above (Centers for Disease Control, 2010). In 2009-2010, the average BMI for U.S. adults was 28.7 for both men and women (Flegal, 2012). Although BMI reliably predicts body fatness, it does not directly measure body fat (Centers for Disease Control, 2010). Thus, obesity can more accurately be described as ≥25% body fat in men and ≥35% body fat in women (Grundy, 2004).

Weight gain occurs when calories consumed exceed calories expended. Thus, when calories consumed exceed the daily energy requirements to support maintenance and activity, the additional energy is stored in the body in the form of fat. When this excess intake of calories occurs over a prolonged period, individuals may reach a state of obesity (Weinsier, et al., 1998).

Body composition is modulated by a combination of two key factors: metabolism, as determined by genetic make-up, and diet and exercise regimens. Studies have shown the existence of such genetic factors that allow certain individuals to inherit "susceptibility genes" for obesity (Weinsier, et al., 1998). Such "susceptibility genes" account for variation in taste preferences, muscle composition, and overall energy requirements and expenditures (Weinsier, et al., 1998). While certain individuals may carry such genes that make them more likely to gain weight, they will not achieve an

overweight or obese state when maintaining a healthy lifestyle that includes consumption of lower fat diets and maintenance of a regular exercise routine (Bouchard, 1991). Thus, since genetic factors alone cannot explain the onset of weight gain and obesity, the increasing trend of obesity in the U.S. and worldwide can be attributed to environmental factors, specifically, reduced physical activity and increased energy intake.

Eating habits that include over-consumption (hyperphagia) and consuming diets high in fat and simple sugars are also contributing factors. In particular, consuming high-fat diets containing greater saturated and trans fats and refined sugars are considered leading contributors to the development of obesity because of the high caloric density of fats and the short-term satiating effects of refined sugars (Astrup et al., 2000). Consumption of high-fat diets also increases plasma levels of low-density lipoprotein (LDL) and plasma insulin, both of which contribute to the health risks associated with obesity (Astrup et al., 2000). The high availability, low cost, and popularity of such high calorie dense foods has contributed to an increase in caloric intake and obesity (Astrup et al., 2000). Caloric intake, combined with a lack of regular exercise (i.e. reduced energy expenditures), results in conversion of these excess calories into fat for storage in adipocytes. Thus, an increasingly sedentary lifestyle is also believed to be a major contributor to the obesity epidemic (Astrup et al., 2000). The prevalence of calorie dense foods combined with a sedentary lifestyle contribute to the prevalence of obesity in our society today.

However, some metabolic differences may increase susceptibility to obesity for certain individuals. Obesity can be viewed as a metabolic syndrome. In addition to excess body fat, obesity can lead to metabolic symptoms such as high blood pressure as well as

elevated blood glucose (≥100 mg/dl) and triglycerides (≥150 mg/dl) (Grundy, 2004). Body weight in non-obese individuals is regulated by multiple interdependent and connected metabolic pathways which sense and regulate energy expenditure and food intake. In obese individuals, this feedback system becomes dysregulated as the body fails to sense an excess energy state and continues a high level of food intake, which is exacerbated by a lower level of energy expenditure. This underlying metabolic dysregulation leads to the persistence of obesity (Friedman & Halaas, 1998). Genetic polymorphisms and quantitative trait loci, both of which are hereditary, have been identified as factors that lead to dysregulation of lipid metabolism and predisposition to excessive weight gain as fat (Du & Feskens, 2010; Sviridov & Nestel, 2007). An extreme example of obesity resulting from a genetic defect in energy regulation is morbid obesity in rodents with mutations in the genes encoding leptin, a hormone regulating appetite and metabolism (Clément et al, 1998). A dysfunctioning energy regulation system encourages the persistence of obesity.

Solutions or Quick Fixes?

The growing obesity epidemic in the U.S. is an ongoing problem with no clear solution in sight. The generally accepted course of action is a combination of steady dieting (i.e. reduced calorie intake), importantly, a reduced intake of high-fat, high-sugar foods, and exercise. Over the past several decades, many commercial supplements and fad diets have been popularized because they have been touted as providing a quick and maintainable weight loss (Miller et al., 2009). However, “quick-fix" dieting is clearly not a promising solution to the obesity epidemic in the U.S. and in other developed countries, with obesity statistics continuing to remain high; in fact, they continue to increase. One

problem with such dieting approaches is the issue of weight cycling, which refers to the continual process of dieting and regaining weight (Thomas et al., 2010). When restricting food consumption during a period of dieting, the body gradually adjusts to the lower intake of calories. However, when an individual stops dieting and returns to previous eating habits, the body remains in this "adapting" state where it becomes more efficient at storing the excess food calories as fat (Summermatter et al., 2007). This metabolic adaptation leads to weight gain and often the individual achieves a body weight that exceeds his or her previous increased weight. This suggests that strictly losing weight to fight obesity is not a maintainable solution.

It would seem that after losing weight, as long as the individual maintains healthy eating habits, this weight cycling can be prevented. However, the body constantly makes metabolic adjustments throughout the different stages of weight gain and loss that alters normal physiology and eating habits. Understanding the derangements that alter the normal energy-sensing mechanisms and consequent redirection of metabolism could be a key toward developing long-term solutions to slow down and reduce the rate of obesity development.

Research Questions, Hypothesis and Objectives

Does diet-induced obesity leave a "nutritional imprint" that tends to maintain a "set point" of increased metabolic efficiency after subsequent weight loss, resulting in a failure to adequately sense energy status in the body? If so, to what extent is the energy sensing enzyme AMP-activated protein kinase involved in this nutritional imprinting as seen through effects on food efficiency and metabolic dysregulation?

We hypothesize that a cycle of diet induced obesity in mice will impart a long-term shift in metabolic efficiency and that this imprinting will display disparate changes in AMPK activation in the liver. It is anticipated that once mice undergo one cycle of obesity and weight loss that AMPK activation will be lower in the liver due to a "thrifty metabolism" geared towards conserving energy. If AMPK proves to play a significant role in this metabolic dysregulation, we further expect the physiological response and food efficiency of obese mice to be altered upon administration of the compound aminoimidazole carboxamide ribonucleotide (AICAR) that activates AMPK.

In order to test this hypothesis, we will achieve the following objectives*:*

1) Determine that diet-induced weight gain and subsequent weight loss leads to a persistent nutritional imprint characterized by changes in metabolic efficiency and energy-sensing that maintains a set point defending the obese state.

2) Determine the role of AMPK in this obesogenic nutritional imprinting phenomenon by manipulating AMPK activation.

Experimental Approach

To elucidate such metabolic changes following diet induced obesity and weight loss, we used mice as the experimental animal model. There are several advantages of using mice as a model for human metabolism. First, mice have many genetic, physiological, and metabolic similarities to humans that have made them one of the preferred models for studying human metabolic processes. Second, as in humans and other complex mammals, mice naturally develop obesity-induced Type II diabetes, hypertension and hyperglycemia (Bergen & Mersmann, 2005). Lastly, mice are relatively

low-maintenance and low-cost mammalian models that develop quickly, which greatly expedites research progress.

While our research builds upon years of prior studies on AMPK and obesity, there has been insufficient research conducted that has investigated the role of AMPK in weight cycling and consequent effects on food intake.

Thus, while it is known that AMPK is involved in energy homeostasis, it is not known whether changes in AMPK activation occur once an obese state is reached. At the same time, it is unknown whether these chronic changes in AMPK underlie long-term control of food intake that characterizes obese individuals who undergo bouts of weight loss and weight gain.

The following explains how this thesis is organized. Chapter 2 is the literature review section which explains the features of metabolic syndrome, various pathways affected by diet induced obesity, the role of an energy regulator called AMP-activated protein kinase, a method for identifying important metabolites within the body and the concept of metabolic imprinting. The subsequent methodology chapter outlines the rationale and procedure for Experiment 1 followed by the rationale and procedure for Experiment 2. Chapter 4 contains the results and discussion for Experiment 1 while Chapter 5 explains the results and discussion for Experiment 2. The final chapter is the conclusion, which summarizes Experiments 1 and 2, ties them together, and elucidates the limitations of this study.

Chapter 2 - Literature Review

Features of Metabolic Syndrome

Overview.

Obesity is thought to be caused primarily by a chronic state of nutrient or "energy excess," i.e. a high energy diet and little physical activity. The result of this is obesity-related metabolic syndrome, characterized by such markers as hyperlipidemia, hyperinsulinaemia, peripheral insulin resistance leading to a reduced ability to clear blood glucose, hypertriglycerideaemia, decreased high density lipoprotein (HDL) cholesterol, and hypertension. This metabolic dysregulation results from high concentrations of lipids in the blood, liver, and muscle. In the obesity state, the levels of stored fat in the form of triacylglycerols (TAG) and non-esterified fatty acids (NEFA) increase. High levels of NEFA result during obesity due to an inability of muscle and adipose tissue to take up NEFA and esterify them to TAG (Aguilera et al., 2008).

Hyperlipidimia and development of insulin resistance.

Under normal conditions, adipose tissue is capable of storing excess energy as fat, but eventually these fat cells become filled, leading to a high level of circulating non-esterified fatty acids (NEFA)—a condition known as hyperlipidemia (Muoio & Newgard, 2006). Hyperlipidemia causes problems for several organs including the pancreas, skeletal muscle, and adipose tissue itself. The high circulating levels of NEFA have been found to disrupt pancreatic secretion of insulin and impair the action of insulin on skeletal muscle (Kahn & Flier, 2000). This dysregulation is a potential cause of insulin resistance which carries with it other problems that exacerbate the already elevated levels of NEFA in plasma. Hyperlipidemia also affects the adipose tissue's natural secretion of hormones,

referred to as adipokines (Muoio & Newgard, 2006). This hormonal abnormality has been observed to worsen the situation by giving false energy signals to the brain. These signals tend to increase, rather than reduce, energy intake. Hyperlipidemia is also thought to cause "ER stress" in the endoplasmic reticulum (de Ferranti & Mozaffarian, 2008). Endoplasmic reticular stress is affected by excess NEFA and has the consequence of increasing cellular insulin resistance. Insulin resistance prevents the cell from extracting glucose from the blood, so when a cell is unable to store energy as fat, levels of NEFA in the plasma rise. Therefore, a cycle of increasing NEFA and insulin resistance may occur.

Insulin Resistance.

During obesity, high levels of NEFA cause tissues, primarily the muscle, to become insulin resistant despite high levels of circulating insulin. One consequence of this reduction in insulin sensitivity is the reduced ability of peripheral tissues such as muscle to uptake and metabolize glucose. NEFA reduce glucose oxidation in muscles through inhibition of pyruvate dehydrogenase via production of acetyl-CoA, the latter being an allosteric inhibitor of pyruvate dehydrogenase. Another reason for the high levels of glucose in the blood is that the increased levels of NEFA stimulate hepatic gluconeogenesis (Aguilera et al, 2008).

NEFA can also interfere in the insulin signaling cascade in skeletal muscle resulting in decreased insulin-stimulated glucose uptake and glycogen synthesis. Insulin resistance may be a result of inflammatory cytokines such as tumor necrosis factor α which activates the nuclear transcription factor kappa B and related inflammatory pathways (Wilding, 2007). In type 2 diabetes, the expression of insulin-regulated glucose transporter 4 (GLUT-4) is reduced in skeletal muscle and significantly reduced in adipose

tissue. Insulin resistance in skeletal muscles is particularly important because muscle uptakes roughly 80% of circulating glucose. However, adipose-specific GLUT-4 knockout mice have impaired insulin sensitivity in the liver and muscle. Food restriction also causes insulin resistance and decreased adipose GLUT-4 expression (Muoio & Newgard, 2006).

Studies have shown that being more than 35 to 40% over an ideal body weight leads to insulin resistance. Normally, insulin suppresses hepatic glucose production and increases glucose uptake by muscle and fat cells. However, after obese individuals lose weight, peripheral tissues remain less responsive to insulin, causing a decrease in glucose uptake and storage (Kahn & Flier, 2000). The sensitivity of tissues to insulin, specifically muscle, decreases by 30 to 40% (DeFronzo & Ferrannini, 1991). The resulting insulin resistance leads to hyperinsulinemia followed by hypertension, hypertriglyceridemia, hypercholestrerolemia, HDL cholesterol, and eventually atherosclerosis (DeFronzo & Ferrannini, 1991).

Lipotoxicity.

Another reason for decreased glucose clearance is the lipid accumulation in skeletal muscles. Long periods of obesity lead to lipid accumulation in non-adipose tissues resulting in reduced glucose uptake, mitochondrial dysfunction, and lipotoxicity. Excess fatty acids lead to lipotoxicity and metabolic syndrome. Lipotoxicity results in impaired hormone sensitivity that eventually leads to the persistence of obesity. One hypothesis for the development of obesity related metabolic syndrome is the inability of white adipose tissues to continue to store fatty acids and the resulting lipotoxicity that occurs in non-adipose tissues. After a period of time, white adipose tissue can no longer

continue to expand, thus resulting in elevated NEFA. When this limit is reached, excess lipids form toxic reactive lipid species, such as diacylglycerols and ceramides. The presence of these toxic reactive lipid species in non-adipose tissues, such as pancreatic β cells, liver, kidneys, heart and skeletal muscles results in toxic responses and increased apoptosis (i.e. programmed cell death). In pancreatic β cells, glucolipotoxicity contributes to β-cell failure in type 2 diabetes. In the hypothalamus, lipotoxicity, increased inflammation as measured by a mediator of metabolic inflammation (IKKβ/NF-κB), and increased endoplasmic reticulum stress affect the regulation of energy homeostasis and lead to obesity (Martínez de Morentin et al., 2010). At high doses, salicylates (aspirin) suppress the activation of the NF-κB transcription factor mediated pathway, thus reversing insulin resistance and hyperlipidemia in obese rodents (Muoio & Newgard, 2006).

All of these metabolic and physiological issues are the result of chronic, often diet-induced, energy excess in the body. The metabolic dysregulation associated with obesity prevents the body from accurately sensing its energy status at the cellular and whole body basis. Obese animals often perceive themselves as being in a low energy or starvation state. The metabolism of an obese individual is geared towards energy conservation leading to the persistence of obesity (de Ferranti & Mozaffarian, 2008).

Pathway Analysis

The key pathways involved in energy generation and energy utilization, such as the Krebs cycle, glycolysis, gluconeogenesis and fatty acid oxidation, are all affected by obesity-induced metabolic syndrome.

Citric Acid Cycle.

As one of the main energy generators, the citric acid cycle, or Krebs cycle, plays a vital role in cellular metabolism. The citric acid cycle is the major pathway through which carbohydrates such as glucose are metabolized. Glucose is broken down through glycolysis into pyruvate, which feeds into the citric acid cycle. In addition to formation through the breakdown of pyruvate, acetyl-CoA is formed via the oxidation of fatty acids. The citric acid cycle forms ATP from the products of glucose and fatty acid metabolism. The relative rates of glucose and fatty acid metabolism are important factors that affect the development of the metabolic dysregulation due to obesity, which will be discussed later in the fatty acid metabolism section.

This cycle is a major component of aerobic cellular respiration that oxidizes acetate to CO_2 through a multi-step and highly regulated process. As a byproduct of these reactions, 3 NADH, an $FADH_2$ and GTP are produced during a complete turn of the cycle, which, through oxidative phosphorylation and the electron transport chain, ultimately yields 12 ATP, the energy currency for cell metabolism (Berg, Tymoczko, & Stryder, 2011).

The first and most crucial step in the citric acid cycle is the aldol condensation of acetyl-CoA with oxaloacetate to form citrate, which is catalyzed by citrate synthase. This reaction, as well as those catalyzed by isocitrate dehydrogenase and α-ketoglutarate dehydrogenase, is highly thermodynamically favorable and irreversible, driving the cycle forward. The high favorability of citrate synthesis is especially important, because the previous step, the conversion of malate to oxaloacetate, actually favors malate, thus

citrate synthesis drives the reaction forward to formation of oxaloacetate via mass action principles (Berg et al., 2011).

The citric acid cycle has many degrees and forms of regulation. The first regulated step is the formation of acetyl-CoA from pyruvate. This reaction is catalyzed by pyruvate dehydrogenase, a multi-enzyme complex, and is irreversible. The reaction is largely regulated by product inhibition such that, as NADH and acetyl-CoA concentration increases within the mitochondria, various enzyme components of the pyruvate dehydrogenase complex are allosterically inhibited (Berg et al., 2011).

Within the cycle itself, there are a few key regulators including acetyl-CoA, succinyl-CoA, the NAD^+/NADH ratio and the ADP/ATP ratio. The availability of acetyl-CoA along with sufficient generation of oxaloacetate determines the rate of the citrate synthase reaction. Succinyl-CoA inhibits α-ketoglutarate dehydrogenase and competes with acetyl-CoA for citrate synthase. As the NAD^+/NADH ratio decreases, indicative of sufficient energy production, NADH inhibits isocitrate dehydrogenase and α-ketoglutarate dehydrogenase, both of which produce NADH. Furthermore, isocitrate dehydrogenase is either allosterically activated by ADP or inhibited by ATP (Berg et al., 2011).

An additional level of regulation occurs as a result of the availability of the substrate for each reaction. Because the citric acid cycle is at the center for several biosynthetic pathways, these substrate pools can be depleted for the production of lipids and amino acids. Some examples of depletion include conversion of acetyl-CoA to fatty acids, transamination of α-ketoglutarate to glutamate and oxaloacetate to aspartate, production of glucose through gluconeogenesis from oxaloacetate, and porphyrin

synthesis from succinyl-CoA. Most of these reactions are reversible and used to refill the substrate pool, known as anaplerotic reactions (Berg et al., 2011).

Due to its importance throughout the body in maintaining an energy balance, it seems logical that aspects of the citric acid cycle would be affected by obesity, which signals a disturbance in energy homeostasis. According to Satapati et al., (2012), Krebs cycle flux is elevated when an animal exhibits insulin resistance, which occurs as a result of obesity.

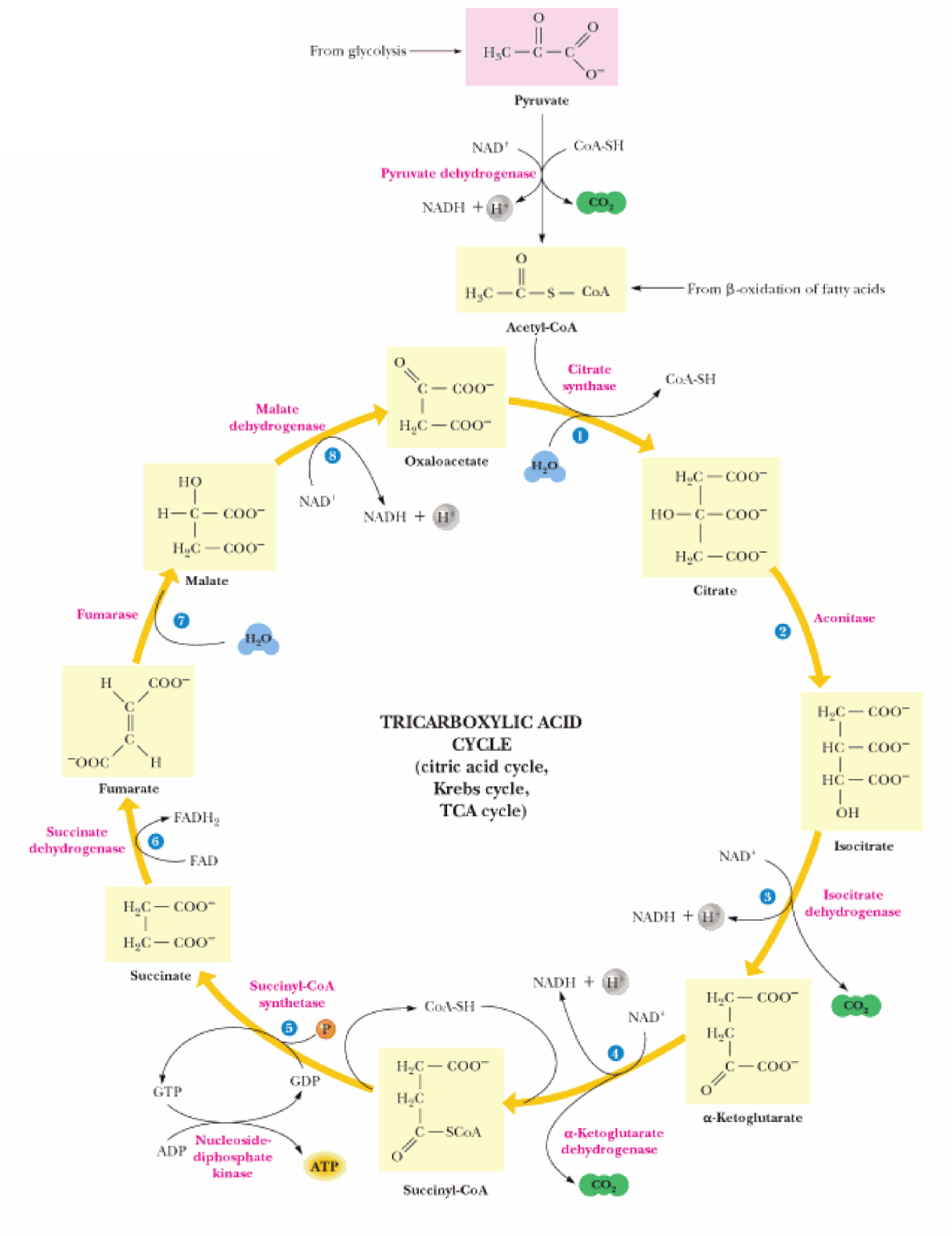

Figure 2-1. Citric Acid Cycle (Campbell & Farrell, 2011)

Gluconeogenesis.

When the amount of stored glycogen and blood glucose in the body falls to low levels, such as during an overnight fast, gluconeogenesis from muscle and dietary derived amino acids and TAG-glycerol from mobilized adipose tissues is enhanced. Both the brain and the nervous tissues are especially dependent upon glucose for normal function. The liver plays a critical role in the synthesis of glucose during fasting as it converts lactate, amino acids and TAG-glycerol to glucose. About 25% of the liver's production of glucose derives from gluconeogenesis during a period of fasting with the remainder derived from breakdown of stored glycogen. In prolonged fasting, gluconeogenesis by the kidneys becomes the major contributor to generation of glucose from amino acids. The carbon skeletons of all amino acids, except lysine, leucine and glycine, can be metabolized to make glucose with the predominant contributors to gluconeogenesis being alanine, glutamate, glutamine, valine and isoleucine (Hall, 2011).

Amino acids take various enzymatic pathways for conversion to glucose. In the case of alanine, it is simply deaminated to form pyruvate, which is an immediate precursor for glucose. Other amino acids require interconversions prior to entry into the Krebs cycle, eventually leading to 3- and 4- carbon skeletons that feed into the gluconeogenic pathway. The main stimulus of gluconeogenesis is the reduction of both sugar and carbohydrates, which causes a decrease in the phosphogluconate and glycolytic pathways to form carbohydrates (Hall, 2011). The reduction in blood glucose is sensed by the anterior pituitary, which then secretes increased amounts of the hormone corticotropin. The adrenal cortex, in response, produces and secretes large quantities of glucocorticoids, in particular, cortisol. Cortisol stimulates the mobilization of amino acids

from skeletal muscles to enhance the supply to the liver and kidneys of amino acids for gluconeogenesis (Khani, 2001). A high proportion of these amino acids are deaminated in the liver (Hall, 2011). **Figure 2-2** shows the pathways that amino acids enter the citric acid cycle and contribute to gluconeogenesis.

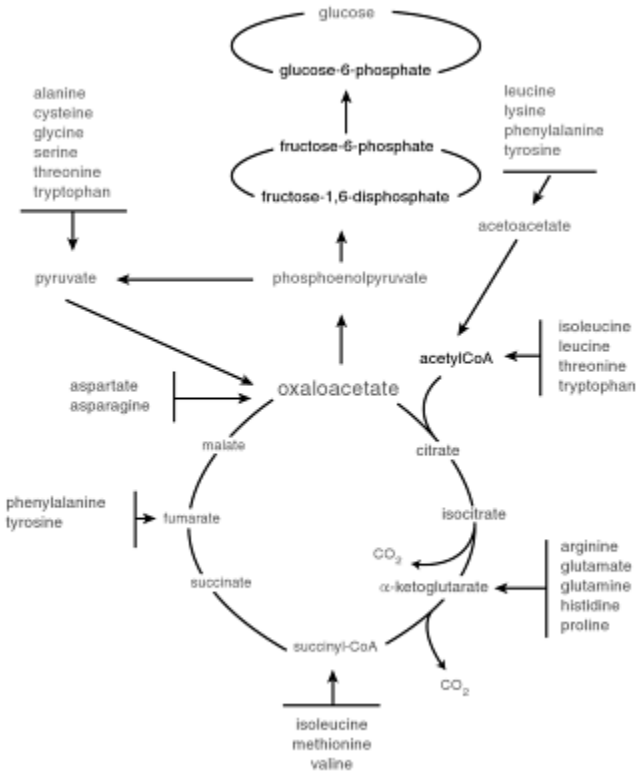

Figure 2-2. The routes of metabolism that amino acids follow to contribute to gluconeogenesis (Cynober, 1995).

Gluconeogenesis and glycolysis pathway.

The net effect of gluconeogenesis is to counter glycolysis (the breakdown of glucose). However, for thermodynamic reasons, gluconeogenesis is not simply the reverse of glycolysis, certain steps must be bypassed. Glycolysis contains three

irreversible steps catalyzed by hexokinase, phosphofructokinase and pyruvate kinase (Hames, 1997). **Figure 2-3** shows the general chemical equation for glycolysis and gluconeogenesis.

Glycolysis

glucose + 2 NAD^+ + 2 ADP + 2 P_i → 2 pyruvate + 2 NADH + 2 ATP

Gluconeogenesis

2 pyruvate + 2 NADH + 4 ATP + 2 GTP →glucose +2 NAD^+ + 4 ADP + 2 GDP + 6 P_i

Figure 2-3. General chemical equation for glycolysis and gluconeogenesis

In bypassing pyruvate kinase, the pyruvate must be converted to oxaloacetate, which is reduced to malate for cytosolic export and then re-oxidized again to oxaloacetate. Phosphoenolpyruvate carboxykinase (PEPCK) phosphorylates and decarboxlyates oxaloacetate to generate phosphoenolpyruvate. From this point, glycolysis is simply reversed chemically up until the point where fructose-1,6-bisphosphate is formed. Fructose-1,6-bisphosphate is dephosphorylated via fructose-1,6-bisphosphatase, which bypasses phosphofructokinase and generates fructose 6-phosphate, an important rate limiting enzyme of the glycolytic pathway. The reversal of glycolysis through the action of phosphoglucose isomerase converts the fructose 6-phosphate into glucose 6-phosphate. Finally, to bypass hexokinase, glucose-6-phosphatase converts glucose-6-phosphate into glucose (Hames, 1997).

Precursors for gluconeogenesis arise from any carbon skeleton whose metabolic pathway leads to either pyruvate or 3-phosphoglycerate (e.g. glycerol and serine). These precursors include lactate, glycerol, propionate (mostly in ruminants) and all amino acids except leucine, lysine, and glycine. Under anaerobic conditions, the muscle metabolizes

glucose to lactate and pyruvate to alanine via alanine amino transferase, whereas glucose is always metabolized (aerobic and anaerobic conditions) by the intestines to alanine and lactate. The recycling of lactate derived glucose for gluconeogenesis is referred to as the Cori cycle, while the use of glucose derived alanine for gluconeogenesis is referred to as the alanine cycle. Glycerol serves as the backbone of TAG and, upon breakdown of TAG in adipocytes, glycerol is released from adipose tissues, taken up by the liver and converted to glucose via formation of 3-phosphoglycerate. Propionate derived from metabolism of odd chain fatty acids, valine, methionine, isoleucine, threonine, and cholesterol, yields propionyl-CoA, which is converted to oxaloacetate, a glucogenic intermediate through a series of reactions in the Krebs cycle. Amino acids (mostly glutamine) formed from muscle catabolism of proteins, during starving or fasting, supply additional precursors for gluconeogenesis (Cynober, 1995).

Regulation of Gluconeogenesis.

The regulation of gluconeogenesis is reciprocal to that of glycolysis. Thus, in general, the negative controls of glycolysis are the positive effectors of gluconeogenesis. Local control includes allosteric regulation by adenine nucleotides. Hence, phosphofructokinase in glycolysis is inhibited by ATP and stimulated by AMP, while fructose-1, 6-bisphosphatase in gluconeogenesis is inhibited by AMP. When intracellular ATP levels are high, glucose is not further broken down to generate ATP, instead it is stored as glycogen in the liver and muscle. Subsequently, when intracellular ATP is low, stored glycogen is broken down to supply glucose and the pathway of gluconeogenesis is reduced dramatically to conserve energy. Global control in liver cells is via the cyclic AMP cascade that is triggered by the hormone glucagon when blood glucose levels are

low. The glucagon induced cAMP cascade stimulates gluconeogenesis, inhibits glycolysis, stimulates glycogen breakdown, and inhibits glycogen synthesis with the net effect of increasing glucose release into the blood from the liver. Protein Kinase A (cAMP-Dependent Protein Kinase) phosphorylates various enzymes and regulatory proteins to initiate gluconeogenesis. Proteins that are phosphorylated by Protein Kinase A include pyruvate kinase and CREB; the former is a glycolytic enzyme inhibited when phosphorylated and the latter is involved in the activation of the transcription of the gene for PEPCK that increases gluconeogenesis. Protein Kinase A also phosphorylates a bi-function enzyme that generates and destroys fructose-2, 6-bisphosphate. Fructose-2,6-bisphosphate stimulates glycolysis and inhibits gluconeogenesis, and it is reduced in liver cells due to the cAMP signaling cascade. Downstream effects cause glycolysis to slow and gluconeogenesis to increase (Hames, 1997).

Obesity, Metabolic Syndrome & Glucose Metabolism.

Several studies have shown an association between fat distribution and increased cortisol secretion (Khani, 2001). It has been observed that in the short term, the increase in cortisol secretion enhances gluconeogenesis, however, long-term effects have yet to be investigated (Khani, 2001). Previous studies also show that leucine, isoleucine, and phenylalanine release from skeletal muscle and blood concentration are greater when cortical concentrations are elevated, hence, increasing the supply of substrates for gluconeogenesis (Khani, 2009). Metabolic syndrome also results in elevated blood NEFA, which accelerates gluconeogenesis since a continuous source of energy (ATP) and substrate is being provided from metabolism of NEFA (Wajchenberg, 2000). Liver AMP-activated protein kinase (AMPK) controls glucose homeostasis by inhibiting gene

expression of gluconeogenic enzymes and thus hepatic glucose production. AMPK suppresses FOXO1 and CRTC2, which increase gluconeogenesis. Hence, gluconeogenesis is reduced by AMPK activation. Studies found that endogenous hepatic glucose production and plasma glucose levels are reduced when AMPK is artificially activated by administration of the AMPK activator 5-aminoimidazole-4-carboxamide ribotide (AICAR) (Zhang, 2009).

Non-Esterified Fatty Acid Metabolism.

NEFA are long-chained hydrocarbons with a terminal carboxylic acid group that serve as the primary source of energy in the body during the resting state (Berg et al., 2011). NEFA are stored as TAG, which are compromised of three moles of fatty acids and one mole of glycerol. These lipid molecules are primarily stored in adipose tissue following absorption as chylomicrons from the gastrointestinal tracts into the lymphatic system. They are processed by the liver and transport HDL and very-low density lipoprotein (VLDL) molecules to target tissues. When energy from stored triglycerides in adipose tissues is required, hormone-controlled lipases catalyze the hydrolysis of adipose TAG, thus releasing NEFA and glycerol. NEFA circulate in blood associated with plasma-albumin, while glycerol is taken up by the liver for gluconeogenesis and reesterification of NEFA to TAG for liver export as HDL and VLDL (Berg et al., 2011).

NEFA are characterized by the length of their hydrocarbon chain, and more importantly, by the presence of double bonds within this chain. Saturated fatty acids contain no double bonds. Palmitic acid is a prominent dietary saturated fatty acid that is stored in adipose tissues (Kien, 2009). Unsaturated fatty acids contain double bonds in their chains. Oleic acid is a prominent monounsaturated dietary fatty acid.

Polyunsaturated fatty acids are those whose chains contain two or more double bonds, the most nutritionally significant of which are oleic acid (omega-9) and the essential fatty acids linoleic (omega-6) and linolenic (omega-3) (Kien, 2009).

The oxidation of NEFA for energy occurs within the mitochondrial matrix. To enable transport across the mitochondrial membranes, fatty acids must be activated. This occurs via esterification of a coenzyme A (CoA) unit to the carboxyl end of a fatty acid on the outer mitochondrial membrane, forming an acyl CoA. To cross the membrane, the CoA group is replaced with carnitine to form acyl carnitine. This reaction is catalyzed by carnitine palmitoyl I (CPTI) and allows the entry of the fatty acid across the intermembrane space via a translocase protein, carnitine acyl carnitine translocase. Carnitine palmitoyl II (CPTII) subsequently replaces the carnitine with a CoA group, resulting in an acyl CoA molecule present in the mitochondrial matrix (Berg et al., 2011).

β-oxidation of saturated fats is a repeated four step process that degrades the acyl-CoA molecule to acetyl-CoA units, which can then enter the citric acid cycle for ATP production. These reactions also produce the reduced species $FADH_2$ and NADH. To oxidize unsaturated fatty acids, additional steps and enzymes are required that result in the production of acetyl CoA units, as well as the three carbon molecule propionyl-CoA when odd chain fatty acids are oxidized— which enters the citric acid cycle after conversion to succinyl-CoA (Berg et al., 2011). The ability of acetyl CoA to enter the citric acid cycle depends on the presence of oxaloacetate with which it condenses; oxaloacetate is produced from pyruvate, generated from glycolysis, and lactate and alanine, synthesized in peripheral tissues. Therefore, under conditions of low intracellular glucose (e.g. during fasting or diabetes), there is an insufficient supply of oxaloacetate

such that acetyl CoA entry into the citric acid cycle is diminished. In consequence, acetyl CoA builds up and is converted to acetoacetate and D-3-hydroxybutyrate (i.e. ketone bodies), which are released by the liver into blood (Berg et al., 2011). Ketone bodies under fasting conditions serve as alternative energy sources for the brain and heart, but if large quantities accumulate in the blood, they can become toxic and reduce blood pH. The latter is sometimes a feature of individuals who are obese and have type 2 diabetes (Kitabchi, Umpierrez, Miles, & Fisher, 2009).

NEFA can be synthesized in the body, both in the liver and adipose tissue, via the process of lipogenesis. Lipogenesis takes place in the cytoplasm of these cells under fed conditions, when energy demands are low and the body attempts to store excess substrates as energy (Jensen-Urstad & Semenkovich, 2011). The process begins irreversibly when acetyl CoA is carboxylated to malonyl CoA by the enzyme acetyl CoA carboxylase (ACC). Eight, two-carbon units are conjugated sequentially to form palmitate (Berg et al., 2011). Oleic acid is formed when palmitate is elongated to stearic acid and then desaturated by stearoyl-CoA desaturase (Kien, 2009). The enyzme malonyl-CoA decarboxylate can convert malonyl-CoA back into acetyl CoA (Muoio & Newgard, 2006).

Abnormally decreased β-oxidation, as well as increased levels of malonyl CoA, are thought to contribute to insulin resistance observed in the metabolic syndrome via lipotoxicity (Muoio & Newgard, 2006). Lipotoxicity occurs when lipid molecules and their intermediates negatively impact insulin signaling (particularly in skeletal muscles), decreasing that tissue's ability to take up glucose. Obese individuals show an increased concentration of intracellular TAG stores in muscular tissue (Muoio & Newgard, 2006).

Intermediates associated with TAG, most notably diacylglycerol (DAG) and ceramide, accumulate when β-oxidation is compromised. The latter have been suggested to negatively impact insulin signaling, and studies have shown TAG levels correlate more closely with insulin resistance than BMI or total adiposity (Muoio & Newgard, 2006).

The type of fatty acids consumed in the diet (i.e. saturated vs. unsaturated), affects the degree of insulin resistance. Studies in humans have shown that diets low in oleic acid and high in the saturated fatty acid palmitic acid lead to insulin resistance via inflammation and the accumulation of intracellular lipids (Kien, 2009). Likewise, glucose uptake by muscle cells in vitro increased when cells were incubated with oleic, pamitoleic (monounsaturated), linoleic, or alpha-linolenic acid (Kien, 2009). Accumulation of unsaturated fatty acids in muscle cell membranes increases fluidity and the number of insulin receptors, the affinity of insulin to its receptor, and glucose transport rate (Jans et. al). Unsaturated fatty acids have also been shown to favor fat oxidation over storage.

AMP-Activated Protein Kinase (AMPK)

AMP-activated protein kinase is a multi-component enzyme that serves an essential first step in the regulation of energy metabolism within all cells in nature. In eukaryotic cells, AMPK activation has pleiotropic effects in many tissues, including adipose tissue, liver, skeletal muscle, and the hypothalamus. AMPK acts as a "metabolic master switch" that serves an essential role in intracellular energy-sensing by detecting cellular energy status in order to maintain energy balance within every cell (Hardie, 2004). AMPK is an intracellular energy sensor that, when activated, induces catabolic processes to rapidly produce more ATP.

AMPK is a heterotrimeric protein consisting of a catalytic alpha, regulatory beta, and gamma subunits. It is allosterically activated by an increase in the intracellular AMP: ATP ratio as well as by phosphorylation on Thr172 by upstream kinases. LKB1 and Calcium/calmodulin-dependent protein kinase kinase (CaMKK) are two upstream regulators of AMPK kinase (Viollet et al., 2006).

Upon activation, AMPK initiates a cascade of catabolic ATP-generating pathways (e.g. fatty acid oxidation and glucose utilization) while switching off anabolic ATP-consuming pathways (e.g. protein synthesis and gluconeogenesis). When enough ATP has been generated to restore balance, ATP competitively inhibits AMP from further activation of AMPK and turns it off (Carling, 2004). In addition to immediate activation of ATP-generating pathways, AMPK also has long-term effects through alteration of gene expression and protein synthesis.

Leptin and Adiponectin as Upstream Regulators of AMPK.

Leptin is a hormone that regulates energy homeostasis by modulating food intake, energy storage, and expenditure (Friedman & Halaas, 1998). In a healthy person, leptin promotes satiety and plays a role in maintaining constant fat stores (Spiegelman & Flier, 2001). Therefore, Ob/ob leptin knockout mice show physiological signs of starvation such as hyperphagia, low body temperature, decreased physical activity, immune function and infertility (Friedman & Halaas, 1998). Leptin resistance during obesity is one mechanism by which energy sensing in obese humans and animals is impaired. While high leptin levels should reduce food intake, and thus obesity, obese humans and animals experience hyperphagia despite high levels of leptin. This implies that the leptin signal fails to reach the brain or that the signal is not transmitted beyond the leptin

receptor (Enriori, Evans, Sinnayah, & Cowley, 2006). In C57BL/6J mice, four or eight weeks of a high fat diet resulted in insulin resistance and a lack of response to intraperitoneal (IP) leptin injections (Prpic et al, 2003). Despite the state of energy excess that exists during obesity, leptin resistance impairs the signaling mechanism(s) to stop eating, resulting in hyperphagia and obesity.

Adiponectin is another hormone secreted by white adipose tissue. It has been shown to stimulate AMPK activation in the peripheral tissues in turn, stimulating fatty acid oxidation. Fasting mice exhibited high adiponectin levels to stimulate AMPK and food intake, and after re-feeding, adiponectin returned to previous fed state levels (Carling, 2004).

Upon activation of AMPK, glucose uptake by myocardial cells increased by upregulation of glucose transporter 4 (GLUT4) (Russell et al., 1999). Leptin has a tissue-specific effect on AMPK: in skeletal muscle, leptin stimulates AMPK whereas in the hypothalamus, leptin decreases AMPK activation (Carling, 2004). These opposite effects of leptin both contribute to its overall effect on energy homeostasis, leading to an increase in fatty acid oxidation in peripheral tissues and reduction in appetite through action on the hypothalamus.

AMPK Inhibits NEFA Synthesis.

NEFA, stored as TAG, are the most significant source of stored energy that generate ATP. When AMPK is activated, NEFA synthesis, an anabolic pathway, is inhibited while NEFA oxidation, a catabolic process, is activated. AMPK reduces NEFA synthesis by inhibiting acetyl-CoA carboxylase (ACC) through phosphorylation, thus reducing the synthesis of malonyl CoA, the initial template for NEFA synthesis. In

addition, because malonyl-CoA is an allosteric inhibitor of the carnitine palmitoyltransferase-1 (CTP1), which is a carnitine shuttle for the transport of fatty acids into the mitochondrial matrix for oxidation, the lower levels of malonyl-CoA result in less inhibition of NEFA oxidation. Meanwhile, the inhibition of ACC stimulates transport of fatty acids into the mitochondria where they are oxidized to generate ATP (Tong, 2005).

AMPK activation in skeletal muscle increases glucose uptake and fatty acid oxidation (Carling, 2004). In the liver, AMPK activation inhibits glucose, fatty acid, and cholesterol synthesis. In adipose tissue, AMPK activation inhibits fatty acid synthesis and increases lipolysis, thus leading to breakdown of TAG and subsequent increased release of NEFA into the plasma (Carling, 2004). These changes in NEFA and TAG concentrations in mice treated with AICAR compared with the Control mice can be measured via metabolomics as described later.

The central nervous system is involved in dietary regulation of appetite and food choices (Morton et al, 2006). Because AMPK is present in the hypothalamus, changes in activation of AMPK also lead to modulation of food intake. Morton et al. (2006) suggested that AMPK does affect food choice. However, the effects of AMPK modulation on food choice are not fully clear, though it is expected that mice with increased AMPK activation will consume more of a high-fat diet if allowed (Morton et al, 2006). What is currently known is that increased AMPK activation in the hypothalamus is associated with increases in energy expenditure through increased fatty acid oxidation, and adipocyte lipolysis, but reduced lipogenesis.

The hypothalamus senses levels of NEFA and other circulating lipids, acting as an energy regulator and initiator of the release of hormones such as leptin, ghrelin, adiponectin, resistin and insulin by other tissues. As mentioned earlier, obesity related hyperlipidemia causes lipid build up and eventually lipotoxicity in non-adipose tissues. In particular, this resulting impairment of lipid metabolism in the hypothalamus affects metabolic homeostasis. Although the mechanism of hypothalamic metabolic regulation is unknown, increased levels of malonyl-CoA in the hypothalamus have been associated with decreased food intake and low body fat in mice. Fasting mice results in AMPK activation and inhibition of ACC in the hypothalamus. The reduced levels of malonyl-CoA and AMPK activation are associated with enhanced appetite and increased food intake and body weight. This indicates a change in metabolic set point where the body attempts to conserve energy. Refeeding decreases AMPK activation in the hypothalamus which leads to reduced food intake (Martínez de Morentin et al., 2010). However, this effect may not be apparent in previously obese mice, which indicates a metabolic imprint. This refeeding period corresponds to the period in which the mice in our study stop dieting and return to a standard diet where they consume food in excess of their non-obese counterparts.

AMPK in the liver.

AMPK plays a major role in the control of hepatic metabolism through short-term effects on phosphorylation of regulatory proteins as well as through long-term effects on gene expression. Activation of AMPK in the liver also leads to the stimulation of NEFA oxidation and inhibition of lipogenesis, glucose production, and protein synthesis (Viollet et al., 2006).

In the liver, AMPK is activated by the metabolic challenges imposed by either a period of fasting or dietary energy restriction (Jiang et al., 2008). In the liver, the transition from the fasted to the fed state is also associated with physiological changes in energy dynamics. The reversal of the metabolic response to starvation includes alterations in enzyme phosphorylation states and changes in the concentration of key regulatory molecules. It has been reported that AMPK coordinates the changes in the activity and expression of a number of enzymes of lipid metabolism during refeeding (Munday et al., 1991; Dentin et al., 2005). Additionally, hepatic AMPK can be regulated by ghrelin, endocannabinoids, glucocorticoids, resistin, and adiponectin (Viollet et al., 2009).

Hepatic AMPK activation can be adjusted and regulated by several drugs such as AICAR (5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside), compound A-769662 (Cool et al., 2006), polyphenols and two major classes of existing antidiabetic drugs biguanides (metformin and phenformin) and thiazolidinediones (TZDs) (Saha et al., 2004). AICAR has been extensively used both in vitro and in vivo to activate hepatic AMPK (Viollet et al., 2006).

AICAR (5-Aminoimidazole-4-carboxamide ribotide).

AICAR (5-Aminoimidazole-4-carboxamide ribotide), an analog of AMP, has been used to pharmacologically activate AMPK. Upon exposure, cells take up AICAR and convert it to AICA ribotide (ZMP). ZMP serves as the analog of AMP, and thus activates AMPK through the same chemical pathway as AMP. AICAR has been shown to affect food intake, body composition, and glucose uptake and metabolism by cells.

Researchers have found that activation of AMPK by administration of AICAR alters body composition by reducing both visceral and subcutaneous adiposity in rats

(Gaidhu et al, 2011). Jessen et al. (2003) chronically administered AICAR to rats and compared glucose transport in skeletal muscle fibers to rats that performed regular exercise, and found that both exercise and AICAR administration improved insulin-stimulated glucose transport, suggesting that AICAR can mimic effects of exercise on glucose clearance.

Previous in vitro studies with rat hepatocytes have shown that AICAR treatment activates AMPK, resulting in reduced fatty acid synthesis and glucose production by inhibition of ACC and 3-hydroxy-methylglutaryl CoA reductase (Buhl et al., 2002). Thus, AICAR treatment has been implicated to suppress gluconeogenesis through downregulation of gluconeogenic enzymes.

AMPK and Insulin resistance.

Insulin-resistance, or the inability of insulin to stimulate peripheral tissue uptake and clearance of glucose, is a hallmark metabolic feature of type 2 diabetes. During hypoxia, AMPK was found to be responsible for the insulin-independent rise in GLUT4 translocation (Jessen et al., 2003). There was also an increase in membrane GLUT4 content upon AICAR treatment. And, long-term AICAR exposure results in a significant decrease in plasma insulin and glucose levels. AICAR treatment also decreased endogenous glucose production in both normal and insulin-resistant obese rats (Halseth, Ensor, White, Ross, & Gulve, 2002). Thus, AICAR may be a potential pharmaceutical regulator of hepatic gluconeogenesis.

In diabetic rats, activation of hypothalamic AMPK may contribute to the rat's hyperphagia and be explained by lower plasma levels of leptin and insulin in diabetic mice (Namkoong et al., 2005). Because AICAR has been shown in many studies to

improve blood glucose concentrations and lipid profiles, AICAR is an attractive pharmacological target for the treatment of Type 2 diabetes.

Metabolomics

In recent years, there has been much interest in developing metabolomics approaches and platforms to allow for the measurement of global metabolic changes in the body as a result of obesity. Metabolomics involves technologies such as nuclear magnetic resonance and mass spectrometry to measure metabolite concentrations, or products of metabolism, in different cells and tissues of the body. Because obesity involves systemic changes in metabolism, metabolomics can be a useful tool to identify dysregulations in specific organs and the whole body, and thus provide a description of the underlying metabolic issues that lead to and maintain the obese state (Gulston et al., 2007). However, it is important to remember that metabolomics only provides a "snapshot" of the organism's metabolic state at the specific timepoint that the organism's tissue sample was collected.

It has been found that obese animals are characterized by certain metabolites, including elevated plasma glucose, fumarate, malate, ribose, carnitine and pyrimidine nucleoside. Obese animals also have low plasma taurine levels (Gulston et al., 2007). There is variation between obese and normal weight animals in lipid composition and tissue composition. For example, the liver of obese rats has a lower ATP/ADP ratio compared to normal weight counterparts (Gulston et al., 2007). Additionally, metabolomics has improved our understanding of the dysregulations that occur in the insulin resistant state. Here, changes in plasma lysine, glycine, citrate, leucine, and

acetate were found to be the important metabolites in identifying insulin resistance (Shearer et al., 2008).

However, an important distinction to make is that some changes in the metabolites may be due to short-term diet effects rather than a state of obesity. It has been found that changes in metabolites related to energy metabolism and glucose usage were caused by the diet, while an obesogenic state caused changes in amino acids and large non-polar molecules (Duggan et al., 2011). Although still a relatively new technology, metabolomics is an inexpensive approach to collect a large amount of data, allowing researchers to distinguish changes in metabolic pathways that characterize the state of obesity.

Metabolic Imprinting

A topic that is still being investigated is what factors determine an individual's body weight set point. One theory is that metabolic imprinting is the mechanism that underpins the establishment of a given "set point". One of the larger challenges to researchers is how to define the biological differences between individuals that seem to naturally carry more weight and those that carry less weight. An explanation to this is the set point theory, which was proposed by Bennet and Gurin in 1982. The theory states that the body has an internal regulator that controls how much fat it naturally tends to store. In other words, an individual's metabolism adjusts to maintain a certain "preferred" weight. This preferred weight is one descriptor of the body's "set point," and explains why individuals who lose weight following diet restriction tend, over time, to regain the lost weight (Kahn and Flier, 2000). This set point is what appears to vary among individuals

of different natural body compositions (e.g. proportion of whole body fat versus lean tissues).

One measure of an animal's set point is their food efficiency. This is simply the amount of weight gained per calorie consumed. If an animal is more efficient in storing food, they will tend to deposit more fat when consuming the same amount of food than a less efficient animal consumes. Naturally, as a biological survival mechanism, the efficiency of storing food as fat is enhanced in times of diet restriction or reduced food availability (de Ferranti & Mozaffarian, 2008). This is the body's biological response to a lack of food. Evolutionarily, this has the benefit of helping an animal survive during the winter when food availability and quality are poor or during famine. This increase in food efficiency indicates a change in the animal's metabolic and physiological set point. However, many food-deprivation and weight cycling studies in animals demonstrate that this change is only temporary—once the animal is again given *ad libitum* access to food, their food efficiency soon returns to normal (Maclean et al., 2004).

Food efficiency is strongly connected with the idea of a set point because some individuals seem naturally more food efficient than others. One may again draw on the example of those who fail to gain much weight and remain "thin," even though their calorie intake is at unhealthy levels. This is a very perplexing problem that remains without a definitive solution in current research.

Metabolic imprinting refers to the permanent changes in biological processes that result from exposure to a specific nutritional environment (Waterland & Garza, 1999). Research has demonstrated that early metabolic programming results in the establishment of set points for physiological and metabolic responses in adulthood. For example,

evidence from epidemiological studies and animal models indicate that maternal health and nutritional status during gestation and lactation have long-term effects on central and peripheral systems that regulate energy balance in the developing offspring (Hanley et al., 2010). Perinatal nutrition also impacts susceptibility to developing metabolic disorders and plays a role in programming body weight set points. Thus, maternal over-nutrition, diabetes, and under-nutrition predispose offspring to an increased risk of developing obesity (Langhans & Geary, 2010). However, while prenatal nutrient restriction in rats increases adult obesity, postnatal nutrient restriction reversed the effect and resulted in normal, lean adults, showing that metabolic imprinting may also be affected by environmental changes after birth (Garg et al., 2012).

Metabolic imprinting may also occur in adulthood as a result of diet-induced obesity. High fat diets can lead to an increased body weight and adipocyte number, which is correlated with elevated food intake and oxygen consumption (Corbett et al., 1986). Mice and rats that were fed high fat diets maintained a consistent obesity condition even when their diet was switched from a high to a low fat diet (Rolls et al., 1980; Guo et al., 2009). When obese rats were subsequently given unrestricted access to the low fat diet, they returned to the same elevated weight and had greater fat stores than the control rats (Rolls et al., 1980). Additionally, obese people who diet or food-restrict tend to gain back the weight that they lose. This may occur because the state of obesity leaves a metabolic imprint, or permanent change in the metabolism of the body, that raises the body's set point. However, among rats who developed diet-induced obesity and were subsequently given unrestricted access to a normal diet, rats bred to be diet-resistant were able to reduce their intake and returned to control weights while others plateaued at elevated

weights (Levin & Keesey, 1998). Therefore, there is an interaction between genetic predisposition and diet in the development of a consistent state of obesity.

Long-term caloric restriction in humans has shown that fatty acid mobilization and oxidation during the fasting state is increased. In addition, calorie restriction led to increased insulin sensitivity (Huffman et al., 2012). A 3-week period of calorie restriction in rodents has previously been shown to lower plasma insulin, triglyceride, and cholesterol levels (Anson et al., 2003). The reduction of these metabolites leads to reduced risk of diabetes and atherosclerosis. Chen et al. (2005) showed that in addition to the reduction of these plasma metabolites, there was increased physical activity in calorie restricted mice presumably due to increased foraging activity. Other studies have shown that calorie restriction reduces glucose levels and elevates ketone bodies (Greene, et al., 2003). Thus, calorie restriction has been shown to have a wide range of health benefits. A previous study conducted by Duarte et al. (2012) which used a high fat diet to induce obesity resulted in increased fatty liver, increased adiposity, and circulating levels of leptin compared to the control mice. Subsequent calorie restriction did not reverse the metabolic changes from the high fat diet induced obesity, except a small decrease in fat mass. However, upon refeeding, this decrease in fat mass was reversed, and the mice maintained the metabolic profiles of obesogenic mice.

Most obese humans seem only to be able to increase their body weight set point permanently, while periods of calorie restricted weight loss are only temporary (Levin, 2010). The change in set point caused by a state of obesity, together with genetic predisposition, causes metabolic changes that work to defend the high weight. Therefore even when obese rats are calorie restricted, their metabolism is adjusted in order to regain

the weight lost, characterized by reduced energy expenditure, increased drive to eat, higher food efficiency, lipid accumulation in adipose tissue, and altered neuronal signals (Maclean et al., 2006). Levin and Keesey (1998) showed that when obese rats that were not diet-resistant were calorie restricted to reach the weight of the control rats, their leptin and insulin levels dropped below the controls. However, when these rats were given unrestricted access to food, their food intake, food efficiency, and leptin and insulin levels rose to obesogenic levels again (Levin & Keesey, 1998). When lean and obese mice are calorie restricted, leptin expression decreases in obese mice but increases in lean mice, suggesting a metabolic imprint associated with their differing diets and weights (Kurki et al., 2012).

Levin (2005) suggests that persistent obesity could be due to neuronal changes in the sections of the brain that regulate metabolism, such as the hypothalamus, amygdala, nucleus tractus solitaries, and striatum. These neurons sense metabolic substrates such as glucose, fatty acids, lactate and ketone bodies, and hormones, and use this information to alter energy intake and energy expenditure. In particular, previous studies have elucidated the connection between the hypothalamus and body weight control. Tanaka et al. (1978) conducted hypothalamic lesion studies in which the ventromedial nucleus was removed from birthing mice. They observed that while 3% of control mice developed obesity, 92% of treated mice whose ventromedial nucleus of the hypothalamus was removed developed morbid obesity, implicating that the hypothalamus plays a crucial role in feeding behavior and weight control.

As with most neurons in the brain, the hypothalamic neurons are constantly being rewired in a process known as synaptic plasticity. Diet during prenatal and postnatal

development can have long lasting effects in the hypothalamus, causing changes in energy regulation (Levin, 2010). Horvath (2006) showed that during high fat diet-induced obesity in mice, the synaptic plasticity of the hypothalamic neurons was influenced by many factors, particularly leptin. This leptin-mediated plasticity of the hypothalamus, therefore, is altered during high fat diet-induced obesity, and the hypothalamus changes in response to leptin released in the obesity state (Horvath, 2006). Additionally, rats that are obesity-prone have dysregulated hypothalamic pathways and a reduced response to leptin (Bouret et al., 2008).

Another important theory relevant to this project is the "thrifty gene" hypothesis. Maintaining energy homeostasis by balancing food intake and energy expenditure through exercise and basal metabolism is crucial for survival (Spiegelman & Flier, 2001). A complex array of physiological processes regulates the rate of catabolic processes that generate energy and anabolic processes that consume energy. These physiological mechanisms maintain body weight and energy stores in homeostasis. In addition to regulating the energy balance of daily activities, such as exercise and food intake, an organism must be able to store energy for prolonged periods of food scarcity. The "thrifty gene" hypothesis describes how natural selection favored the advantageous traits of effective energy storage during periods of excess to allow survival during periods of famine (Spiegelman & Flier, 2001). In order to maintain energy homeostasis, metabolism is regulated at the gene transcription and translation, cellular and tissue, and whole organism levels.

For optimal health and survival of humans and animals, a balance between catabolic and anabolic processes must be maintained. Catabolic processes include

carbohydrate, amino acid and fatty acid oxidation. Anabolic processes, such as fatty acid, protein, and cholesterol synthesis, are necessary for normal cellular division and growth, and for health and survival. In humans, it is critical to the maintenance of a constant body weight that the balance between these energy-generating and energy-consuming processes be maintained, otherwise, under-nutrition or overweight and obesity will ensue.

Chapter 3 - Methodology

Experiment 1 Rationale

The aims of this experiment were: 1) to characterize the developmental timeline for the attainment of obesity and related metabolic derangements in C57BL/6J mice and 2) to determine whether establishment of this obese state and resultant metabolic syndrome entrains an 'imprint' that establishes certain metabolic set points (e.g. AMPK activation, glucose and fatty acid metabolisms) that remain despite a period of weight loss.

Experiment 1 Methodology

University of Maryland's Institutional Animal Care and Use Committee (IACUC) approved all experimental procedures (R-11-42). Male C57BL/6J mice (n = 54, Charles River, Wilmington, MA) were acquired at 3 weeks of age. Upon arrival, mice were housed in plastic cages in groups (n = 6 mice/group) during which they were fed *ad libitum* a standard diet (TD.06416, TekLad), had free access to water provided by an automatic dispenser system and were kept on a 12 hour light/12 hour dark cycle in the Department of Animal and Avian Sciences Animal Wing at the University of Maryland. At 8 weeks of age, 48 mice were placed into individual plastic cages and randomly allocated to either a Control or Diet Induced Obesity (DIO) Group. The Control mice were fed a standard diet (2018 Teklad Global 18% Protein Rodent Diet). The standard diet had an energy density of 3.1 kcal/g, with 24% of energy from protein, 18% from fat, and 58% from carbohydrates. The DIO mice were fed a high fat diet (Teklad Rodent Diet TD.06414). The high fat diet had an energy density of 5.1 kcal/g, with 18% of energy from protein, 60% from fat, and 21% from carbohydrates. The remaining mice (n = 6),

which served as baseline controls, were given a glucose tolerance test (GTT) after which blood and tissues were collected and analyzed as described below.

The experiment lasted 13 weeks (**Figure 3-1**). After 4 and 8 weeks on both Control and experimental diets, groups of mice (n=6, n=6) were fasted for 4 h, after which they were given the GTT and subsequently euthanized by CO_2 asphyxiation, and blood and tissues were removed for later analysis. At week 8, the remaining mice in the Control group continued to consume the basal diet *ad libitum* while the remaining mice in the DIO group were food restricted (20% less energy intake) on the basal diet for 2 weeks and then allowed to consume the basal diet *ad libitum* for the final 3 weeks of the study. Subsequently, at weeks 10 and 13, groups of mice (n=6, n=6) were fasted for 4 h after which they were given the GTT and subsequently euthanized by CO_2 asphyxiation, and blood and tissues were removed for later analysis. See below for descriptions and explanations of laboratory analyses.

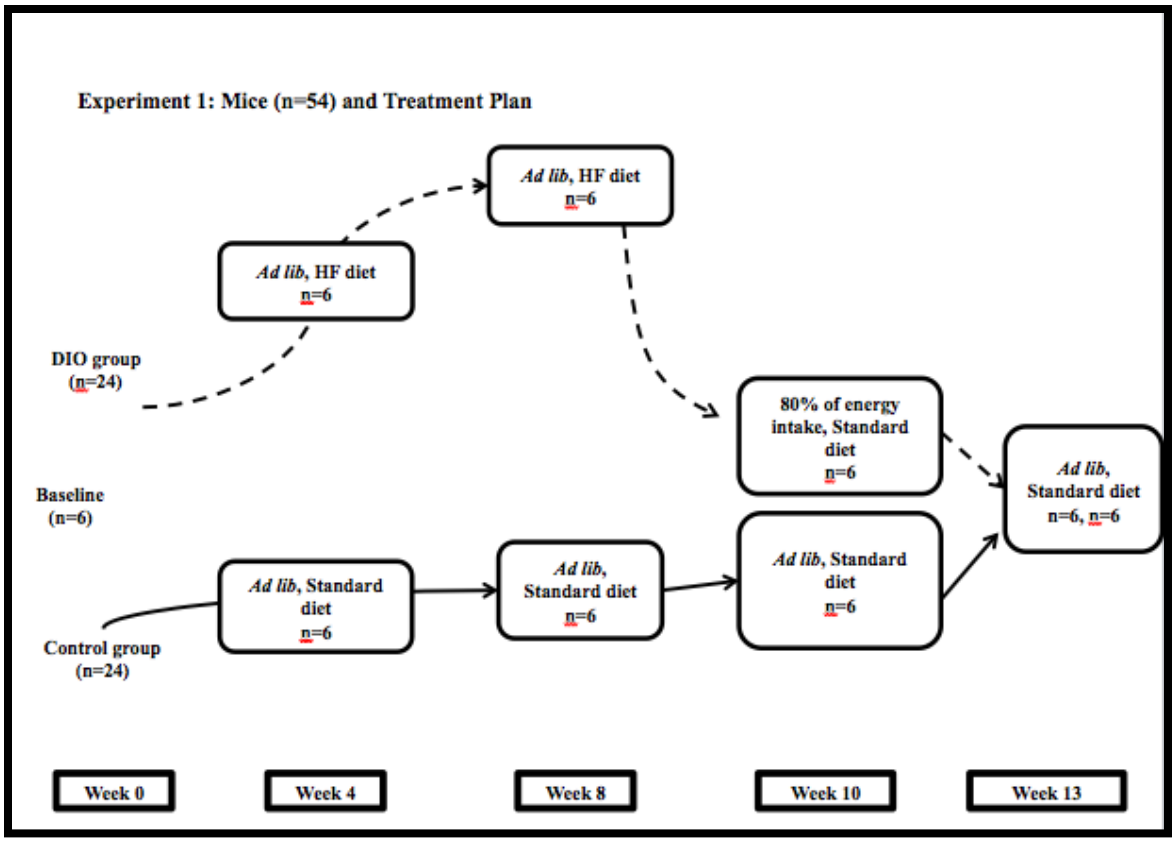

Figure 3-1. Experiment 1 design and treatment arrangements.

Experiment 2 Rationale

The aims of this experiment were: 1) to characterize the influence of AMPK activation on the obesogenic state of the C57BL/6J mice and 2) to determine whether increasing AMPK activation affects the metabolic profile encountered due to obesity and subsequent weight loss.

Experiment 2 Methodology

University of Maryland's Institutional Animal Care and Use Committee (IACUC) approved all experimental procedures (R-11-42). Male C57BL/6J strain mice (n=48), (Charles River, Willmington, MA) were acquired at 6 weeks of age. Upon arrival, mice were housed in stainless steel cages (n=6 mice/cage) during which they were fed *ad libitum* a standard diet (TD.06416, TekLad), had free access to water provided by an

automatic dispenser system and were kept on a 12 hour light/12 hour dark cycle in the Department of Animal and Avian Sciences Animal Wing at the University of Maryland. At 8 weeks of age, 48 mice were placed into individual plastic cages and randomly allocated to either the standard (Control, n = 18) or the high fat (DIO, n = 24) diet. The composition of the standard (2018 Teklad Global 18% Protein Rodent Diet) and the high fat diets (Teklad Rodent Diet TD.06414) were the same as in Experiment 1. The remaining mice (n=6) served as baseline controls and were given a glucose tolerance test (GTT) and blood and tissues were collected following euthanasia as described below.

The Control and DIO groups were fed their respective diets *ad libitum* for 8 weeks (**Figure 3-2**). In Experiment 1, the results indicated that 8 weeks was a sufficiently long enough period to induce a state of obesity and to reduce the ability of the mice to clear blood glucose as determined by the GTT. After 8 weeks on standard and high fat diets, mice (n=6) from each dietary group were fasted for 4 h, after which they were given the GTT and subsequently euthanized by CO_2 asphyxiation. Blood and tissues were removed for later analysis as described below. The remaining mice in the DIO group were allocated to three treatment groups (n = 6 per group) that received either 1) the DIO *ad libitum* plus intraperitoneal (IP) administration of buffered (pH 7.4) sterile saline, 2) the DIO *ad libitum* plus IP administration of 5-amino-4-imidazole carboxyamide riboside (AICAR, 0.5 mg/g body weight), or 3) the standard diet at 80% of previous metabolizable energy intake plus IP administration of buffered sterile saline. The mice remaining from the Control group continued to be fed the standard diet *ad libitum* and were allocated two treatment groups (n = 6 per group) that received either 1) IP administration of buffered (pH 7.4) sterile saline or 2) IP administration AICAR (0.5

mg/g body weight), Mice received the IP injections of buffered (pH 7.4) sterile saline (0.28 mL per injection) or AICAR (dissolved in 0.28 mL saline per injection) three times per week for 2 weeks. After 2 weeks, mice were fasted for 4 h, after which they were given the GTT and subsequently euthanized by CO_2 asphyxiation. Blood and tissues were removed for later analysis as described below.

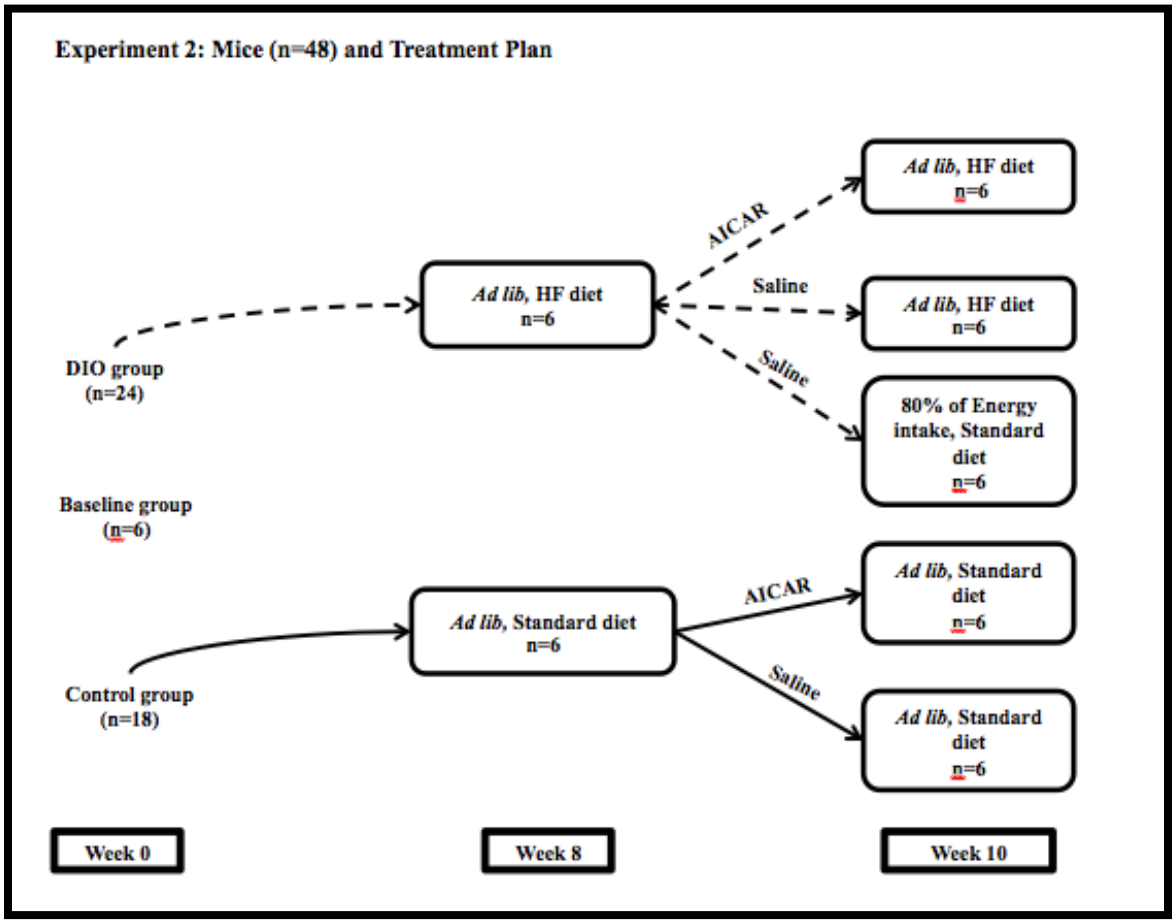

Figure 3-2. Experiment 2 design and treatment arrangements

Treatment and Analysis for Experiments 1 and 2

The subsequent sections outline the treatment and analysis performed on mice at the aforementioned time points labeled in **Figures 3-1** and **3-2**. Mice in Experiments 1

and 2 were subjected to the following methods relating to feeding, body weight analysis, glucose clearance, tissue collection, AMPK analysis, fat content and metabolomics.

Feeding.

When mice were fed *ad libitum*, a known amount of feed was placed in food hoppers at the beginning of each week. Hoppers were checked daily and extra feed was added as needed. The amount of feed consumed by each mouse was recorded at the end of the week. The food hoppers were designed to allow easy access to feed by the mice yet prevent feed spillage.

During the calorie restriction phase for the mice previously fed the HFD, a fixed amount of feed was placed into a glass tube feeder and feed remaining the next day was weighed and recorded. The feeder consisted of a glass test tube with a smoothed hole near the bottom to allow easy access yet prevent feed spillage.

Body Weight.

At the end of every week, all mice were weighed and body weight was recorded. A small beaker (250 mL) was placed on a standard laboratory scale. The mouse was placed inside the beaker, and the weight was recorded in grams (0.1 g).

Glucose Tolerance Test (GTT).

The GTT was performed on mice (n = 5-6) from both the Control and DIO mouse groups at 4, 8, 10, and 13 weeks to determine changes in pancreatic function (i.e. insulin secretion) and the ability of tissues (mainly muscle) to clear blood glucose. The GTT is a standard test conducted to diagnose type 1 diabetes and the development of metabolic syndrome associated with obesity. The GTT was performed after fasting the mice for 4 hours beginning at the onset of the light cycle. Prior to intraperitoneal injection of the

glucose dose, a sterile surgical blade was used to nick the tip of the tail to acquire a drop of blood for measurement of baseline blood glucose concentration using a hand held glucometer (AlphaTRAK, Abbott Labs, Inc.). Immediately after the baseline blood glucose sample, a bolus dose of a sterile solution of glucose (2g glucose/kg body weight) was administered by intraperitoneal injection. At 30, 60, 90 and 120 min after the glucose bolus, glucose concentration was measured in a drop of blood from the tail by gently removing the clot over the incision and massaging the tail vein (Andrikopoulos, Blair, Deluca & Fam, 2008).

Euthanasia and Tissue Collection.

After the GTT, mice were placed individually into a sealable polycarbonate chamber and the chamber purged with CO_2 until breathing had stopped for at least one minute. Euthanasia by CO_2 asphyxiation leads to rapid death without severe distress or pain (Hackbarth, Kuppers & Bohnet, 2000). After euthanasia, the peritoneal cavity and posterior vena cava of the mice was exposed by blunt dissection through the abdominal cavity and a blood sample (approximately 0.5 ml) withdrawn into a tuberculin syringe (27 ga needle, 0.5 mL) containing ethylenediaminetetraacetic acid (EDTA) to prevent blood clotting. Whole blood was aliquotted (150 µL) into three tubes and placed on ice prior to storage at -20 °C. Removal of this quantity of blood also ensured death by exsanguination. Immediately following blood sampling, the mice were decapitated and the whole liver was removed, weighed, sliced into 200-400 mg amounts, and wrapped in aluminum foil prior to plunging the sample into liquid nitrogen. The empty carcass (minus visceral organs, lungs and heart) was weighed, placed into a ziplock freezer bag

on ice, and within 2 h stored at -20 °C. Liver samples were also stored at -20 °C for metabolomics and AMPK analyses.

Measurement of AMPK activation.

AMPK activation was measured in liver samples from mice. Once removed from the freezer, liver samples were kept on ice. For processing, liquid nitrogen was poured over a sample (approximately 0.6-0.7 g) to allow the sample to be crushed. The crushed sample was transferred to a 2 mL snap cap tube placed in ice, and 0.5 mL of extraction solution added followed by homogenization for 1 min (Brinkman POLYTRON Homogenizer, Brinkman Instruments, Inc., Westbury, NY). The protein extraction solution consisted of 20 mM Tris-HCl, 150 mM NaCl, 1 mM EGTA (ethylene glycol tetraacetic acid), 1% Triton-X-100, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride in isopropanol, and 1x Halt^{TM} Protease and Phosphate Inhibitor Cocktail (Thermo Scientific). After homogenization, samples were centrifuged at 15,000 ×g for 10 min at 4 °C. The supernatant was removed and stored at -20 °C.

Protein concentration in liver extracts was determined using the Bradford assay. A standard curve was constructed for each 8 by 12 well plate. A standard curve of bovine serum albumin at 0, 8, 16, 24, 44, and 64 μg/ml was used. One sample from each treatment group was diluted 1:50, 1:250, 1:500, and 1:1000 to establish the proper dilution that falls on the standard curve. For samples from week 8 and 10 mice, a 1:500 dilution was required whereas for week 13 samples a 1:1000 dilution was necessary. To each protein extract (160 μl of diluted sample) was added 40 ul of Coomassie Brilliant Blue G-250 dye and absorbance measured at 595 nm using a UV-Vis spectrophotometer. All samples were run in triplicate.

For determination of phosphorylated AMPK α (AMPK activation) in liver, about 50 µg of total protein was analyzed (AMPK α [pT 172] immunoassay kit, Invitrogen Corp., Camarillo, CA) according to the manufacturer's protocol. Absorbance was recorded at 450 nm using a microplate reader (ThermoElectron, San Jose, CA). Final AMPK activation was calculated using standards supplied with the kit.

Fat Content.

The fat content of the carcasses was determined by Soxhlet extraction. The carcass consisted of the decapitated and eviscerated mouse. Frozen carcasses were pulverized under liquid nitrogen in a freezer mill (model 6850, Spex Certiprep, Metuchen, NJ), weighed and lyophilized to dryness (FreeZone 12 L Freeze Dry System, Labconco Corp, Kansas City, MO). After drying (3-4 days), samples were weighed for calculation of carcass water and dry matter contents. Fat extractions were performed in triplicate on 1 gram samples.

Briefly, samples (1 g) were weighed into a porcelain extraction thimble, covered with a piece of gauze and continuously extracted in petroleum ether over approximately 18 h. The process extracts all compounds dissolvable in ether, including tissue triglycerides, fatty acids and other lipid compounds (e.g. phospholipids). After extraction and complete evaporation of residual ether, the whole thimble was weighed and recorded. The difference between the initial and final weight of the thimble represents the loss of lipid, which was then extrapolated to a fresh (wet) carcass weight after correction for water loss following lyopholization.

Metabolomics.

Metabolomics analysis on whole blood and frozen liver was performed on all samples. Polar and non-polar metabolites (e.g. fatty acids, glucose, amino acids, Krebs cycle keto-acids, glycolytic intermediates) were extracted and derivatized for gas chromatography-mass spectrometry (GC-MS, Agilent, Palo Alto, CA) analysis.
For blood samples, to a known weight of thawed chilled whole blood (0.1 g) was added a known weight (0.05 g) of a solution containing the internal standard norleucine (1.3 mM). Next, ice-cold methanol (1 mL) was added, and the samples were vortex mixed for 15 min on an orbital shaker. At room temperature, the methanolic supernatant was separated following centrifugation (15,000 ×g, 15 min at 4 °C), transferred to a glass v-vial and reduced to dryness under a gentle stream of nitrogen gas.

For liver samples, to a known weight of chilled liver (0.05 g) was added a known weight (0.05 g) of a solution containing the internal standard norleucine (3.8 mM). Next, an ice-cold mixture of methanol: chloroform: water (5:2:2) was added (1 mL), and the samples were homogenized on ice for 2 min. The samples were vortexed for 15 minutes, the organic supernatant was separated following centrifugation (15,000 ×g, 15 min at 4°C), transferred to a glass v-vial, at room temperature, and reduced to dryness under a gentle stream of nitrogen gas.

For both the blood and liver dried extracts, metabolites were derivatized by the additions of 60 uL of O-methoxylamine in pyridine (30 mg/mL) and 60 uL of N,O-bistrimethylsilyl trifluoro-acetamide (containing1% trimethylchlorosilane) with heating in a microwave (200 W for 90 seconds). Derivatized metabolites were separated by GC (HP6890 GC, HP50+ midpolarity capillary column) and full scan monitoring (50-650

molecular weight) of metabolite ions was performed on an MS (HP5973 Mass Selective Detector) under electron impact mode.

Individual metabolite data was extracted from RAW GC-MS spectra using AMDIS Version 2.71 (http://chemdata.nist.gov/mass-spc/amdis). Metabolite values were normalized to norleucine internal standard using G3835AA MassHunter Mass Profiler Professional (Agilent Technologies, Santa Clara, CA) following manufacturer's software instructions. Metabolites were identified in Mass Profiler Professional using the Fiehn Library (G1676AA).

Statistical Analysis

Differences between data were analyzed using Student's t-tests, and in the case of unequal variance, a Welch's t-test. Body weight, food and energy intake, body composition, and glucose clearance were analyzed in excel using Welch's 2-tailed t-test, with $P < 0.05$, as well as Chi Squared tests. Metabolomics data was analyzed in SAS/STAT® Software. Experiment 1 metabolomic data was analyzed using pooled student's t-test or Satterthwaite t-test. Experiment 2 metabolomic data was compared using Tukey multiple mean comparison test with ANOVA.

Chapter 4 - Experiment 1 Results and Discussion

Weight and Energy Consumption

Weight gain and Food efficiency.

The high fat diet successfully induced a steady weight gain in the DIO group up to week 8 (**Figure 4-1**). From weeks 4 to 8, the rate of weight gain was higher in the DIO group compared to the Control group. Previous studies with mice fed a similar HFD diet demonstrated that this pattern of body weight and fat gain continues beyond 8 weeks. The stability of this growth rate relative to the Control group (**Figure 4-2**) results from the higher energy density of the HFD (5.1 kcal/g), (i.e. about the energy-density of an average brownie). The DIO group gained weight mainly in the form of fat as opposed to protein (**Figure 4-9**), confirming that consuming the HFD diet for 8 weeks was adequate to achieve a state of obesity.

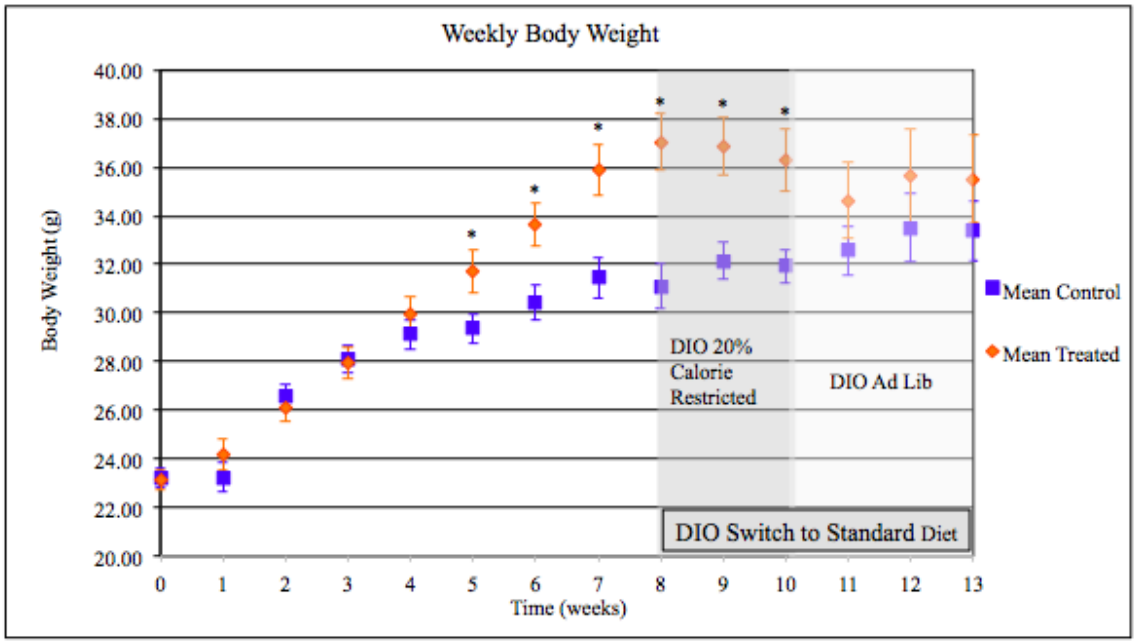

Figure 4-1. Experiment 1: Weekly body weight. Control mice were allowed *ad libitum* intake of the standard diet (SD) from week 0 to 13. From weeks 0 to 8 the DIO mice were allowed *ad libitum* intake of the high fat diet, from weeks 8 to 10 the DIO mice were fed the SD diet at 80% of caloric intake compared to the *ad libitum* period and from weeks 10 to 13 the DIO mice were allowed *ad libitum* intake of the SD. Individual data points represent the means of: Control: n=20 for weeks 1-4, n=14 for weeks 5-8, n=9 for weeks 9-10 and n=5 for weeks 11-13; DIO: n=21 for weeks 1-4, n=15 for weeks 5-8, n=10 for weeks 9-10, and n=5 for weeks 11-13. * indicates a significant ($P<0.05$) difference between groups. Error bars represent the standard error of the mean.

After switching to the SD diet and being fed at 80% of energy intake at the beginning of week 9, the DIO group immediately and steadily lost weight, indicating that the mice were not able to sustain their high body fat when fed the Control diet.

We quantified how well the two groups stored energy from food as body weight (i.e. how readily the body gained weight under a normal diet). This measurement, called "food efficiency," would help detect an obesity-induced change in the body's preferred amount of fat.

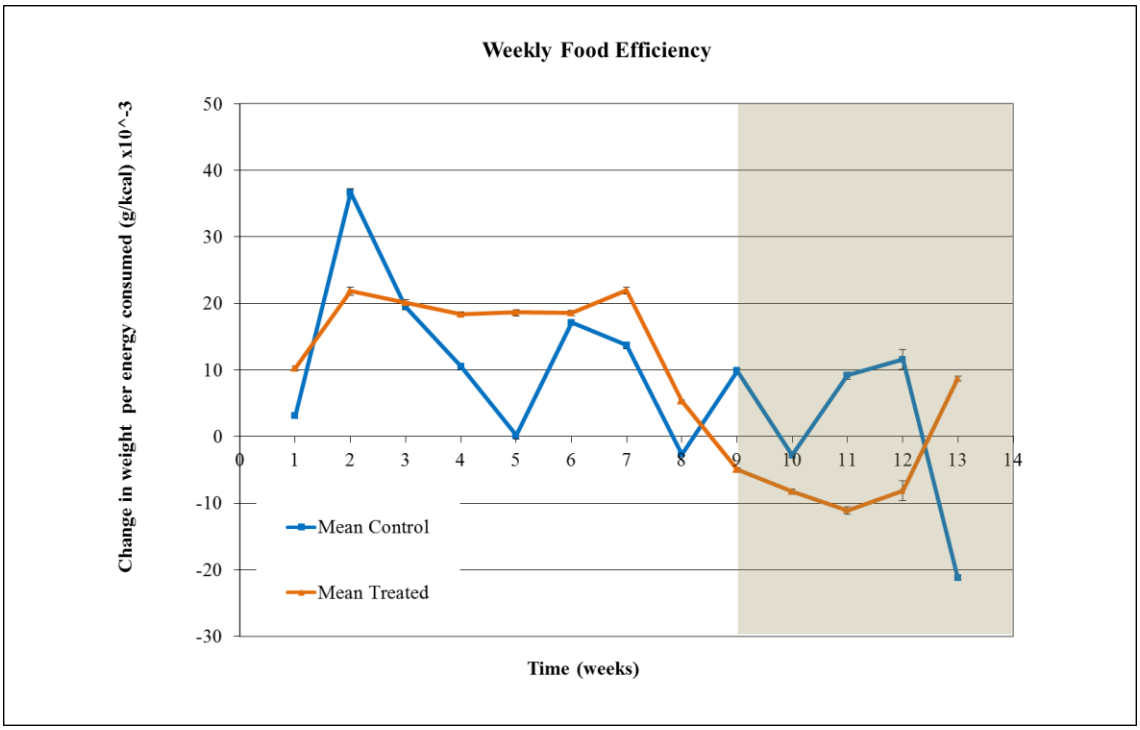

Figure 4-2. Experiment 1: Weekly food efficiency. Values represent the ratio of weight gained to calories consumed per week. Refer to Figure 4-1 for the descriptions of the 3 dietary periods for the DIO mice. Individual data points represent the means of: Control: n=20 for weeks 1-4, n=14 for weeks 5-8, n=9 for weeks 9-10 and n=5 for weeks 11-13; DIO: n=21 for weeks 1-4, n=15 for weeks 5-8, n=10 for weeks 9-10, and n=5 for weeks 11-13. Error bars represent the standard error of the mean.

We expected that following the period of food restriction, the DIO mice would retain a "metabolic imprint" that would eventually lead them to naturally continue to store dietary energy more efficiently once the mice were offered *ad libitum* intake of the SD diet.

The food efficiency plot in **Figure 4-2** reports this as the ratio: $\frac{\text{change in average weight during week}}{\text{average energy consumed during week}}$. However, this ratio is not meaningful when the caloric intake is very different between the groups; the DIO mice have higher food efficiency values up until the diet switch only because they are rapidly gaining weight as a result of their diet. The higher values do not necessarily imply that they are naturally better at

storing fat. Food efficiency as a measure of a natural ability to store fat is likewise not very meaningful during the weight loss period, when the ratio is negative (**Figure 4-2**). The weight of the DIO group would need to stabilize before food efficiency could demonstrate a metabolic imprint (by consistently remaining higher than the Control group's food efficiency). However, because the body weight of the food restricted DIO mice fed *ad libitum* never fully stabilized over the 3-week period, the food efficiency measurements failed to fully capture the metabolic imprint of the DIO group. The eventual increase in food efficiency over the final week of *ad libitum* intake, however, is promising because it means that weight loss was slowing and eventually reverting back to weight gain—possibly reflecting an imprint that predisposed the post-obese mice to maintain a higher body weight. Unfortunately, our study did not continue longer than the 3 weeks of *ad libitum* intake of the SD diet to further emphasize possible long-term changes in body weight and fat set points. The potential of obesity to cause this kind of metabolic imprinting will need to be investigated over longer periods of time than what was possible in the present study.

The nature of the diet: Food Intake vs. Energy intake.

When comparing food intake (**Figure 4-3**) to caloric intake (**Figure 4-4**), the difference between the two diets immediately becomes clearer.

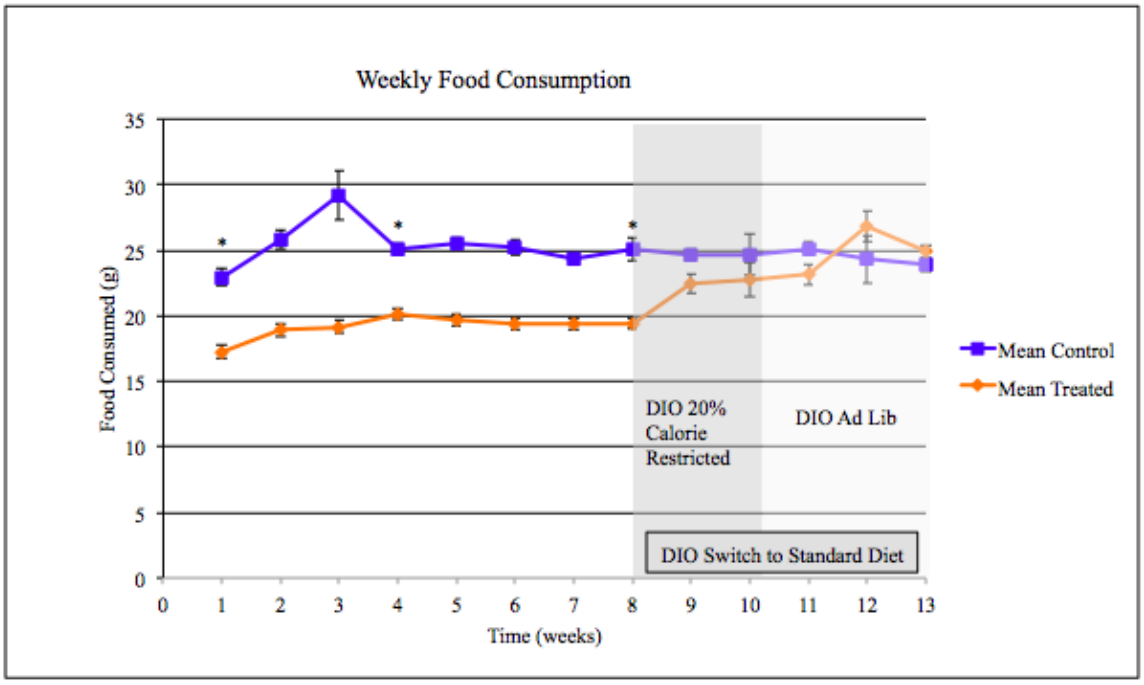

Figure 4-3. Experiment 1: Weekly food consumption. Weekly values represent the average intake (as-is basis) of each group. Refer to Figure 4-1 for the descriptions of the 3 dietary periods for the DIO mice. Individual data points represent the means of: Control: n=20 for weeks 1-4, n=14 for weeks 5-8, n=9 for weeks 9-10 and n=5 for weeks 11-13; DIO: n=21 for weeks 1-4, n=15 for weeks 5-8, n=10 for weeks 9-10, and n=5 for weeks 11-13. * indicates a significant ($P<0.05$) difference between groups. Error bars represent the standard error of the mean.

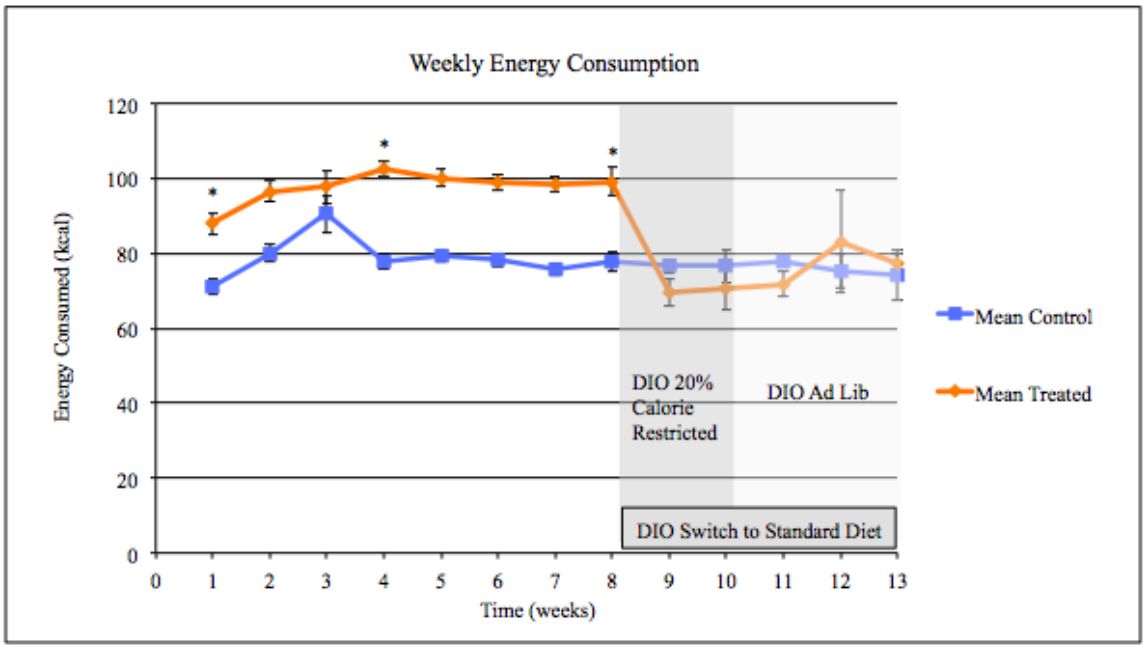

Figure 4-4. Experiment 1: Weekly energy consumption. Values represent the energy (kcal) consumed per week for each group. Refer to Figure 4-1 for the descriptions of the 3 dietary periods for the DIO mice. Individual data points represent the means of: Control: n=20 for weeks 1-4, n=14 for weeks 5-8, n=9 for weeks 9-10 and n=5 for weeks 11-13; DIO: n=21 for weeks 1-4, n=15 for weeks 5-8, n=10 for weeks 9-10, and n=5 for weeks 11-13. * indicates a significant ($P<0.05$) difference between groups. Error bars represent the standard error of the mean.

The DIO group consumed less food than the Control group during the first eight weeks, but consumed much more energy. This is possible due to the very high energy density of the DIO diet: 5.1 kcal/g for HF diet versus 3.1 kcal/g for the SD diet. This difference in energy density resulted in the DIO mice consuming less compared to the Control group. Thus, when the DIO mice were energy restricted after the switch to the SD diet, their weekly food intakes increased by 3.5 g (week 9, **Figure 4-3**) to a level that was similar to the Control mice.

Our methodology intended a two-week caloric restriction period (on the SD) starting the beginning of week 9 and then a switch to *ad libitum* feeding of SD for the next three weeks. Our plot of food intake however reveals that DIO mice did not eat

significantly more once given *ad libitum* access to the diet. For this reason, we executed an effective methodology, which consists of an eight week HFD feeding period followed by a five week "diet phase" for DIO mice.

Energy consumed over body weight.

There is also a relationship between food consumption and body weight (**Figure 4-5**). Each point represents the mass of food consumed and body weight of a single mouse during one week. The positive correlation coefficients, Control R=0.45 and DIO R=0.63, demonstrate a trend towards heavier (not necessarily DIO) mice consuming more food, as anticipated. The slopes of this relationship are nearly the same between Controls (slope=0.48) and the DIO (slope=0.49), implying that a given difference in body weight will, for each group, correspond to the same difference in food intake. Since the Control mice (blue line) lie above the DIO points (orange), the average food consumed per gram body weight is not equal between the groups; the Controls each consumed more per gram body weight.

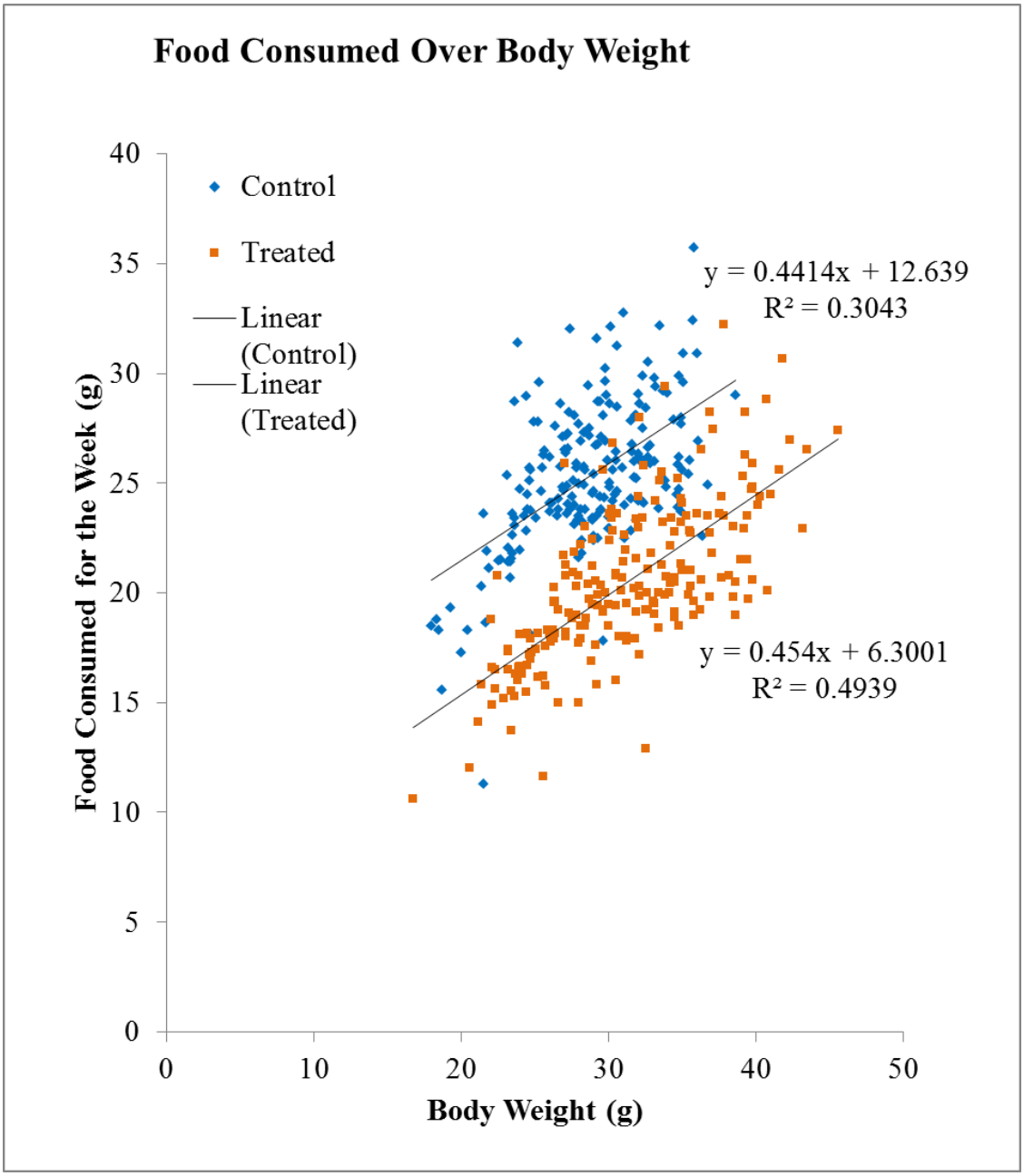

Figure 4-5. Experiment 1: The relationship between body weight and food consumed. This correlation relates food mass consumed in one week to body weight at the end of that week. Each value represents a single mouse at a given week, and mice at all time points are plotted. Mice are generally observed to eat in proportion to their body weight for both groups.

In a plot relating energy (kcal) consumed to body weight (**Figure 4-6**), the difference in dietary energy density resurfaces. The DIO points now lie above the Control group points; DIO mice took in more calories per gram body weight than Controls, on

average. This scaling effect on the energy also causes the slope of the DIO group linear fit to be notably larger. The larger slope means that a given difference in body weight will correspond, within the DIO group, to a larger difference in caloric intake. Likewise, within the Control group, the same difference in body weight would correspond to a lesser difference in caloric intake. This fact has significant implications for an understanding of obesity and will be addressed later.

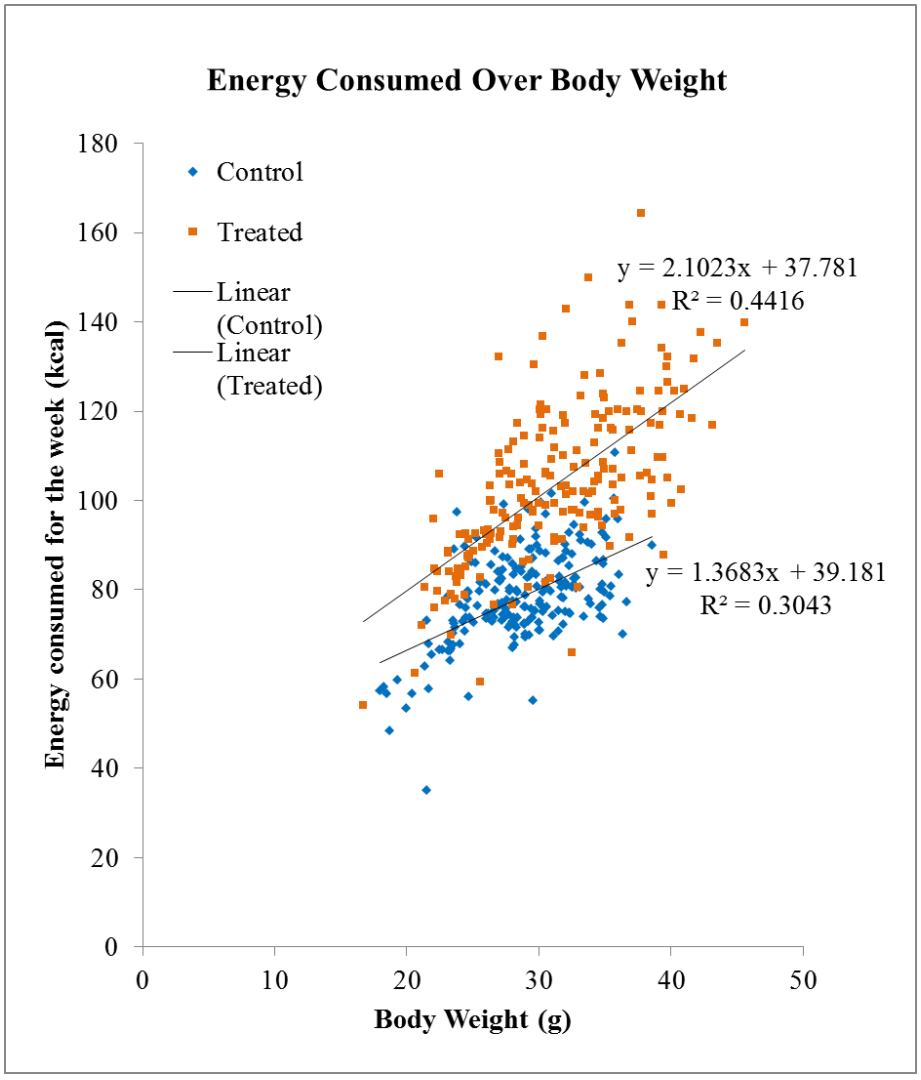

Figure 4-6. Experiment 1: Energy consumed correlated to body weight. Different dietary energy densities resulted in a scaling effect on the slopes of the plot. Between a light and heavy mouse in the DIO group, there is a greater difference in energy intake.

Finally, in a plot relating the ratio $\frac{\boldsymbol{energy\ consumed}}{\boldsymbol{body\ weight}}$ to body weight (**Figure 4-7**), we find that the kcals consumed per gram body weight tends to decrease as body weight increases. That is, although heavy (not necessarily DIO) mice ate more food, they consumed a relatively smaller proportion of their body weight compared to lighter mice.

Taken together, these trends (**Figures 4-5**, **4-6**, **4-7**) may shed some light on the mechanism underlying the development of obesity. It is assumed that when provided *ad libitum* access to food, mice will consume to the point of satiation. How much food is satiable for each mouse is more or less proportional to their body weight, demonstrated by **Figure 4-5**. However, when the heavier mice in the DIO group ate to satiety (i.e. intake proportional to their body weight), they consumed energy disproportionately due to the high energy density of the diet. Thus, since food (mass) consumption is proportional to body weight and an energy-dense diet causes a scaling effect on energy intake, when the more energy-dense diet is fed, there is a greater caloric excess for heavy mice than for light mice. This fact is best demonstrated in **Figure 4-7**. If we consider the linear fit for the Control group to be a healthy ideal—a prescription for healthy energy consumption based on body weight—we see that there is a greater deviation from this ideal for heavy members of the DIO group than for light members, suggesting that a high energy diet is worse for heavy individuals because it tends to result in a greater deviation in caloric intake from a healthy ideal. These conclusions can help explain how obesity can develop; for heavy individuals, a satiable amount of food often translates to a disproportionately greater intake of calories—*if* that food is energy-dense.

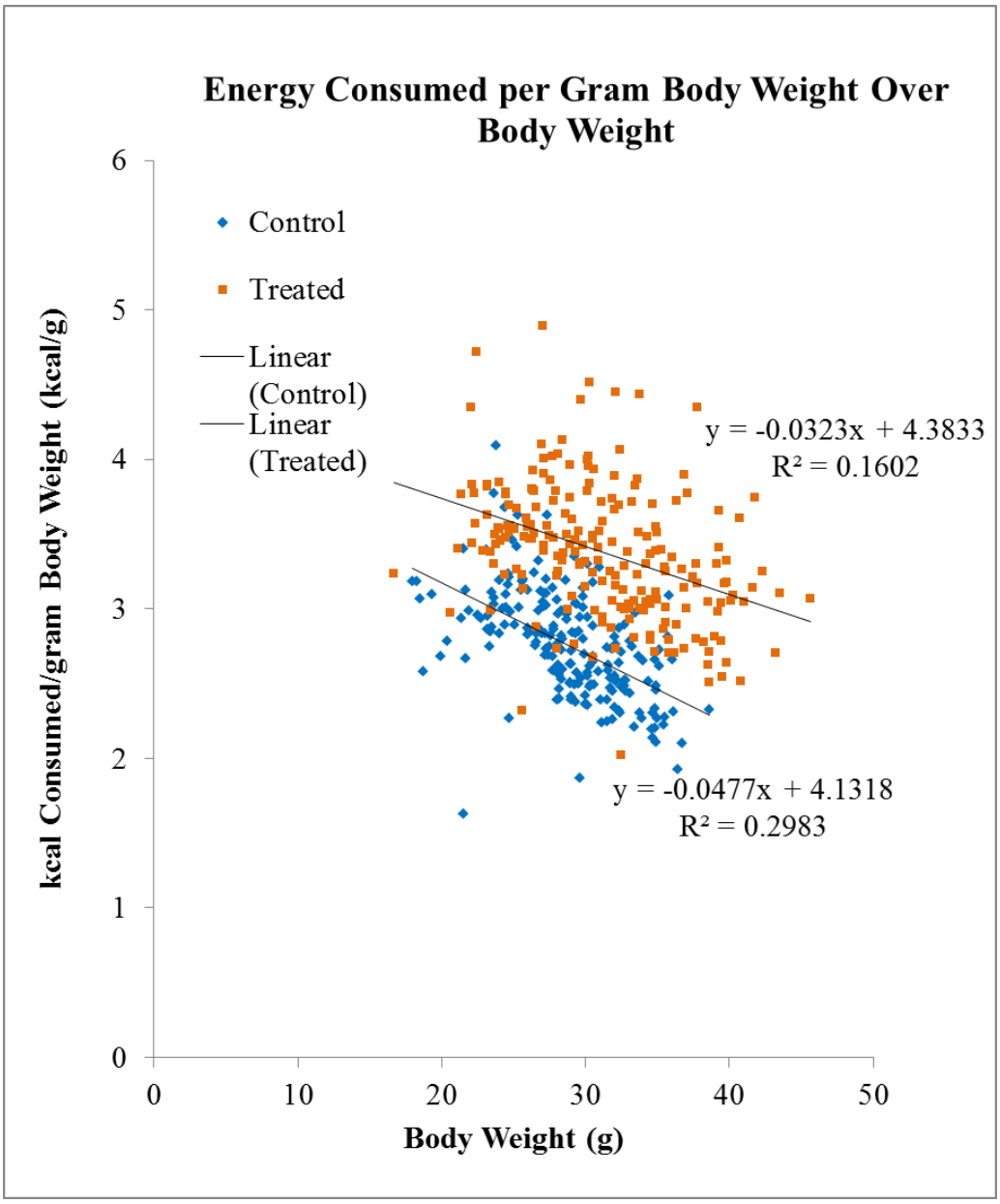

Figure 4-7. Experiment 1: Energy consumed per gram body weight *vs.* body weight. The plot indicates that as mouse body weight increased, they consumed less energy per unit body weight. For DIO mice on the heavy end, there is a greater upwards deviation from their Control counterparts. If one considers the Control group's linear fit a healthy ideal, this implies that a HFD is worse for heavier mice.

Body composition

Group totals over time.

Changes in body composition (lean dry mass, water and fat mass) are illustrated in **Figure 4-8**. For both groups, lean dry mass remained largely constant over the course of the 13 week experiment such that changes in weight were attributable to fluctuations in body fat and water mass. At week 4, there was not a significant difference in fat content between the DIO and Control groups. By week 8, however, the DIO group displayed a higher ($P < 0.005$) proportion of body fat (DIO, 43.34% vs. Control, 22.66%) and, in turn, a lower body water percentage. The DIO group's rise in fat mass with no change in lean dry mass from week 4 to 8 suggests that the steady weight gain shown in **Figure 4-1** was due almost exclusively to accumulation of fat mass. Evidently, consumption of the HFD diet by the DIO mice resulted in a linear increase in fat mass ($R = 0.996$), while lean mass gain remained constant. The slope of a linear fit over the 8 weeks of consuming the HFD diet (**Figure 4-9**) indicates an average growth rate of 1.80 (± 0.08) g per week. A chi^2 test comparing data to the fit yields $P=0.90$, indicating excellent agreement of DIO weight gain with a linear model.

Following two weeks of calorie restriction, while the DIO mice lost body weight, body fat percentage was not affected. Because the Control group increased their body fat content over this same period to 32.47% ($P<0.005$), there was a large but not significant difference between the DIO and Control groups over the 2 week period the DIO mice were energy restricted (DIO 41.13%, Control 32.47%, $P=0.087$).

After 3 weeks of consuming the SD diet *ad libitum*, there was a reduction ($P<0.05$) in body fat content of the DIO mice compared to values of the DIO mice at the

end of the initial 8 week period of consuming the HFD diet. This surprising decrease in body fat even after 3 weeks of *ad libitum* intake of the SD diet may be due to the continued weight loss of the DIO group during the first 2 weeks of consuming the SD diet *ad libitum*, during which the DIO mice maintained the same caloric intake as during the calorie restriction period. Over this same time period, the Control mice reduced their body fat content from 32.57 to 27.62%. It is unclear why the Control group showed a spike in fat content at week 10.

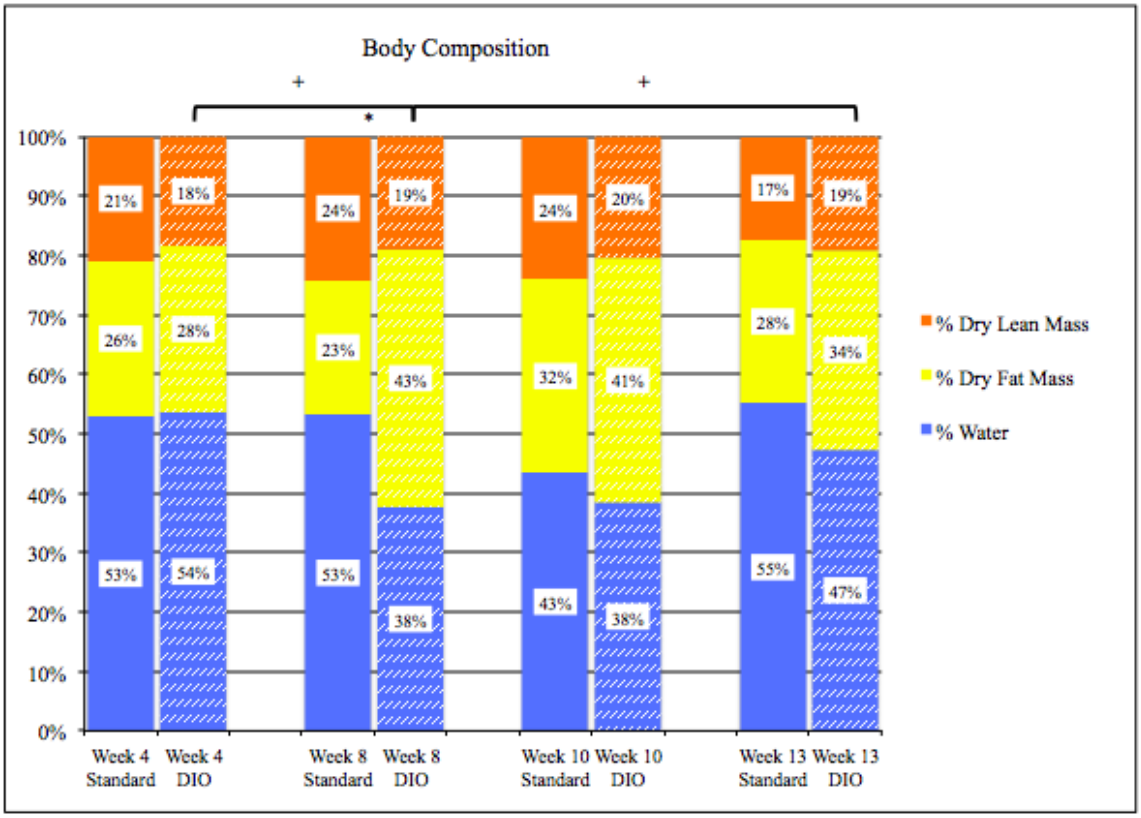

Figure 4-8. Experiment 1: Body composition. Dry lean mass represents dehydrated and fat-extracted non-visceral tissue, fat mass represents all ether extractable lipids and water mass is the loss in carcass weight after samples were lyophilized. DIO mice had a greater ($P<0.005$) proportion of body weight as fat mass after consuming the high fat diet for 8 weeks compared to Control mice consuming the standard diet and compared to the DIO mice at week 4 and week 13. This excess fat impinges on body water while dry lean mass is fairly constant.

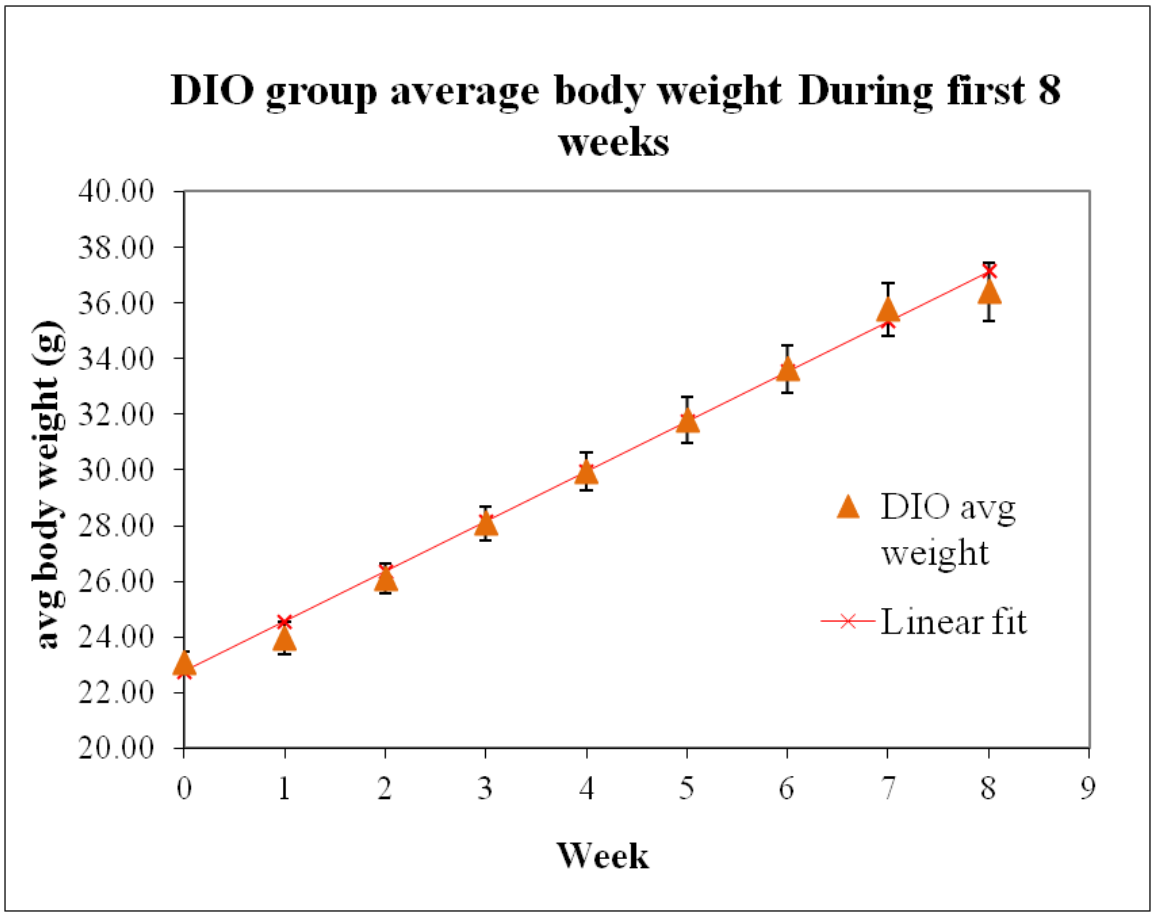

Figure 4-9. Experiment 1: Linear weight gain of DIO mice when consuming the high fat diet. A χ^2 test of linear fit yields P = 0.9; R=0.996. This constant growth rate is hypothesized to be primarily due to fat gain. Error bars represent the standard error of the mean.

Glucose Clearance

The following figure shows the results of the glucose tolerance test at weeks 4, 8, 10, and 13. The blood glucose concentration at each time point represents the mean (n=5-6) value for each group.

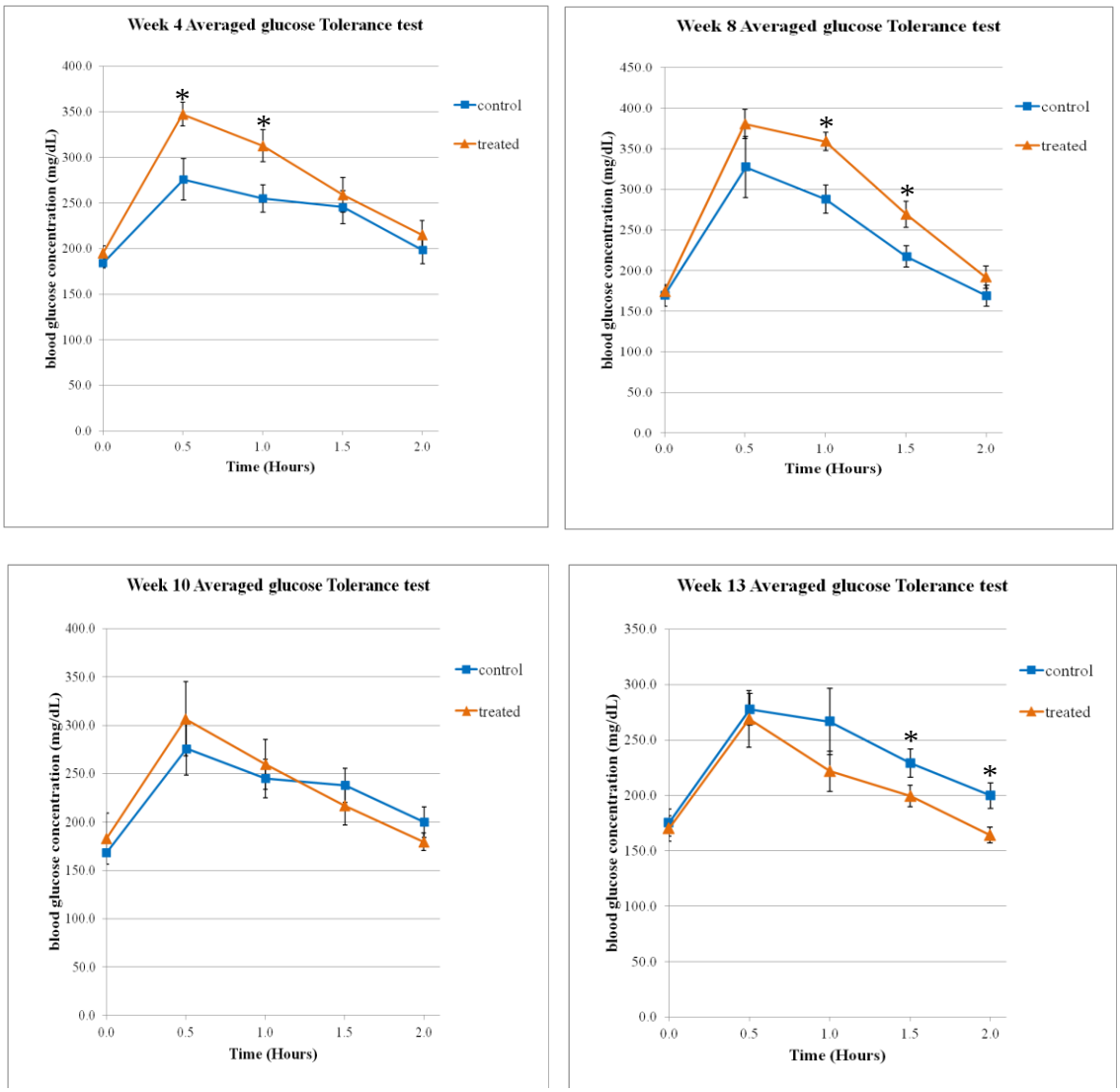

Figure 4-10. Experiment 1: The mean of blood glucose concentrations following administration of 2g/kg glucose dose. Higher ($P < 0.05$) blood glucose concentrations were observed in DIO mice at weeks 4 and 8 compared to Control mice, clearly indicating impairment in the ability of the DIO mice to clear blood glucose. The DIO curve appears to be translated downward for the two test points after the diet switch (weeks 10, 13), indicating a progressively improving glucose clearance. Error bars represent standard error of the mean. (Control n=4-6, DIO n=6)

Chi-squared tests were used to evaluate the goodness of fit of the DIO mice to the Control mice at every data collection point. The curves differed ($P < 0.001$) at all time points except week 10. Upon integration, we saw a downward trend in blood glucose levels of the DIO group following their switch to the SD diet and energy restriction for 2

weeks. From weeks 8 to 13, the DIO groups' glucose concentration curve crosses over that of the Control group (**Figure 4-10**). The maintenance of the downward translation of the curve indicates a steady increase in glucose clearance in the DIO group. Hence, the DIO group developed a greater ability to clear blood glucose following the 2-week period of energy restriction on the SD diet. However, by week 13, the DIO glucose curve appears to over compensate given that the curve is lower than the Control curve at all time points after t=0. This downward deviation is significant (χ^2=18.0, P=0.001) and may represent a hysteresis effect caused by the period of obesity.

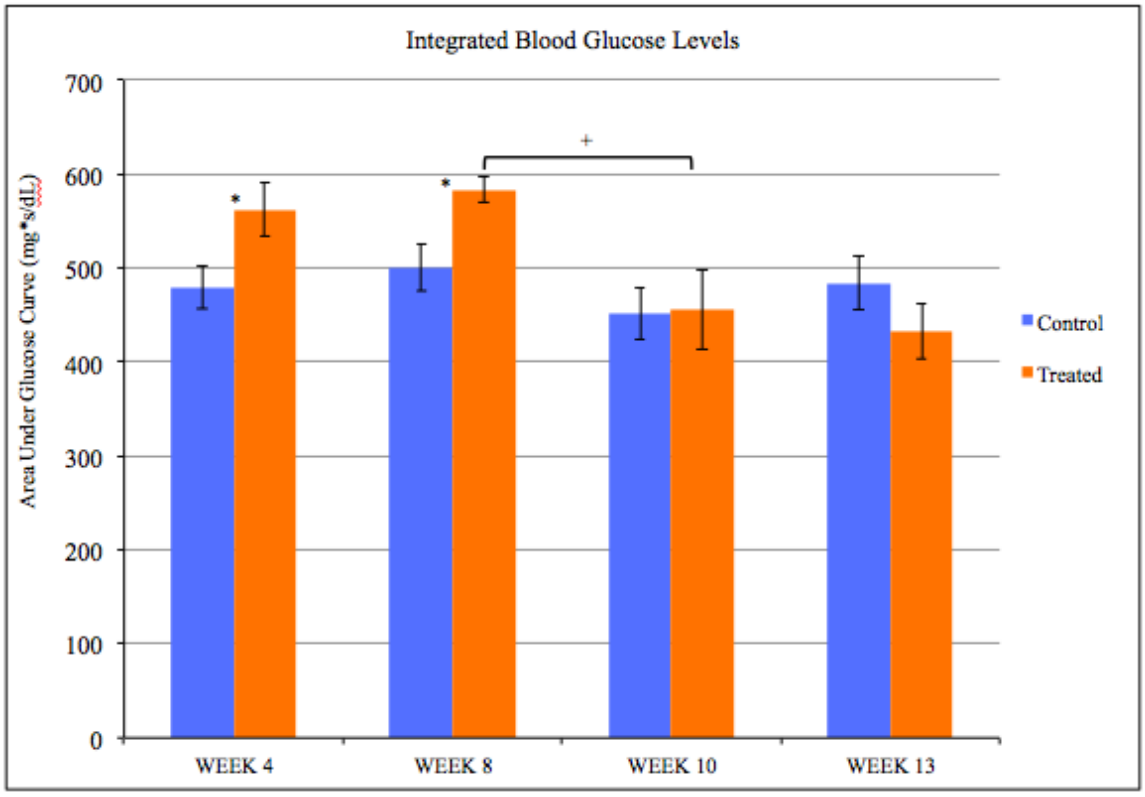

Figure 4-11. Experiment 1: Area under the curve of blood glucose concentrations during the 120 min period following administration of a 2g/kg glucose dose. Glucose clearance for the Control mice remained similar throughout the 13 week experiment. In contrast to the Control mice, glucose clearance by the DIO mice at weeks 4 and 8 was lower (P<0.05). After 2 weeks of calorie restriction, however, the DIO mice improved (P<0.05) their ability to clear blood glucose. There was no significant difference between the Control and DIO groups at week 13. Error bars represent the standard error of the mean. (Control n=4-6, DIO n=5-6)

Figure 4-11 provides another comparison between the DIO and Control groups. Each bar represents the area under the curve of the data from **Figure 4-10**. Higher values indicate a reduced ability to clear blood glucose, a sign of insulin resistance. At weeks 4 ($P < 0.05$) and 8 ($P < 0.005$), the DIO group had higher integrated values compared to the Control group, thus the DIO mice were displaying symptoms of the metabolic syndrome of obesity. By contrast, there were no differences in glucose integrated values between the DIO and Control groups at weeks 10 and 13, (i.e. 2 and 5 weeks after the diet switch). The DIO integrated values were not different when comparing week 4 to 8 and week 10 to 13. However, the values decreased ($P < 0.005$) for the DIO group between weeks 8 and 10 when the DIO mice were switched from *ad libitum* consumption of the HFD diet to energy restricted intake of the SD diet.

The results agree with our previous expectations of the effect of a high fat diet and high body fat on glucose clearance. After 4 weeks on a high fat diet, the DIO group exhibits significantly elevated glucose intolerance, a sign of diet induced insulin resistance. The glucose intolerance progresses as the group gains weight, from week 4 to 8. Finally, the sudden reduction in the DIO group's dietary NEFA after week 8 induces a rapid improvement in glucose clearance implying that a change in diet has powerful effects on the body's ability to metabolize glucose.

Glucose Clearance and Body Composition.

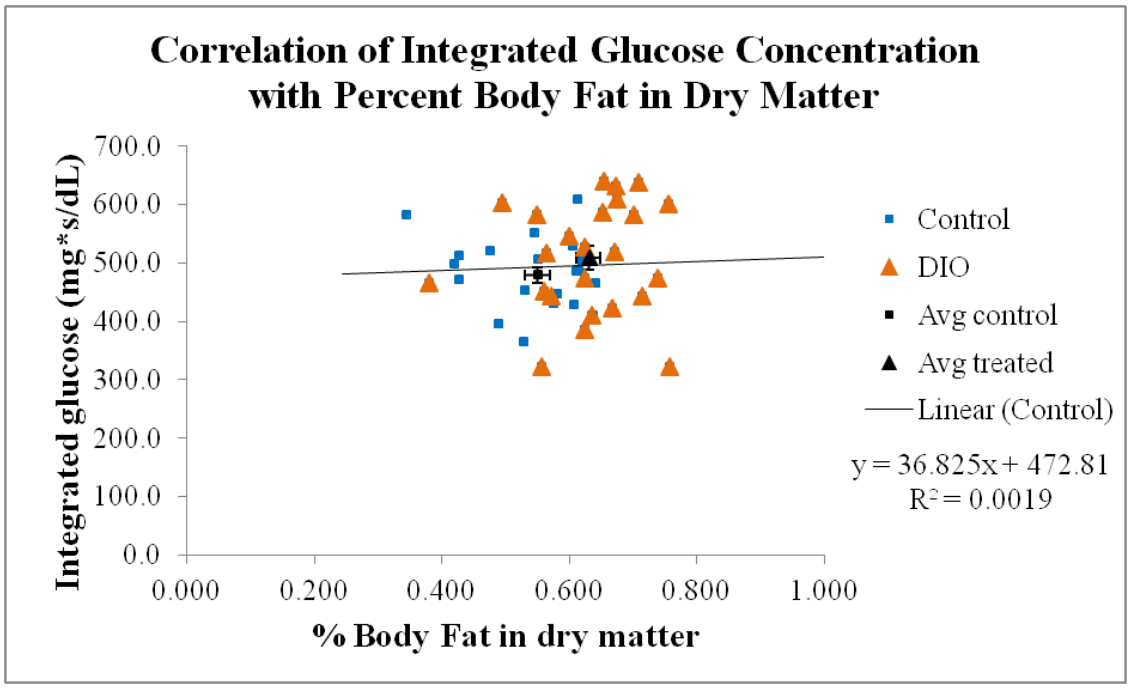

Figure 4-12. Experiment 1: Correlation of integrated glucose concentration with percent body fat in dry matter. There is virtually no correlation between these two variables. Group means are only significantly separated in the dimension of percent body fat. Error bars represent standard error.

Although the glucose clearance trend correlates strongly with increases and decreases in weight and percent body fat, investigating whether the glucose clearance values are tied to the fat stores of mice requires a plot relating integrated glucose values to percent body fat (**Figure 4-12**). Each point represents an individual mouse's integrated glucose curve value and percent body fat by mass in the dehydrated carcass. The plot reveals a poor relationship (R=0.0019) and a relatively low, positive slope.

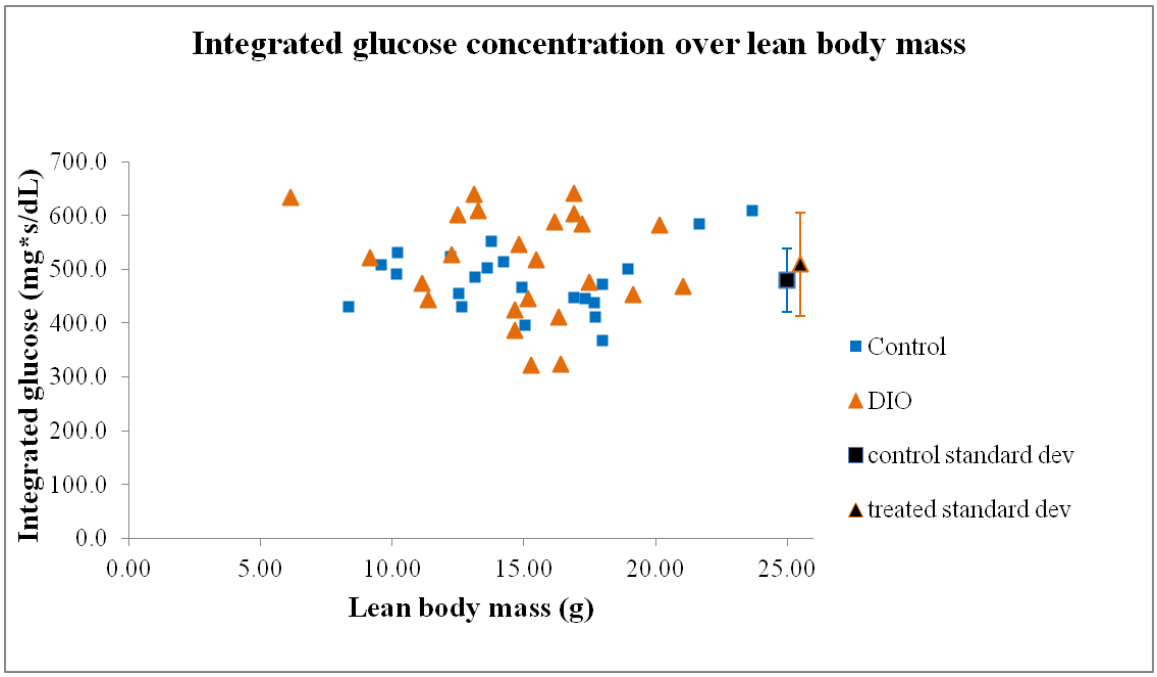

Figure 4-13. Experiment 1: Integrated glucose concentration per unit lean body mass. We observe no correlation between these two variables. Groups are not separated in the dimension of lean body mass. Black marks are placed to the side to illustrate group means (in glucose integrals) and relative variances. Error bars represent standard error.

Glucose clearance correlates poorly with lean body mass, the sum of dehydrated lean mass and water mass (**Figure 4-13**). Since blood glucose is primarily taken up by muscle, a major form of lean tissue, the integrated curve values should decrease as lean mass increases. However, there is virtually no correlation. Due to the lack of dependence on lean mass, the glucose concentration measurements were not standardized. Any attempt to standardize glucose concentration values to lean mass would increase the slope of the data since mice with lean mass have presumably lower readings of blood glucose concentrations.

Figures 4-12 and **4-13** and **Figures 4-8**, **4-8**, and **4-11** above show a lack of a strong correlation between glucose clearance and percent body fat or lean mass on an individual basis and group average basis respectively. The DIO group's glucose clearance

dramatically improved, with little change in body composition. The results suggest that the diet composition, particularly the rich saturated fat content in the HFD, likely exerts a greater metabolic influence on glucose clearance. The overcompensated glucose clearance in the DIO group can be attributed to a hysteresis effect (i.e. imprinting) due to the diet history of the DIO group. Weight and body composition are vastly different at week 10, after 2 weeks of diet, however, glucose clearance equalizes between the two groups. The analysis of dietary fat on glucose clearance and liver NEFA was investigated using metabolomics in subsequent sections.

Metabolomics

Review of Diet Composition.

The standard diet and HFD have energy densities of 3.1kcal/g and 5.1kcal/g, respectively. For mice in the Control group, caloric intake is divided into three parts to reflect healthy nourishment for mice. The Control group derives 24% of calories from protein, 58% from carbohydrates, and 18% from fat. For the DIO mice, 18% of calories come from protein, 21% from carbohydrates, and 60% from fat. The high dietary fat content consists mostly of saturated fats that are broken down into NEFA, which subsequently accumulate in the liver, blood, muscle, and adipose tissue. The results indicate that the amount of hepatic NEFA varies with diet composition and correlates significantly with the state of glucose clearance.

Dietary fat, glucose clearance, and insulin resistance.

The accumulation of triglyceride stores in muscle and elevated amounts of circulating NEFA are strongly associated with impaired insulin action. However, postulates that describe the influence of high lipid availability on glucose metabolism

vary greatly. Literature suggests an impairment of glucose transport due to the down regulation of GLUT-4, decreased activation of insulin receptor tyrosine kinase, overstimulation of glucose production by the liver, and inhibition of glucose oxidation through a variety of pathways (Kahn & Flier, 2000).

Research studies acknowledge the competition for respiration between glucose and fatty acids, which leads to a reciprocal relationship between their metabolisms (Randel 1999). Elevated NEFA and muscle triglyceride stores cause increased fatty acid oxidation, which impairs whole-body glucose oxidation and induces insulin resistance though a number of mechanisms (Storlien et al., 2006). Authors have shown that insulin sensitivity is inversely correlated with muscle triglyceride accumulation. Conversely, high blood glucose tends to inhibit fatty acid oxidation (Storlien, Jenkins, Chisholm, Pascoe, Khouri, & Kraegen, 1990). Fatty acids also play a central role in the development of insulin resistance. Prolonged exposure of islet β-cells to high circulating NEFA leads to attenuation of the secretory response to glucose. NEFA also induce the downregulation of GLUT-4 in myocyte membranes and thus prevent rapid glucose uptake. Finally, insulin resistance may also be caused by elevated transport of NEFA into mitochondria (Kien, 2009).

Liver NEFA and glucose clearance on an individual basis.

In individual mice, the results showed a weak and unexpectedly negative correlation between total NEFA levels (a sum over all NEFA investigated) and glucose clearance (**Figure 4-14**).

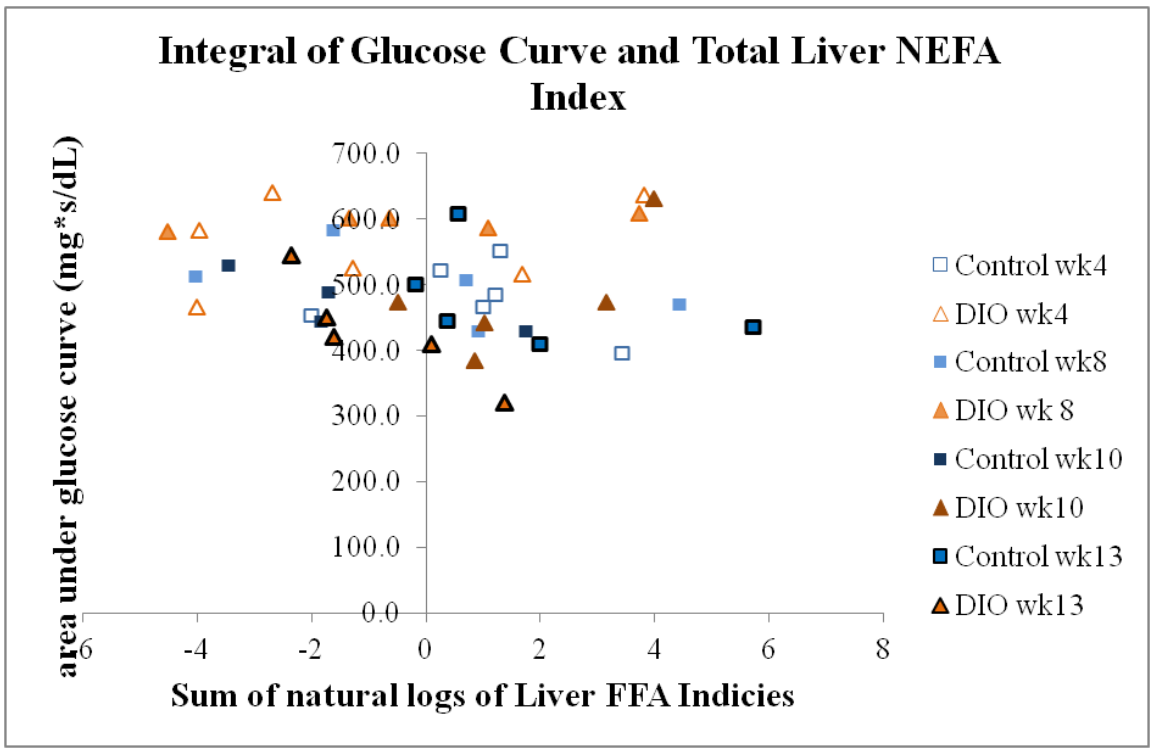

Figure 4-14. Experiment 1: Integral of glucose curve and total liver NEFA index. Total liver NEFA index (a sum) is intended to be an indicator of generally elevated NEFA concentrations.

Since higher glucose curve integrals represent a relative inability to clear glucose, a positive correlation with total NEFA levels in both groups is expected. However, the outcome of this correlation may be complicated by the fact that not all dietary NEFA produce a deleterious effect on glucose clearance. Polyunsaturated fats such as linolenic (Ω-3) and linoleic acids (Ω-6) can, when introduced into a diet rich with saturated fats, lead to improved insulin sensitivity and may even prevent the development of insulin resistance (Storlien, Jenkins, Chisholm, Pascoe, Khouri, & Kraegen, 1990). Our experiment indicates a large variance in total NEFA within a given group (e.g. DIO week 8 or Control week 8); this is possibly due to the high sensitivity of metabolomics to conditions preceding measurement. Despite the lack of a positive correlation between the total NEFA in an individual mouse and its glucose curve integral, group averaged NEFA

levels (covered in next section) reveal significant changes during dieting that correlates strongly with the improved glucose clearance of the DIO group. Reduced circulating NEFA, and subsequent improved insulin sensitivity, suggests an explanation for the rapid improvement in DIO glucose clearance.

NEFA over time.

We observed the largest group differences in liver NEFA at weeks 4 and 8. **Figure 4-15** shows group average metabolite peak areas for the four fatty acids investigated. At the first two test points (pre-diet phase), DIO livers have notably higher levels of linolenic, palmitic, and linoleic acids. They have lower levels of oleic acid. After two weeks on the standard diet (week 10), several DIO fatty acid levels aligned with Control levels, none, however, proved significantly different (**Figure 4-18**). This is clearly the result of the severe reduction in dietary fat intake of the DIO group. The realignment of DIO liver fatty acid levels after dieting and relative fixity of their fat stores demonstrates that dietary fat intake contributes heavily to liver NEFA levels (**Figure 4-18**).

Palmitic acid is the main component of dietary saturated fat and adipose tissue stores. The observed significantly elevated DIO concentrations of palmitic acid are thus consistent with our expectations for a diet high in saturated fat. An excess of palmitic acid in both blood and liver fat stores is associated with the development of insulin resistance. **Figures 4-10** and **4-15** demonstrate a high correlation between the DIO group's relative glucose clearance and concentration of palmitic acid in liver. Glucose clearance worsens at test points where palmitic acid is high. The shift in Control levels

versus DIO levels of palmitic acid mirrors that of glucose clearance, although neither trend is significant.

Oleic acid is a *cis* monounsaturated fatty acid and essential dietary fat component. Several mono- and poly- unsaturated fatty acids, including oleic acid, have been shown to markedly improve insulin sensitivity when included in a diet high in saturated fat. Diets low in oleic acid have been associated with insulin resistance. The relatively high Control group levels of oleic acid and low DIO levels are likely due to respective diet composition. The lack of this essential fatty acid in HFD for the first 8 weeks may have contributed to insulin resistance in the DIO group (**Figure 4-15**).

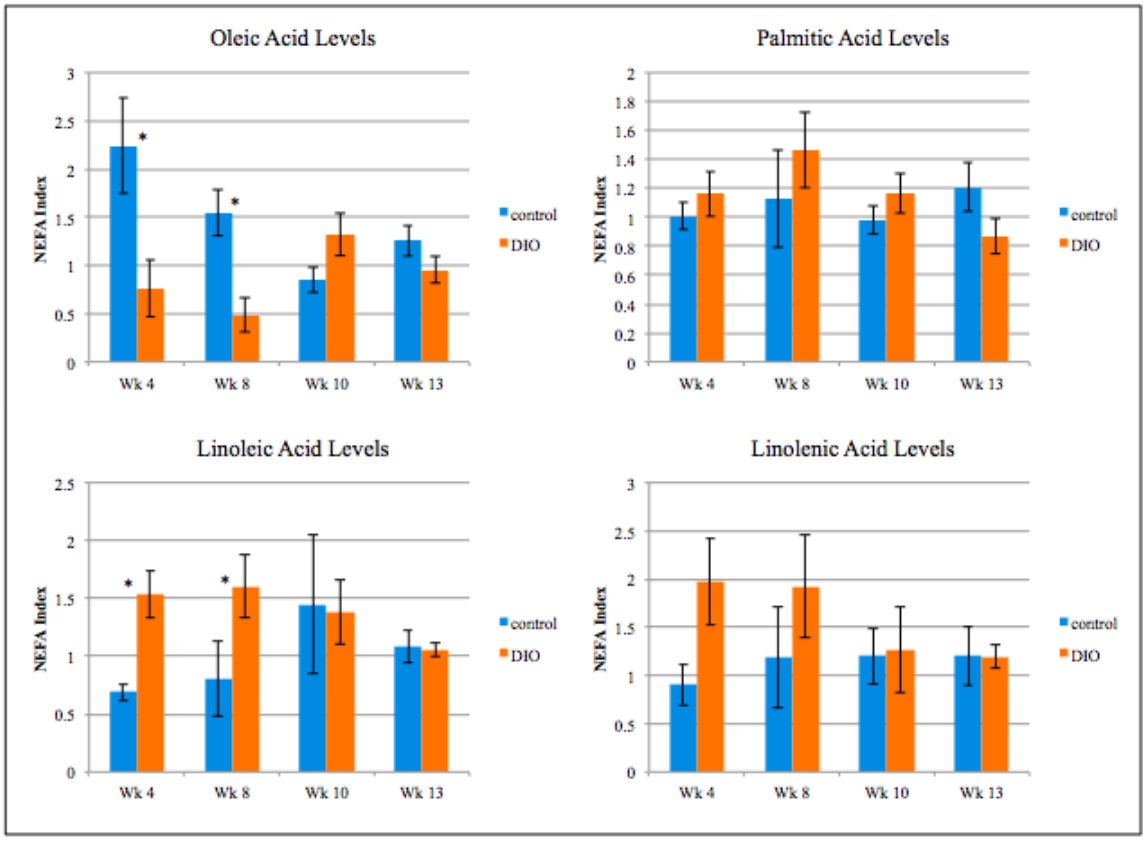

Figure 4-15. Experiment 1: Relative levels of liver non-esterified fatty acids. Bars represent the means (Control n=4-6, DIO n=5-6) of each group and error bars represent the standard error of the mean.

Principle Component Analysis of NEFA metabolic profiles.

To gain an understanding of the NEFA profile of the group as a whole, we performed a Principle Component Analysis (PCA) of the collection of individual NEFA profiles. In PCA, each mouse's profile is represented by a point in an n-dimensional space where the position along each dimension is the liver concentration of a different fatty acid (n=6, fatty acids). The space is compressed mathematically into a 3-dimensional space where the original points are represented, with some loss of information. This assumes that within a group, proximity of NEFA levels along any set of dimensions will emerge as spatial clustering in the 3-dimensional "projection." Hence, when members of a group are similar in at least some of their NEFA levels, the 3D representation will manifest a spatial nearness.

Figure 4-16 shows the 3D representations of individual NEFA profiles of mice at each test point. The plus signs represent the center of mass of each group, and the spheres represent the standard deviation of the points from their center of mass. The spatial separation of the centers of mass (in distance) is used as a metric to evaluate differences between the NEFA profiles of the groups as a whole. Therefore, greater separation of the groups' clusters signifies a greater difference between their respective metabolic profiles. More tightly clustered groups (smaller spheres) have members that are more similar in their NEFA levels.

This depiction of data allows the ability to test significance in the difference between our groups' metabolic profiles. We found that Control and DIO clusters are significantly spatially separated, and thus have significantly different NEFA profiles, at all weeks except week 10. The corresponding P-values are: P_4=0.0087, P_8=0.0568,

P_{10}=0.109, and P_{13}=0.0761 using α=0.10. The constant α is set at 0.10 because spatial clustering is difficult to produce unless several metabolites differ between the groups. The distance between the "means," or in this case the centers of mass, produces a test statistic for a Welch's T-test. The standard deviations are the sphere radii allowing statistical testing of the spatial separation of data clusters in 3D.

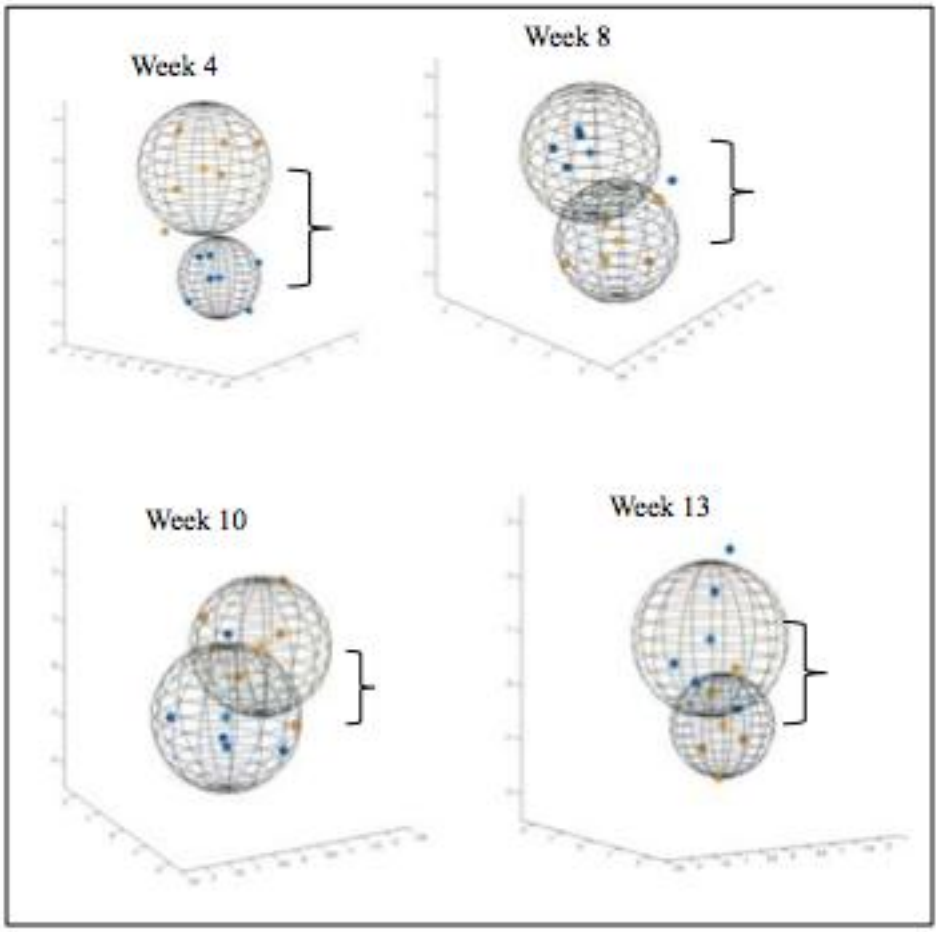

Figure 4-16. Experiment 1: Three-dimensional projections of metabolic profiles at each test point. All axes are effectively dimensionless. Plus signs are the centers of mass for each group, and sphere radius represents the standard deviation of points from their center of mass. Each point represents an individual mouse's metabolic profile (its position determined by the unique amounts of each NEFA in its liver sample). Tighter clustering of a group represents closeness of the members' NEFA profiles. Spatial separation of centers of mass implies systematic deviation of the group members from each other. Groups are significantly different at weeks 4, 8, 13 ($P<0.1$).

The PCA analysis reveals that members of the Control group differ systematically from their DIO counterparts in NEFA levels. For a given NEFA, members of each group tend to have similar levels amongst themselves and to differ systematically from members of the other group. That is, members of a group vary together across multiple

NEFA. This is the best evidence the difference in glucose clearance seen at weeks 4, 8, and 13.

Energy Pathways and Glucose Metabolism

Lactic Acid.

The trend of hepatic lactic acid levels from weeks 4 to 13 was established in a similar fashion as the fatty acid trends previously discussed. This trend was indicative of notable metabolic differences established between the DIO and Control groups, persisting during food restriction and increasing during subsequent *ad libitum* dieting periods.

Hepatic concentrations of lactic acid were similar for the DIO and Control groups at week 4, as shown in **Figure 4-17**. By week 8, when differences in energy intake, body weight, body fat proportion, and glucose clearance were largest compared to the Control group, hepatic lactate levels deviated slightly but in opposite directions relative to week 4. After the 2 week food restriction period of the DIO group, hepatic lactic acid levels remained fairly constant in both groups relative to their respective levels in week 8. After 3 weeks of *ad libitum* intake of the SD by the food restricted DIO group, hepatic lactic acid levels were significantly higher ($P < 0.0005$) than in the Control group. Similarly, metabolomics analysis of blood showed that lactic acid was also higher ($P < 0.002$) in blood of the DIO group after 3 weeks of *ad libitum* consumption of SD diet compared to the Control group.

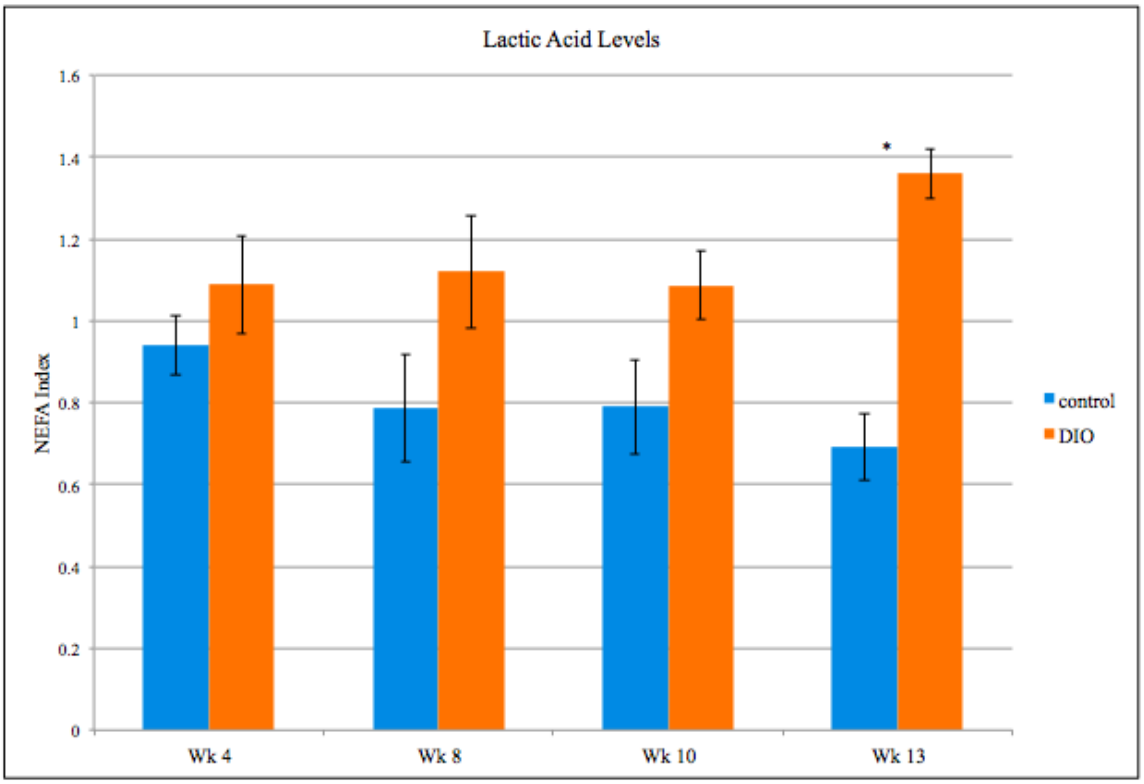

Figure 4-17. Experiment 1: Liver lactic acid levels. Liver lactic acid was higher ($P<0.05$) in the DIO mice at week 13 following a 3 week period of consuming the standard diet *ad libitum*. Error bars represent standard error. (Control n=4-6, DIO n=5-6)

Prior studies have shown that the concentration of lactate in blood and liver is elevated in DIO mice compared to their lean counterparts (Xie, Waters, & Schirra, 2012). This elevation of lactate was suggested to occur because anaerobic glycolysis is up-regulated in obese mice, which may cause the elevation of lactate levels. Also, lactate release from adipose tissue is increased because of increased mass in obese individuals.

Hepatic gluconeogenesis is up-regulated during fasting or periods of starvation when blood glucose levels are low. And, in animal models of insulin resistance and metabolic syndrome brought about by DIO, hepatic gluconeogenesis is also up-regulated. Blood lactate, derived mostly from muscle metabolism of glucose, is a major gluconeogenesis precursor taken up by the liver as part of the Cori Cycle. Elevated

lactate suggests an up-regulation of lactate uptake by the liver. It has been suggested that blood lactate may even serve as a suitable marker for overall dysregulation of hepatic glucose production (Xie et al., 2012). While the latter would seem to be compatible with our findings of elevated blood glucose (P=0.057) but lower hepatic glucose (P=0.0512) in the DIO group at week 13 (decreased hepatic glucose signifies a need for up-regulated glucose production), it is at odds with the fact that glucose clearance improved once DIO mice were food restricted and subsequently fed *ad libitum* the SD diet for 3 weeks. However, because of the persistent elevation and increase over time of lactate, and the elevation of other gluconeogenic substrates (see below), gluconeogenesis as a viable indicator of derangement in the obese state should be considered a potential indicator of persistent metabolic dysregulation.

With respect to liver AMPK, it is notable that AMPK activation in the DIO was considerably elevated after 3 weeks of consuming the SD diet *ad libitum*. As noted above, when hepatic AMPK is activated, gluconeogenic gene expression and hepatic glucose production are reduced. Thus, we would have hypothesized that if there is higher hepatic AMPK activation of the *ad libitum* fed DIO mice, signaling the need for ATP generation, then gluconeogenesis would be reduced to allow for the partition of substrates toward the Krebs cycle for oxidation. The fact that hepatic levels of gluconeogenic substrates remain elevated through week 13 suggests some disruption in this progression prevents oxidation of Kreb's cycle intermediates. Perhaps some upstream or downstream components of the AMPK signaling pathway and/or metabolic pathways regulated by AMPK (i.e. gluconeogenesis) have been altered long term such that the metabolic

signature of the obese state continues to persist even when the mice were fed a standard lower fat diet.

Previous studies have shown that NEFA concentration is positively associated with greater rates of gluconeogenesis, possibly due to increased ATP and NADH production via pyruvate carboxylase activity, a key enzymatic step to ensure high rates of acetyl-CoA and thus fatty acid oxidation (Consoli, Nurjhan, Reilly, Bier, & Gerich, 1990). Our findings that several liver fatty acids are persistently elevated in the DIO group seem to support this view. Such patterns of dysregulation may be involved in some general change in metabolic set point that is suggested by our findings. Again, specific components in these pathways as well as others regulating gluconeogenesis must be examined to further confirm this view.

Succinic Acid, Malic Acid and Pyruvic Acid.

Metabolite	Week 4	Week 8	Week 10	Week 13
lactic acid	-	-	-	0.0017
succinic acid	-	-	0.0882	0.0214
β-hydroxybutyric acid	-	0.1455	-	-
glucose 1	-	-	-	0.0571
glucose 2	-	-	-	0.0907
glutamic acid	-	-	0.0789	-
3-phosphoglyceric acid	-	0.0648	-	-
tryptophan	-	-	0.0834	-
palmitoleic acid	0.0004	0.0008	0.0156	-
oleic acid	0.1241	-	-	0.0563
linoleic acid	-	-	-	0.019
palmitic acid	0.0815	0.0771	0.0342	0.0407
aspartic acid	0.1446	0.101	0.12	-
lysine	-	0.1394	-	-
tyrosine	-	-	0.0839	0.0333
methionine	0.0708	0.0315	-	-
glycine	0.0095	-	0.025	-
malic acid	-	-	-	0.057
alanine	-	-	-	0.0411
hydroxyaspartic acid	-	-	-	0.1257
pyruvic acid	-	-	-	0.1214

	down regulated in DIO
	up regulated in DIO

	significant difference
	nonsignificant trend
-	no significant difference

Figure 4-18. Experiment 1: Metabolome profile of mouse livers. Only those metabolites that were statistically different or tended (dotted) to be different between groups are shown. Values represent the level of significance when comparing DIO and Control mice at weeks 4, 8, 10 and 13. Metabolites shaded orange were higher in the DIO group, while those shaded blue were lower in the DIO group. (Control n=4-6, DIO n=5-6)

Metabolite	Week 4	Week 8	Week 10	Week 13
lactic acid	-	-	0.1057	0.0004
succinic acid	0.03	-	-	-
glucose 1	0.0651	-	-	0.0152
glucose 2	0.0273	-	-	0.01
glutamic acid	0.0253	-	-	-
palmitoleic acid	0.0003	0.0231	0.0352	0.0543
oleic acid	0.0174	0.0072	0.1167	0.1413
linoleic acid	0.0009	0.0431	-	-
linolenic acid	0.0683	-	-	-
palmitic acid	-	-	-	0.0916
serine	0.0818	-	-	0.0063
tyrosine	0.0483	-	-	-
methionine	-	0.0876	-	-
valine	0.0944	-	-	0.0079
threonine	0.0358	-	-	-
glycine	0.0011	-	-	-
oxalic acid	-	-	-	0.0145
alanine	-	0.092	-	-
phenylalanine	0.0948	-	-	-
glutamine	0.0961	-	-	-
beta-alanine	-	-	0.0729	0.0219
glycerol 1-phosphate	-	-	-	0.1305

	down regulated in DIO
	up regulated in DIO

	significant difference
	nonsignificant trend
-	no significant difference

Figure 4-19. Experiment 1: Metabolome profile of mouse blood. Only those metabolites that were statistically different or tended (dotted) to be different between groups are shown. Values represent the level of significance when comparing DIO and Control mice at weeks 4, 8, 10 and 13. Metabolites shaded orange were higher in the DIO group, while those shaded blue were lower in the DIO group. (Control n=4-6, DIO n=5-6)

Metabolomics analysis of blood revealed that concentrations of succinic acid (P=0.0214) and malic acid (P=0.057) were higher in the DIO group compared to the Control group at week 13 (**Figure 4-20**). And, though not reaching a level of significance, pyruvic acid was numerically higher in the blood of DIO mice in week 13. Succinic acid was numerically higher in the blood of DIO mice in week 10. All three of these metabolites are intermediates of the Krebs Cycle, and that these were elevated in the blood of DIO mice fed the SD diet *ad libitum* suggests that the Krebs cycle fluxes in tissues, perhaps the liver, have been reduced.

Previous metabolomic research with DIO mice has observed elevated succinate and pyruvate after 12 weeks of feeding a HFD (Duggan, Hittel, Hughey, Weljie, Vogel, & Shearer, 2011). In that study, diet-induced metabolic changes were clearly distinguishable in obese mice even after they were subsequently switched to a standard

balanced diet for one week. Although both succinate and pyruvate were found to be diet-dependent in that study, our study corrected for the influence of diet by food restricting the DIO for 2 weeks on a standard diet and then allowing these mice to consume the standard diet *ad libitum* for three weeks. This discrepancy requires further investigation but possibly suggests that some obesity induced metabolic dysregulation preceding the potential weight gain during the rebound period is causing the energy-related metabolism to behave as if the animals were consuming a high-fat diet (and hence a diet-dependent response in various metabolites).

Blood pyruvate was also found to be elevated in a metabolic profiling study of 74 obese subjects versus 67 lean subjects (Newgard, 2009). These authors implicated the higher pyruvate in plasma as a possible contributor to the impairment in glucose clearance. This possibility is discussed further later in the paper.

Subsequent studies are needed to confirm the cause and role of both elevated plasma pyruvate and succinic acid in DIO metabolism, although their presence in the literature is encouraging. No previous studies have found notably elevated malate in DIO obese individuals. The relation of malate to DIO metabolism, if any, can therefore not be discussed further without further study.

Alanine.

In addition Krebs Cycle intermediates, we also observed elevated blood alanine ($P=0.0411$) in the DIO group following *ad libitum* consumption of the SD diet for 3 weeks.

In the studies of Duggan et al. (2011) and Newgard et al. (2009) mentioned above, plasma alanine was also found to be elevated in the DIO mice and in obese

subjects. After blood lactate, blood alanine is a significant precursor removed by the liver for gluconeogenesis, as part of the alanine cycle. In a study of insulin-resistant diabetic patients, plasma alanine was elevated by 40% compared to non-diabetic patients, and the recycling and use of plasma alanine for glucose synthesis was greater by one-third (Consoli et al., 1990). Herein, the elevated blood alanine observed in the DIO mice fed the SD diet *ad libitum* provides further support that gluconeogenesis is elevated and that, perhaps there is a persistent metabolic dysregulation present.

Newgard et al. (2009) suggests that elevated plasma alanine may arise from increased BCAA catabolism and glutamate accumulation. In conjunction with increased pyruvate formation, these authors hypothesized that this elevation in plasma alanine may negatively impact glucose clearance.

AMPK Activation in the Liver

There is an increase in AMPK activation in DIO mice from week 8 to week 10 (P=0.056), and from week 10 to week 13 (P=0.0104) (**Figure 4-21**). At week 13, liver AMPK activation is elevated in the DIO group compared to the Control group (P=0.0035). This elevation in AMPK activation in DIO mice even after 3 weeks of *ad libitum* feeding on a balanced standard diet shows that mice are perceiving a low energy state, i.e. a high AMP:ATP ratio. One possible explanation for why mice fed *ad libitium* falsely perceive a low energy state is because of the metabolic imprint left by diet induced obesity and weight loss. Previous literature confirms this inclination to regain body fat after weight loss, increased food intake, thrifty metabolism (decreased energy expenditure), and increased fat storage in adipose tissues (Summermatter et al., 2007).

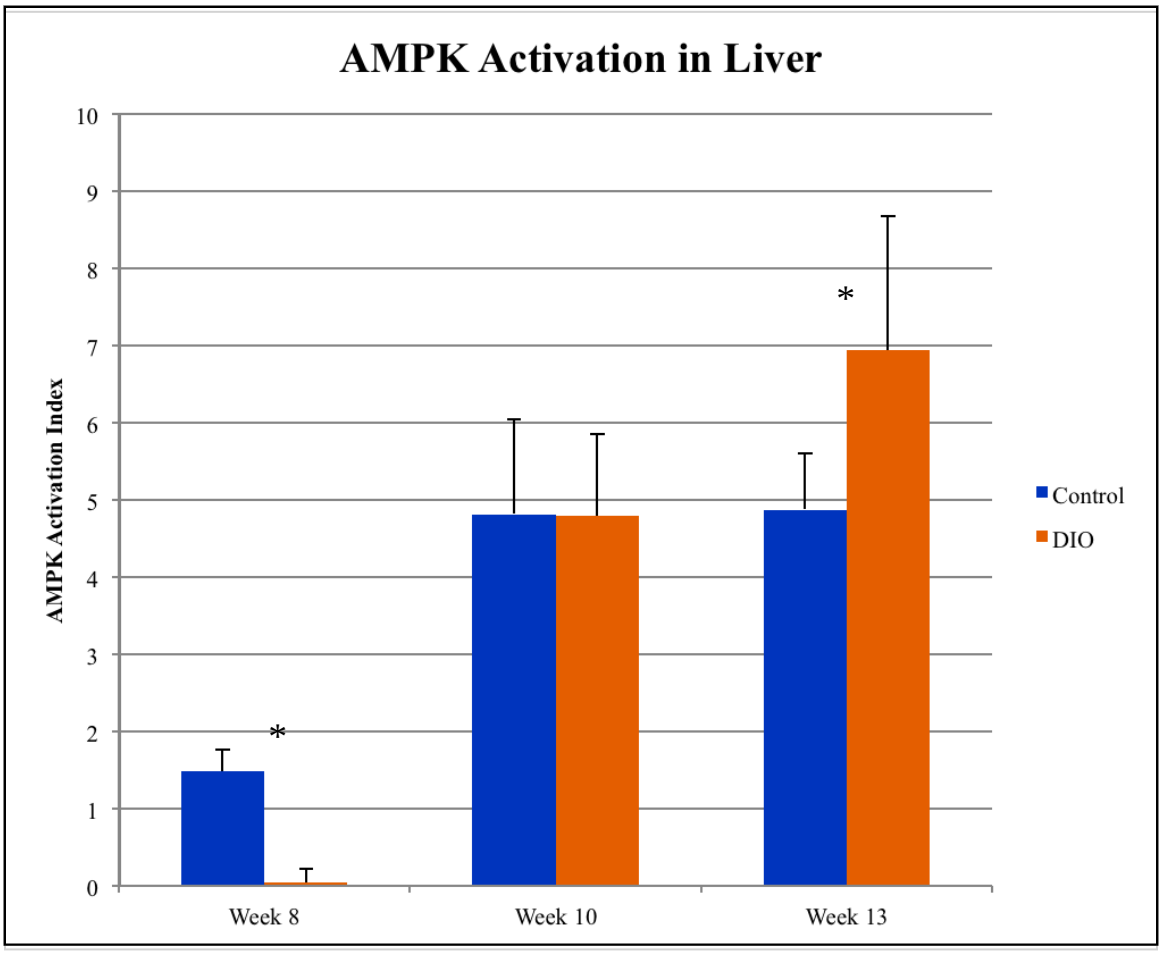

Figure 4-20. Experiment 1: AMPK activation in the liver at weeks 8, 10, and 13. Liver AMPK activation was greater ($P<0.05$) in DIO mice at weeks 8 and 13 compared to Control mice at those times. Error bars represent the standard error of the mean. (Control n=4-5, DIO n=5)

Relationships to food intake and body composition.

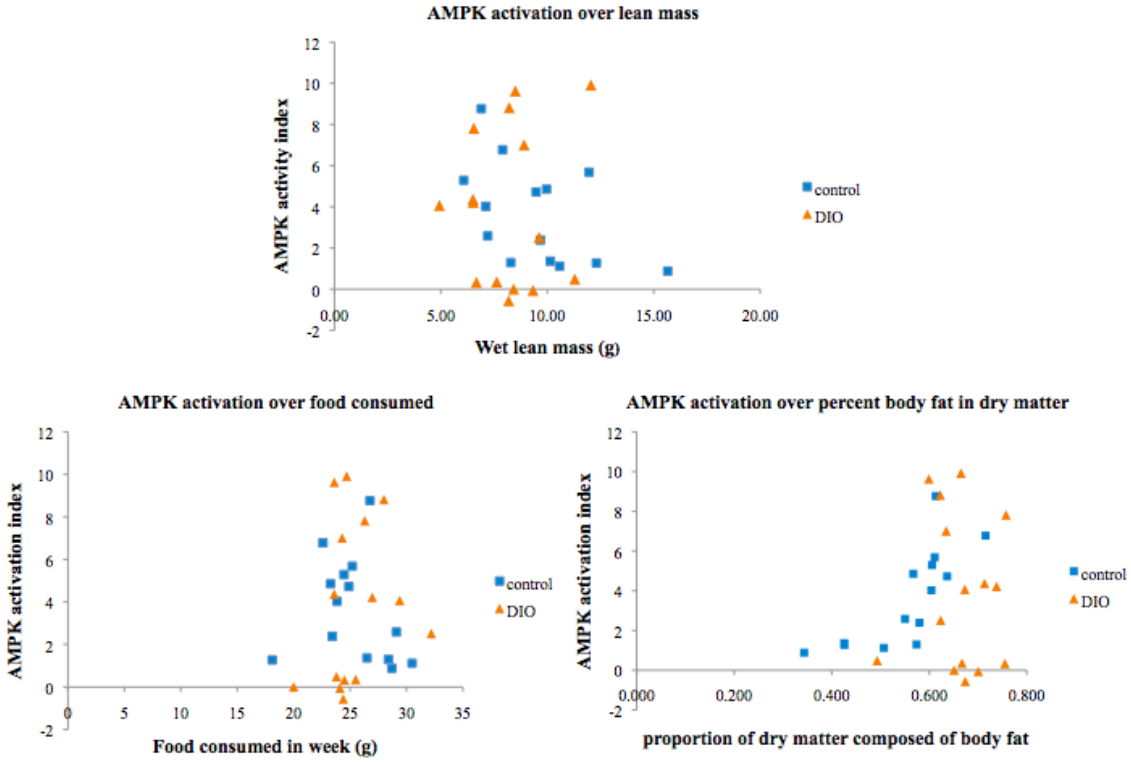

Figure 4-21. Experiment 1: Correlation between liver AMPK activation and either food intake, lean body mass, or percent body fat. Liver AMPK activation did not correlate with any of these parameters.

AMPK activation was compared on an individual mouse basis to percent body fat in dry matter, wet lean mass, and food intake (**Figure 4-21**). Strong correlations were not observed with any of these variables.

AMPK activation is most responsive to changes in the intracellular AMP:ATP ratio due to external metabolic stresses such as exercise or fasting. This short-term variation in AMPK activation affects the measured value of AMPK activation. Thus, AMPK activation can vary due to transient low energy conditions such as food restriction. The overall energy status of the body such as an abundance of energy due to

obesity or long-term nutrient scarcity due to a famine is also a factor in AMPK activation (Osler & Zierath, 2008).

Food intake should not be presumed to be a strict function of AMPK activation (or vice versa). A number of upstream hunger and satiety factors such as ghrelin and leptin, which target many downstream effector proteins other than AMPK are also important. The variable most closely associated with food intake is in fact body weight (**Figure 4-5**). The lack of AMPK correlation with food intake, lean body mass, and body fat percentage does not however assure a total lack of dependence. A more complete explanation of AMPK's potential role is postponed until a later section. Finally, it is possible that the potential dependence of AMPK on each of these bulk measurements is too small to detect within the limits of our (relatively large) uncertainty.

AMPK and glucose clearance

In performing a similar correlation with glucose curve integrals, we found no strong relationship (**Figure 4-22**). The DIO group's average AMPK activation increases from week 8 to 10 alongside its glucose sensitivity (**Figures 4-20** and **4-11**). This relationship between AMPK activation and glucose clearing has been shown in previous studies where AMPK activation is associated with increased glucose uptake by the liver and decreased hepatic gluconeogenesis (Carling, 2004). The fluctuation in Control mice AMPK seems without adequate explanation given our data. We would expect it to be rather constant, and the result remains unclear.

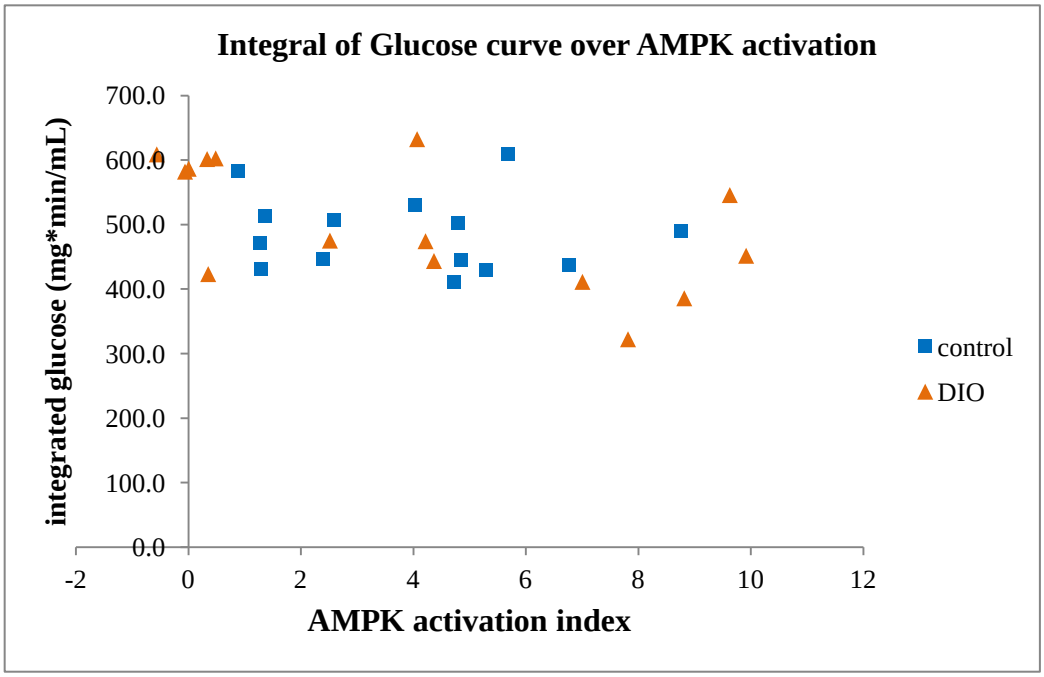

Figure 4-22. Experiment 1: Integral of glucose curves over AMPK activation. No dependence of glucose clearance on AMPK activation was observed for either group.

AMPK Dysregulation factors: Leptin resistance.

We may be able to explain the link between AMPK activation and glucose clearance by gathering the aspects of metabolic syndrome of which both glucose intolerance and AMPK malfunction are symptoms. Leptin resistance is one such feature. It is a disruption of the normal function of the hormone leptin, which has direct consequences for AMPK, a downstream effector protein, as well as glucose absorption. Leptin resistance may be responsible for the high food intake during obesity despite the abundance of energy available.

Leptin is an adipocyte-derived hormone that is released following a meal and functions as a satiety factor and promoter of lipid oxidation. Its circulating concentration is directly proportional to body fat mass, and it has a role in preventing fat accumulation in non-adipose tissue. Obese individuals very commonly suffer from leptin resistance.

Similar to insulin resistance, leptin resistance is characterized by high circulating concentrations of leptin and a blunted metabolic response to peripherally and/or centrally injected leptin.

Several studies have demonstrated that rodents fed a high fat diet will develop a resistance to leptin that appears in as little as 16 days and is very progressive (Enriori et al., 2007). In leptin resistant mice, both endogenous and high-administered doses of leptin fail to reduce appetite, do not decrease energy expenditure, and do not reduce body fat (Van Heek et al., 1997).

Desensitization to leptin is often conceived as resulting from repeated exposures to high circulating concentrations of leptin. These abnormally high levels are created when adipocytes naturally secrete more leptin in response to a high fat diet. The chronic elevation of circulating leptin has been shown to create a deficiency in adipocyte leptin receptors (ObRb) in peripheral tissues, leading to reduced metabolic response to leptin. The effect is not necessarily permanent and can be ameliorated by diet modification. Van Heek et al. (1997) found that food restriction and fasting produce a rapid decline in the normally stable concentration of circulating leptin. Observing this trend over time, Enriori et al. (2007) found that the leptin resistance accrued after 20 weeks on a high fat diet (at which point mice are fully obese) is completely reversible within at most 17 weeks after returning to a standard diet.

The body composition results shows that differences in body weight among DIO mice are largely due to increased adipose tissue mass (a combination of hypertrophy and hyperplasia of adipocytes). The state of DIO body fat at the peak of obesity is sufficient to presume correspondingly high levels of circulating leptin and we may safely consider

them leptin resistant. This assumption of leptin resistance is supported by the fact that elevated leptin is only symptomatic of leptin resistance, and several similar studies utilizing diet induced obesity have induced leptin resistance by 8 weeks.

Minokoshi et al. (2002) have discovered that leptin mediates its functions (fatty acid oxidation, hunger suppression, and prevention of lipid accumulation in non-adipose tissues) primarily via AMPK. This was until recently an unestablished pathway. Leptin promotes AMPK activation by phosphorylation of the α2 subunit of AMPK. This finding lead to the realization that leptin acts directly on muscle, utilizing AMPK to inhibit Acetyl-CoA carboxylase (ACC), and thus promotes fatty acid oxidation during ATP shortage. Leptin is thereby found to be a principle upstream modulator of AMPK activation (Minokoshi et al., 2002).

Leptin resistance, initially caused by a deficiency in ObRb receptors, should then correspond to lower AMPK activation in both muscle and liver (Minokoshi et al., 2002). At least, it should impose a limit on the maximum activity of AMPK during states of rest (strenuous exercise stimulates AMPK). The DIO mice leptin resistance at week 8 is therefore one way to explain their low AMPK levels.

Once DIO mice are switched to a standard diet, AMPK activation is expected to increase. Following the diet switch, DIO mouse body fat is in a state of flux; the mice are shedding excess fat that apparently can no longer be maintained under the standard diet. In this state of weight loss, AMPK plays a critical role in orchestrating fatty acid oxidation and other catabolic processes required for lipid utilization. AMPK levels in DIO mice may have increased after the change to a standard diet due to the increased

demand for ATP, combined with an improved leptin sensitivity caused by the switch to a standard diet (**Figure 4-20**).

AMPK and glucose intolerance

If diet-induced leptin resistance does impose an upper limit on AMPK activation, this dysregulation may offer one clue into the relative inability of DIO mice to metabolize glucose. AMPK plays a central role in the transition from carbohydrate to lipid utilization during fasting and exercise. This transition is essential to a healthy metabolism, and the body's ability to affect it is in fact a common gauge of metabolic fitness. Osler and Zierath (2008) refer to the idea of "metabolic flexibility," or the ability to switch readily between glucose utilization and NEFA oxidation, to characterize metabolic fitness. Obese individuals are characteristically inflexible and do not make acute transitions in substrate utilization. They will not, for example, experience a sharp rise in glucose utilization and storage following a meal, whereas fit individuals will. Metabolically fit persons are also able to switch readily to NEFA oxidation during states of exercise, while obese individuals are not. The ability to activate AMPK, which orchestrates the steps needed to effect NEFA oxidation, factors heavily in the capacity for transitions in energy source. Leptin resistance-induced limitations on this ability will therefore contribute to the state of an individual's metabolic fitness.

In particular, a limitation on AMPK will likely contribute to the inability to uptake glucose. Insulin resistance, as opposed to leptin resistance, is recognized as the main cause of glucose intolerance. While both conditions are operating in obese individuals, leptin resistance-induced AMPK inactivity may influence insulin sensitivity. Schimmack et al. (2006) reported that decreases in malonyl-CoA and NEFA due to

AMPK activation are associated with reductions in fatty acyl-CoA, diacylglycerol, and ceramides. Accumulation of these lipid metabolites inhibits glucose metabolism and is correlated with insulin insensitivity (Schimmack, DeFronzo, & Musi, 2006).

Chapter 5 - Experiment 2 Results and Discussion

Food and Energy Intake

Weekly energy intake by the DIO mice fed the HFD for 8 weeks was higher ($P<0.01$) than that of the Control mice fed the SD diet. From weeks 4 to 5, the DIO group showed a large spike in food and energy consumption, which gradually reduced to intakes close to those of the DIO group from weeks 1 to 3. This trend, suggestive of an increase in energy demand and decrease in satiety in the DIO group over a two week period, is not readily explainable. In Experiment 1, the food and energy intake of the DIO group remained fairly constant over the 8 week period when the HFD was offered.

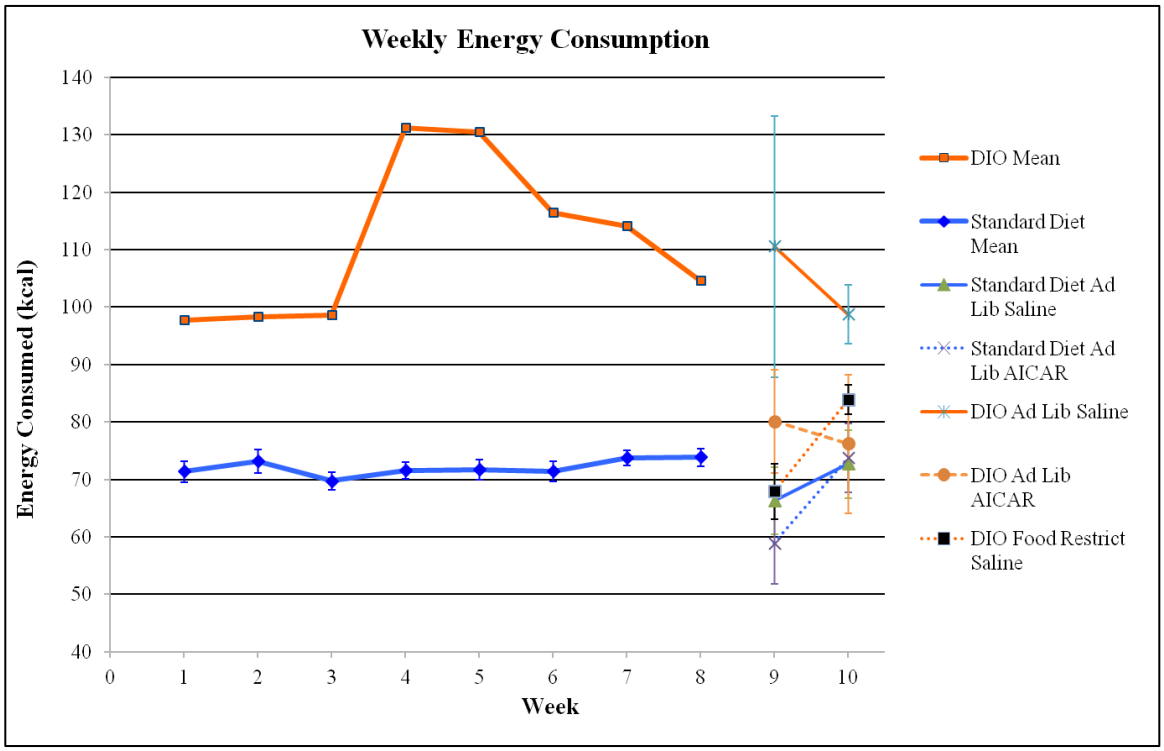

Figure 5-1. Experiment 2: Weekly energy consumption. Energy consumed was constant for the Control group through to week 8, while energy intake was greater for DIO mice particularly from weeks 3 to 5. A decrease in energy consumption was observed in all groups from week 8 to 9, except for the DIO mice consuming the standard diet *ad libitum* and injected with saline (placebo). Individual data points represent the means of: Control: n=14 for weeks 1-8, and n=4-5 for weeks 9-10; DIO: n=21 for weeks 1-8 and n=5-6 for weeks 9-10. Error bars represent standard error.

Energy and food intake tended (P = 0.086) to be reduced in the DIO *ad libitum* AICAR administered group compared to the DIO *ad libitum* saline group at week 10. Thus, altering AMPK activation in the whole body with AICAR changed the mice's perception of energy demands and satiety. It is important to note that intravenous, oral, and intraperitoneal administration affects all tissues except the brain, since AICAR cannot considerably cross the blood-brain barrier (E. Spangenburg, personal communication, November 11, 2012). Thus, the effects on AMPK activation in other tissues resulted in effects on food intake via indirect neuronal and/or hormonal mechanisms. This trend suggests an ability of AMPK to alter the body's perceived energy status, part of a generalized metabolic set point. This data also lends support to our assumption that the weight loss trend observed in the DIO *ad libitum* AICAR group would continue this trend beyond the two weeks of AICAR treatment (**Figure 5-2**).

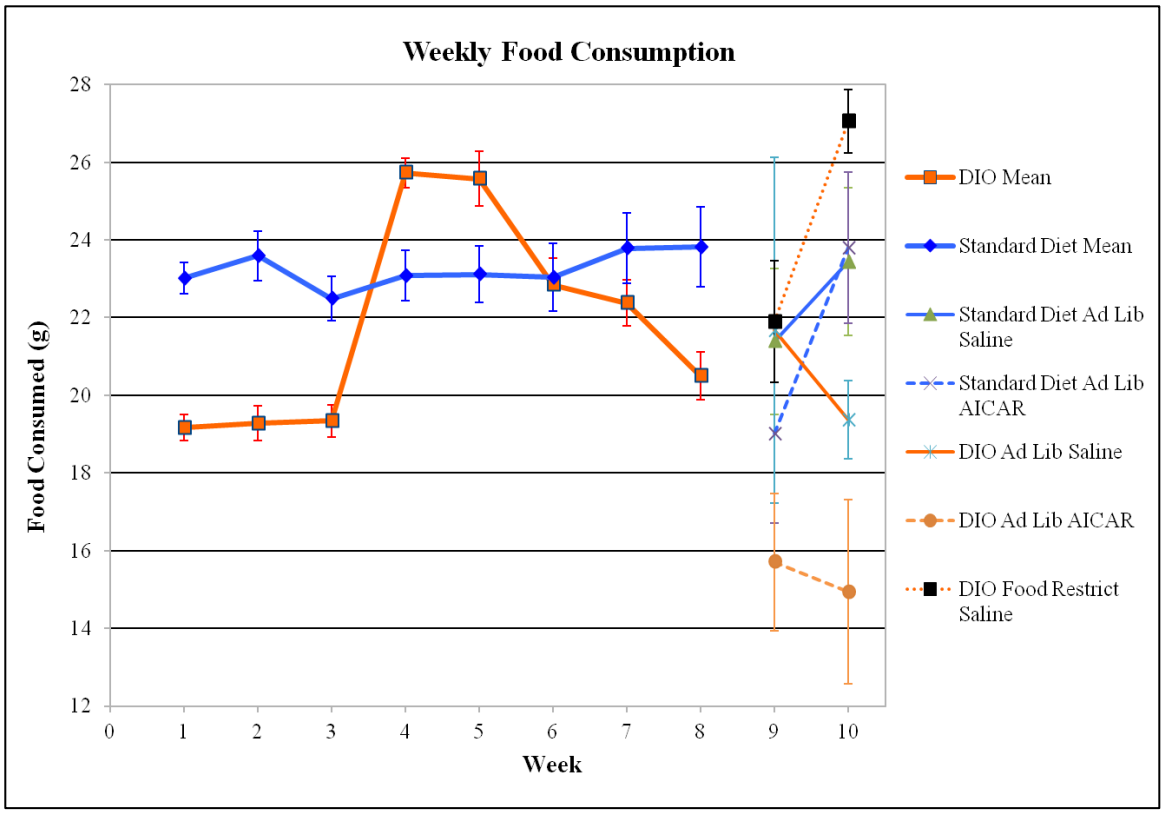

Figure 5-2. Experiment 2: Weekly food consumption. The Control mice consumed the standard diet (SD) *ad libitum* throughout the experiment, and from week 9 to 10 the Control mice were given an IP injection (3 times/wk) of either saline or AICAR. The DIO mice consumed the high fat diet (HFD) *ad libitum* for 8 weeks, after which they were allocated to 1 of 3 treatment groups for 2 weeks: HFD *ad libitum* + saline IP, HFD *ad libitum* + IP AICAR, or SD at 80% of energy intake + IP saline. Individual data points represent the means of: Control: n=14 for weeks 1-8, and n=4-5 for weeks 9-10; DIO: n=21 for weeks 1-8 and n=5-6 for weeks 9-10. Error bars represent the standard error of the mean. For the Control mice, food consumption was constant through to week 8, while food consumption by the DIO mice was greater, particularly from weeks 3 to 5. A decrease in food intake was observed for all groups from week 8 to 9 except the DIO *ad libitum* saline group.

It is firmly established in the literature that regulation of food intake and satiety involves hypothalamic AMPK; as previously discussed, its inhibition is associated with decreased food intake. AICAR's ability to cross the blood brain barrier is minimal, in fact, the amount (< 1%) is not substantial enough to observe its effects in the periphery (Marangos et al., 1990). So while AICAR may enter the hypothalamus and affect hypothalamic AMPK, it would not reach beyond this central effect (Gaidhu et al.,

2011).This makes sense given that elevation of hypothalamic AMPK by AICAR would actually increase food intake. It also must be noted that hepatic AMPK levels measured via ELISA were inconclusive due to experimental error and therefore the efficacy of administered AICAR cannot be undoubtedly established.

It is unclear in the literature how peripherally injected AICAR affects food intake, as most related studies administer it via intracerebroventricular injection. In fact, few studies regarding the effects of AICAR on organisms as a whole have been conducted (Daignan-Fornier & Pinson, 2012). Several compounds, including nutrients and hormones, have been established as suppressors of hypothalamic AMPK and therefore lead to decreased food intake: leptin, elevated glucose, insulin, α-lipoic acid, and others (Xue & Khan, 2006). Similar studies in the future measuring hypothalamic AMPK must be conducted to further elucidate this observed effect.

Energy and food consumption did not significantly change in the week 10 SD *ad libitum* AICAR group compared to the week 8 group. It is interesting that the effect of AICAR on body and food consumption was more prominent in the DIO group than the SD group, as was observed in respect to weight change (see below). This suggests that the effect of AICAR may have a diet-dependent component. No support has been established for this in the literature.

Another possibility for this disparate effect between the DIO and Control groups may involve AMPK, the target of AICAR, as a metabolism-dependent rather than diet-dependent element. It superficially appears that AMPK may sense energy status relative to some "programmed" metabolic baseline; the DIO group is metabolically distant from this baseline, while the Control group is at or near it. AMPK activation would then be

regulated accordingly, or perhaps just more effectively, in the DIO group, resulting in a near return to the baseline level of perceived energy metabolism via changes in satiety and food intake (assuming those are the established intact pathways of AMPK). This return to the metabolic baseline would most likely involve other metabolic regulators and energy sensors.

Ideally, such an effect would lead to similar states of "perceived" energy metabolism between the DIO *ad libitum* AICAR and Control *ad libitum* AICAR groups (which both have increased AMPK). Our results happen to show such a trend. No statistically significant difference in energy consumption between the DIO *ad libitum* AICAR and Control *ad libitum* AICAR groups remained at week 10, while a statistically significant difference did persist between the DIO *ad libitum* saline and Control *ad libitum* saline groups (P=0.001). However, while our results apparently match such a prediction, a lack of direct evidence, an absence of a proposed mechanism, and too small of a sample size make this concept merely a hypothesis that should be further investigated in similar future studies.

Body Weight Changes

As in Experiment 1, feeding the HFD replicated the diet-induced changes in body weight and adiposity compared to mice fed the SD diet. After 8 weeks of consuming the HFD, mice in the DIO group were allocated into three groups receiving the following treatments for 2 weeks: 1) HFD *ad libitum* and saline injection, HFD *ad libitum* and AICAR administration, and SD food restricted (FR) and saline injection. The final body weights of the DIO group fed the HFD *ad libitum* and given saline injections were significantly higher than those of the DIO group fed the HFD *ad libitum* and given

AICAR injections group (P<0.05). This suggests that activation of AMPK by AICAR improved to some extent the ability to lose weight in spite of an *ad libitum* diet. Had this trend been allowed to continue beyond a two week period, AICAR may have proved to be an effective method in body weight reduction. The fact that the SD FR group did not deviate significantly from the HFD *ad libitum* saline group suggests that the tendency to maintain a set weight (i.e. body weight set point) within this two week period is not effectively changed by diet or energy intake. Future studies must be conducted to confirm if AICAR administration over a longer period of time would be successful.

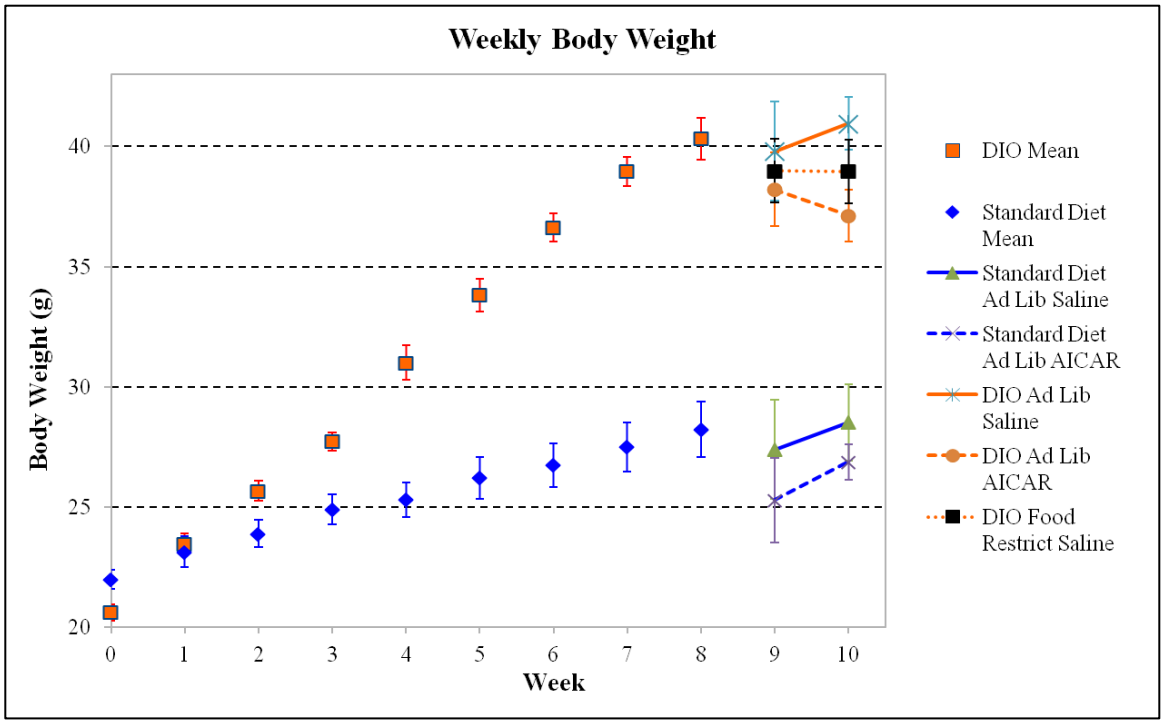

Figure 5-3. Experiment 2: Body weights. Refer to Figure 5-2 for descriptions of groups and treatment assignments. From week 2 onwards, DIO mice weighed more (P<0.05) than Controls DIO mice administered AICAR for 2 weeks weighed less (P<0.05) than DIO mice given saline. Individual data points represent the means of: Control: n=14 for weeks 1-8, and n=4-5 for weeks 9-10; DIO: n=21 for weeks 1-8 and n=5-6 for weeks 9-10. Error bars represent the standard error of the mean.

At the beginning of week 9, mice in the Control group were split into 2 groups over the final 2 week period: SD *ad libitum* saline and SD *ad libitum* AICAR. Like all DIO groups, both Control groups exhibited a small drop in weight over the first week. This phenomenon is not easily explained (it may be due to the effective methodology of injections, perhaps causing the mice to eat less due to stress or some related factor) and warrants further investigation.

Over the second week both Control groups steadily regained weight. Neither group showed a statistically significant weight change over that period. In addition, unlike in the DIO groups, no significant change in weight was seen between the SD *ad libitum* saline and SD *ad libitum* AICAR groups at the end of the two week period. This trend further suggests a diet-dependent or metabolic-dependent effect of AICAR, as its trending weight reduction effect observed in the DIO groups was not seen with the Control groups.

Body Fat Composition

Fat proportion was calculated for week 8 and 10 time points. As in Experiment 1 at week 8, this value was significantly higher in the DIO group (P=0.0018). This suggests, as previously noted, that the weight gain experienced in the DIO group over 8 weeks was primarily fat mass accumulation due to the high fat content of the diet.

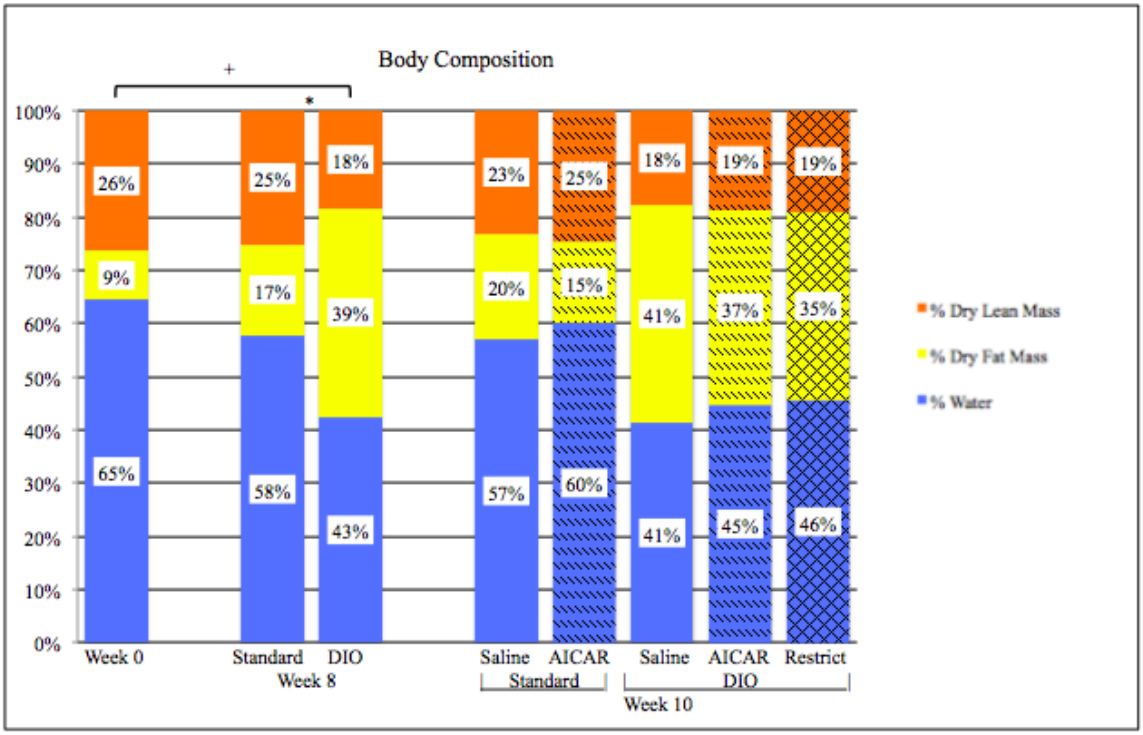

Figure 5-4. Experiment 2: Body composition. Refer to Figure 5-2 for descriptions of groups and treatment assignments. By week 8, DIO mice had a greater ($P<0.05$) proportion of body fat. Fat proportion does not change significantly within either group from week 8 to week 10. Individual data points represent the means of: Control: n=14 for weeks 1-8, and n=4-5 for weeks 9-10; DIO: n=21 for weeks 1-8 and n=5-6 for weeks 9-10. Error bars represent the standard error of the mean.

At the end of 10 weeks, the three DIO groups showed no significant change in fat proportion relative to each other or to those of week 8. Likewise, no significant change in fat proportion was seen among the Control groups. Our results show that AICAR is ineffective in decreasing fat mass over a 2 week period. Such findings suggest a possible disruption in AMPK pathways related to fatty acid metabolism and storage. Prior studies have shown that AMPK increases fat oxidation via inhibition of ACC, as well as increasing mitochondrial biogenesis and inhibiting fatty acid synthesis (Xue & Kahn, 2006). AMPK can also directly reduce adiposity by inducing adipocyte apoptosis, inhibiting lipolysis, and down-regulating adipogenic genes (Dagon, Avraham, & Berry,

2005). Further investigation is therefore required to elucidate any possible deregulation up- or down- stream of these pathways, and whether or not residual DIO effects are at least partially responsible

Insulin Resistance

As previously discussed, insulin resistance is a primary sign of metabolic syndrome resulting from obesity. Average glucose tolerance test levels and integrated glucose levels were completed for each group and subgroup in weeks 8 and 10. The integrated glucose level in the DIO group at week 8 was significantly greater than that of the Control group (P=0.0025), similar to the results from Experiment 1 (**Figure 5-5**). This shows that HFD diet-induced obesity significantly increased systemic insulin resistance and suggests the onset of metabolic syndrome did occur by this time.

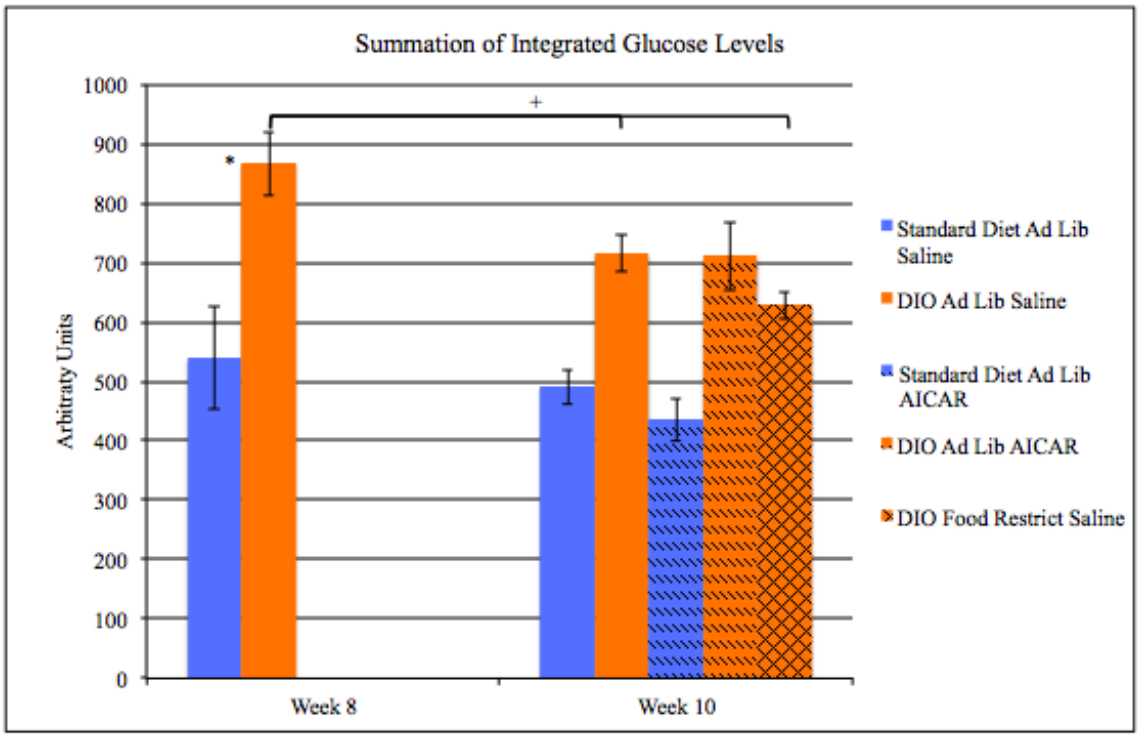

Figure 5-5. Experiment 2: Area under the curve of blood glucose concentrations during the 120 min period following administration of a 2g/kg glucose dose. Refer to Figure 5-2 for descriptions of groups and treatment assignments. Glucose clearance was lower in DIO mice after 8 weeks of consuming the high fat diet compared to Controls. Glucose clearance improved ($P<0.05$) in the DIO mice at week 10, but was not different between treatments administered to the DIO mice. Individual data points represent the means of: Control: n=14 for weeks 1-8, and n=4-5 for weeks 9-10; DIO: n=21 for weeks 1-8 and n=5-6 for weeks 9-10. Error bars represent the standard error of the mean.

All DIO and SD groups showed some improvement in glucose clearance in week 10 versus week 8. Integrated glucose levels across the DIO groups remained higher than those of the SD groups. Interestingly, of the DIO groups, only the FR and *ad libitum* saline groups showed a statistically significant improvement ($P=.0033$, $P= 0.0191$ respectively), while the *ad libitum* AICAR group did not. There was no statistical difference in glucose clearance between any of the DIO groups at week 10. Likewise, the SD *ad libitum* saline group showed a statistically significant improvement ($P=0.0054$)

while the SD *ad libitum* AICAR group did not. There also was no statistical significance between the SD groups at week 10.

This data suggests that AICAR administration at a dose of 0.5 mg/g body weight three times a week over a two week period did not produce any sizeable improvement in insulin sensitivity. AMPK, as previously mentioned, inhibits hepatic gluconeogenesis. In addition, prior studies have suggested that increased muscle AMPK activation indirectly improves insulin resistance via ACC inhibition, leading to a decreased resting level of Malonyl Co-A and less production of inflammatory lipid intermediates (Muoio & Newgard, 2006). Our findings suggest possible disruptions in these or related pathways, similar in nature to those discussed involving NEFA and body fat metabolism. AICAR administration over a longer time frame may have lead to improvements in fatty acid metabolism that precede improvements in glucose clearing.

Non-Esterified Fatty Acids

Week 10 hepatic metabolomic data reveals some significant differences in several metabolites related to fatty acid metabolism between the DIO AICAR and saline groups. Palmitic acid was significantly higher (P=0.0109) in the DIO AICAR group than in the DIO *ad libitum* saline group at week 10 (**Figure 5-6**). While the SD diet contained only 0.7% palmitic acid, the HFD diet contained much higher levels of palmitic acid due to large amount of lard (31%) in the diet. As previously discussed, high levels of palmitic acid are associated with lipotoxicity and insulin resistance. This therefore reassures our findings of no significant improvements in fat composition or insulin resistance in the DIO AICAR group from week 8 to week 10. Upon further inspection, these results are actually peculiar given that body composition was not significantly different between the

two groups and the DIO AICAR group actually showed decreased food and energy intake.

Oleic acid was significantly lower (P=0.0029) in the DIO AICAR group compared to the DIO saline group (**Figure 5-6**). As discussed earlier, oleic acid is associated with improved insulin resistance and decreased lipotoxicity. This serves as further evidence of dysregulated fatty acid metabolism in the DIO AICAR group compared to the saline group. These findings again show that AICAR has failed to effectively improve the underlying metabolism stemming from DIO. The reason fatty acid metabolism actually appears to be worse in the AICAR group than the Saline group is not readily explained and requires further testing.

Metabolite	Group A vs. B	Group C vs. D	Group C vs. E	Group D vs. E	Group B vs. D
proline	-	-	-	-	0.001
glutamic acid 3	0.0018	-	-	-	0.122
glucose-6-phosphate 1	0.0424	-	-	-	-
serine	-	0.0015	-	-	0.045
aspartic acid	0.0916		-	-	0.1326
tyrosine	-	0.0751	-	-	-
methionine	-	-	-	0.1413	-
valine	-	0.1245	-	-	-
succinic acid	-	-	0.0013	-	-
fumaric acid	-	-	-	-	0.0786
malic acid	-	0.0723	-	0.0132	-
palmitic acid	-	0.0109	-	-	-
palmitoleic acid	-	-	0.0011	0.007	-
oleic acid 1	-	0.0029	0.001	0.0115	-
linoleic acid	-	0.0043	-	-	0.0355

	Group X vs. Y
	Up regulated in group X
	Down regulated in group X

	significant difference
	nonsignificant trend
-	no significant difference

Group A: Control Saline
Group B: Control AICAR
Group C: DIO Saline
Group D: DIO AICAR
Group E: DIO Saline Food Restricted

Figure 5-6. Experiment 2: The liver metabolome at week 10 after saline, AICAR and feed restriction. Only those metabolites that were statistically different or tended (dotted) to be different between groups are shown. Values represent the level of significance when the specific planned comparisons were made. Metabolites shaded orange were higher in the DIO group, while those shaded blue were lower in the DIO group (n=4-6)

Chapter 6 - Conclusions

Experiment 1 Summary

The experiments reported in this thesis investigated the perturbations in body composition, blood glucose clearance, hepatic metabolism (metabolomics), and hepatic AMPK activation following DIO, weight loss, and weight regain in a mouse model. In Experiment 1, following 8 weeks of consuming a HFD *ad libitum* to establish DIO, we monitored changes in body composition and the above metabolic parameters after a 2 week period of calorie restriction on the SD diet and then again after 3 weeks of *ad libitum* consumption of the SD diet. Due to effective methodology (discussed above), reduced food intake was observed throughout these 5 weeks, establishing a 5 week diet phase.

Throughout the first 8 weeks, DIO mice gained weight at a constant rate and weighed, on average, 18.4% more than Control mice fed the SD over the same period. This difference was primarily due to increased fat mass. By week 4 and continuing through to week 8 of feeding on HFD, body fat proportion was also significantly higher in the DIO mice. Feeding the HFD significantly elevated hepatic palmitic, linoleic, and linolenic acids, while reducing hepatic oleic acid levels in the DIO group compared to the Control group by week 8. Glucose clearance by the DIO mice was reduced relative to the Control mice by week 4, and this trend continued through to week 8.

We also examined a variety of correlations between various metabolic and physiological measurements throughout this 8 week period of feeding the HFD. For example, while the relationship between body weight and food intake was the same for both groups, the energy-dense HFD skewed energy intake of heavier mice toward

disproportionately higher values. HFD feeding thus had a greater negative impact on heavy mice.

After 2 weeks of calorie restriction, body fat proportion in the DIO mice decreased and, despite consuming the SD diet (lower energy-density and less fat) *ad libitum* for a further 3 weeks after the restriction period, body fat proportion of the DIO mice increased relative to the Control mice. Hepatic NEFA levels in the DIO mice also decreased throughout these two diet periods, approaching levels similar to the Control mice. However, principle component analysis of four NEFA levels (see above) showed spatially separated clustering of the DIO and Control groups at weeks 4, 8, and then again at week 13, indicating a persistent systematic deviation in hepatic NEFA between the DIO and Control groups.

Glucose clearance markedly improved throughout the diet phase in the DIO group. By the end of the 3 week period of consuming the SD diet *ad libitum* glucose clearance was higher in the DIO group compared to the Control group. This improvement in glucose clearance likely resulted from improved skeletal muscle insulin sensitivity as a result of the reduction in circulating NEFA concentrations.

Hepatic AMPK activation was higher in the Control group compared to the DIO group after 8 weeks of consuming the HFD diet *ad libitum*, as would be expected under conditions of excess energy intake. After 2 weeks of energy restricting the DIO group, hepatic AMPK activation increased, however, AMPK activation only increased to the same level as in the Control mice. Of particular note, after 3 weeks of consuming the SD diet *ad libitum*, hepatic AMPK activation in the DIO mice increased, rather than decreased. It would have been expected that, once the DIO mice were allowed to

consume *ad libitum* the SD diet, hepatic AMPK activation would be reduced since the mice were allowed to consume the SD diet to meet their energy requirements. That hepatic AMPK activation increased more than 2-fold suggests that the DIO mice were incapable of sensing energy intake but rather sensed the SD diet as being inadequate in meeting energy needs. Indeed, hepatic AMPK activation was considerably lower in the Control compared to the DIO mice even though the Control mice had also consumed the SD diet *ad libitum*. Clearly, there was a defect in the ability of DIO mice to properly "sense" energy intake relative to whole body energy needs.

Improved glucose clearance by the DIO mice throughout the energy restriction and SD diet *ad libitum* periods, along with a trend towards improvements in body composition and fatty acid metabolism, suggests a pronounced and abrupt shift away from adiposity and metabolic syndrome in the DIO mice. These trends first became apparent following the 2 week energy restriction period by the DIO mice. A detailed explanation of these findings is difficult to formulate given only basic metabolic measurements, but hepatic AMPK activation and glucose clearance after the 3 week SD diet *ad libitum* period by the DIO mice suggests that the mice may have some form of "metabolic overcompensation" and may indicate that DIO mice were in a state of weight flux throughout these two dietary periods which possibly resulted in rapid oxidation of blood NEFA.

Experiment 2 Summary

Experiment 2 investigated the role of diet and AMPK in modulating the DIO perturbations observed in Experiment 1. Following 8 weeks of consuming the HFD *ad libitum*, mice in the DIO group were allocated to three diet and treatment regimes for 2

weeks: 1) HFD *ad libitum* plus injection of the AMPK activator AICAR, 2) HFD *ad libitum* plus injection of saline and 3) SD diet fed to 80% of energy requirements plus injection of saline. The Control mice were allocated to two treatments: 1) SD diet *ad libitum* plus injection of AICAR and 2) SD diet *ad libitum* plus injection of saline.

Comparisons in body composition, glucose clearance, and hepatic NEFA suggested that administration of AICAR to DIO mice had limited effects on the metabolic indicators associated with DIO. No significant differences in body fat proportion or glucose clearance were found between the DIO mice given AICAR and saline. Hepatic metabolomics analysis revealed that palmitic and linoleic acid levels were significantly elevated, while the oleic acid level was significantly lower in the DIO mice administered AICAR compared to the DIO mice administered saline.

There was a tendency for energy consumption by DIO mice administered AICAR to be reduced compared to the DIO mice administered saline. Consistent with this finding, body weight was significantly lower after the two week treatment period compared to the DIO mice administered saline injections. The latter suggests that elevated AMPK activation in peripheral tissues may play a role in modifying food intake behavior and therefore energy-sensing status in DIO mice. It is important to note that AICAR is unable to pass the blood-brain barrier appreciably, thus the effects on food intake did not result from a direct action on the brain. Literature regarding AMPK and food intake has focused solely on its role in the hypothalamus, thus future studies must be conducted to investigate the potential role of AMPK in other tissues that ultimately modify food intake.

No significant changes in body weight, energy consumption, body fat proportion, or glucose clearance were found between the Control mice administered AICAR or saline. The effects of AICAR on energy consumption and body weight observed in the DIO groups, yet absent in the Control groups, suggest additional metabolic factors influencing and relaying the effects on AMPK activation to metabolic and physiological mechanisms. Certainly the type of diet, (i.e. low versus high fat), and thus the state of obesity, appears to have been responsible for altering the response to enhanced AMPK activation. Another possibility involves different activity of up-stream regulators of AMPK between the DIO and Control mice, reflective of differences in baseline metabolism.

Experiment 1 and 2: Possible Roles of AMPK in DIO Metabolic Dysregulation

Elevating AMPK activation by administration of AICAR in Experiment 2 was clearly ineffective in inducing the trends of metabolic improvement following DIO. In Experiment 1, differences in glucose clearance and body composition improvement after 8 weeks of feeding on HFD and after 2 weeks of food restriction on SD are most notable. The key differences between the two experiments are the diet and energy consumption changes that occur in DIO mice. The DIO mice in Experiment 1 were calorie restricted on SD, while the DIO mice, administered AICAR in Experiment 2, continued consuming the HFD diet *ad libitum*.

Our results show that the HFD elevated hepatic NEFA and increased body fat percentage. Calorie restriction on the SD diet tended to reverse these effects in Experiment 1. These effects persisted in Experiment 2 as HFD *ad libitum* feeding was continued despite a modest decrease in energy consumption and elevation of AMPK

(which functions to increase fatty acid oxidation to generate ATP). These findings suggest continual dietary fat intake may be the primary feature that allowed for the perpetuation of the metabolic dysregulation by preventing potential benefits from elevated AMPK activation. Such a perturbation likely involved up-stream regulators or cofactors necessary for proper long-term functioning of AMPK related pathways. Some compounds that are potentially involved will be speculatively discussed; however, future studies are required to confirm the involvement of such compounds in our observed results.

One possibility worthy of discussion here is the involvement of gene transcription processes related to AMPK signaling and fatty acid metabolism, as effects at these levels endure over longer periods of time. It has been shown that long-term promotion of fatty acid oxidation by activation of AMPK requires peroxisome profilerator-activated receptors (PPAR) and the transcriptional factor PPAR-γ co-activator-1 α (PGC-1α) (Lee et al., 2006). These factors, which are stimulated by exercise and calorie restriction, regulate genes involved in mitochondrial biogenesis, oxidative phosphorylation, gluconeogenesis, and other energy requiring pathways (Canto & Auwerx, 2009). Their expression is promoted by AICAR administration, and PGC-1α is phosphorylated directly by AMPK when activated. When inhibited via siRNAs, the effect of AICAR on fatty acid oxidation *in vitro* diminishes (Lee et al., 2006).

Studies involving PGC-1α suggest a possible mechanism of resistance to AMPK activation due to HFD. Diets high in palmitic acid have been shown to down-regulate the expression of PGC-1α (Kien, 2009). We have established that palmitic acid is elevated in DIO mice throughout the 8 weeks of consuming the HFD *ad libitum*. Hepatic NEFA

analysis in Experiment 2 also revealed elevated palmitic acid in the DIO mice administered AICAR. Persistently high palmitic acid may, therefore, reduce PGC-1α expression. As noted earlier, PGC-1α is required for long term AMPK-mediated fatty acid oxidation, the process needed to reduce these palmitic acid levels still being ingested with the HFD. This scenario creates a persistent condition of high NEFA levels, leading to continued lipotoxicity and insulin resistance by peripheral tissues such as skeletal muscle. Further investigation utilizing PGC-1α assays in our methodology is required to elucidate the possible role of this scenario following DIO.

Leptin resistance, discussed earlier, may also play a primary role in the metabolic trends observed through DIO and subsequent periods of calorie restriction and AICAR administration. Yu et al. (2004) found that both muscle and liver AMPK activation are reduced in leptin-receptor deficient Zucker rats fed a SD diet *ad libitum*. Such findings further suggest that calorie restriction may have played a key role in metabolic improvements observed solely in Experiment 1 because, as previously mentioned, it has been shown that calorie restriction greatly increases leptin sensitivity. Therefore, it is also possible that leptin resistance may have played a role in the persistent metabolic perturbations observed in Experiment 2 when AICAR was administered to the DIO mice fed the HFD. However, Yu et al. (2004) suggests that there is no direct role of leptin sensitivity in determining the effectiveness of AICAR in improving metabolic syndrome: Zucker rats fed a SD diet *ad libitum* and administered AICAR had improved glucose clearance and reduced triglyceride content in muscle and liver compared to corresponding Controls.

Another regulatory component worthy of investigation is Ghrelin. Briggs et al. (2013) demonstrated that the propensity for weight rebound following calorie restriction in previously DIO mice may involve changes in ghrelin sensitivity. They suggested that a "high body weight" set point is maintained following calorie restriction by restoration of ghrelin sensitivity, which is composed of an increase in both ghrelin production and ghrelin-responsive neuronal populations in the hypothalamus. They found that ghrelin-knockout mice that were calorie restricted following DIO exhibited less of a rebound in weight gain compared to Control DIO mice.

Limitations of the Study

In Experiment 1, the poor correlation between several pairs of variables may reflect poor data rather than a lack of relationship. For example, the correlation between AMPK and integrated glucose values showed little covariance. We would expect some increase in AMPK activation to correlate with the observed increase in glucose clearance (**Figure 4-27**). Because group average glucose levels rise and fall as expected with fair precision, we can assume high variability with some error in AMPK values from ELISA. Future studies should conduct more extensive ELISA analysis, a logistical impossibility for our current study, to increase accuracy and precision.

The plot relating integral glucose values to NEFA indices is also disconcerting, showing a vaguely negative trend when a positive one is expected (**Figure 4-15**). We can stipulate that there is a limitation in the ability of the "total NEFA index" (equal to the sum of the logs of all NEFA indices for an individual mouse) to act as an overall gauge of circulating NEFA concentrations. This is due to variation in the metabolomic mass spectrometry readings where some metabolites are registered as being disproportionately

higher, skewing the average sum values for a given set of metabolites. Furthermore, metabolomic data for both Experiment 1 and 2 was overall relatively sparse, with many key DIO related metabolites absent from the data (such as Citrate, Taurine, Choline, Leucine) or disproportionately present in samples between groups and between weeks. This lack of data limits our ability to correlate an underlying metabolic state with DIO induced symptoms of metabolic syndrome and perceived energy status. The incompleteness of our metabolomic data was most likely due to incomplete derivatization and lack of separation in the GC-MS column, an issue that can be addressed in future related metabolomic studies.

Given our methodology, the differing effects of calorie restriction and diet on the efficacy of AICAR in Experiment 2 cannot be distinguished. To do so, our study should have included an additional DIO group in Experiment 2 that was calorie restricted but remained on HFD.

Conclusions

Our study aimed to better model the metabolic changes through DIO and subsequent dieting in order to establish a better understanding of this progression and what contributes to a propensity for weight rebound once dieting has ended. We also aimed to investigate the potential role of AMPK in these processes. We attempted to address these goals in a novel approach, correlating various metabolic and physiological measurements to track metabolic trends through DIO and subsequent dieting. We also used liver and blood metabolomic analysis to correlate both specific metabolite changes and generalized shifts in overall metabolite composition to the aforementioned

relationships. Finally, we tracked changes in hepatic AMPK throughout this period and further evaluated its role by activating AMPK with AICAR following DIO.

While our results and correlations were generally incomplete given the imperfections of our AMPK and metabolomics data, we clearly observed distinct metabolic changes throughout the period of DIO and subsequent dieting, implicating important roles for calorie restriction and elevated hepatic AMPK in these trends. Furthermore, we clearly demonstrated the inability of AICAR to effectively combat DIO-related metabolic dysregulation. These findings suggest AICAR is not likely to be an effective quick-fix solution to obesity and metabolic syndrome as recently popularized in the media (Zarembo, 2008).

Together our findings, and more importantly, our methods for studying DIO and subsequent dieting provide a promising approach to establish a more detailed model that can be further built upon in future studies. Further studies can also establish the response of AMPK throughout this period, specifically how its effect is mediated by diet and energy consumption and any associated up or downstream mediators of AMPK that become dysregulated throughout this period. Hopefully such work will contribute to developing future treatments that are more effective in combating obesity and changes in metabolic set point that may lead to weight rebound.

Appendix A- Methodologies

Appendix A.1: Soxhlet Fat Extraction

Note: Soxhlet Fat Extraction is run in triplicate, with an 18 sample per day maximum.

1. Label and weigh ceramic thimbles, three per mouse. Record Weights.
2. Using a metal scoopula, place about 1g of dried, milled powder mouse into each thimble.
3. Record weight of thimble and sample.
4. Stuff small piece of gauze into top of thimble, sealing in powder.
5. Record weight of thimble, sample, and gauze.
6. Bundle thimbles together in large piece of gauze, no more than three thimbles tall. Secure with rubber bands.
7. Place into Soxhlet extraction system for at least 12 hours, preferably overnight.
8. Record weight of thimble, sample, and gauze after extraction.
9. Dispose of contents of thimble, and clean with air duster and soap water to be used for next sample. Let dry fully.

Appendix A.2: Blood and Liver Metabolomic Extraction

Make internal standards. Internal standard selection should represent the compounds that are not in the samples.

1. Thaw blood or liver tissue on ice. Try to avoid getting sample warmed to room temp.
2. Use 1.8-2 mL snap cap Eppendorf tube
3. Weigh and record weight of sample (0.1 g for blood and 0.05 g for liver) into tube, weigh and record **Sample Internal standard** solution (0.1 g, Norleucine @ 1.275 mM for blood and 3.8 mM for liver). For blood, add 1 mL ice-cold methanol, place on vortex-shaker for 15 min and centrifuge at 15,000 rpm for 15 min at room temperature. For liver, add 1 mL ice-cold distilled H_2O:methanol:chloroform (2:5:2), homogenize on ice for 1 min, then place on vortex-shaker for 15 min and centrifuge at 15,000 rpm for 15 min at room temperature.
4. For both types of samples, pipette extract into a 2 mL V-vial, and blow down under N_2 gas without heat. This may take 15-30 min. Vortex sample midway through blow down step to prevent water being trapped under lipids and dry upper layer. Once completely dry, proceed to step #5.
5. Add 60 uL O-methoxylamine in pyridine (30 mg/mL) and then add 60 uL BSTFA + 1% TCMS, cap and vortex thoroughly (30 secs). Micro-wave for 2 minutes at power level 2 (200 W)

6. Transfer from V-Vial to 0.5 mL eppendorf tube and centrifuge at 15,000 rpm for 15 min at room temperature to remove debris if debris is observed at bottom of V-Vial.
7. Transfer the derivatized sample (avoid sucking up debris at bottom of tube) into GC vial insert and inject onto GC-MS using MethOxime method.

Preparation of O-methoxylamine in pyridine solution:
According to the volume for all the samples, weigh a certain amount of O-methoxylamine and dissolve it in pyridine for final concentration of 30 mg/ml. Vortex vigorously until dissolved. Slight heat can also be applied to aide in dissolving. This concentration can also be adjusted in terms of the sample amount.

Preparation of standard solution and retention index solution:
1. For liver, make a stock internal standard solution containing 10 mg Norleucine/20 mL double distilled H_20 (3.825 mM)
2. For blood samples, dilute stock 1:2 (ie. 3 fold dilution) with double distilled H_20 (ie. 1.275 mM)

Appendix A.3: ELISA Procedure for AMPK Measurement (Life Technologies, 2013)

1. Determine the number of 8-well strips needed for the assay. Insert these in the frame(s) for current use. (Re-bag and seal extra strips and frame. Store these in the refrigerator for future use.)
2. Pipette 100 µl of the Standard Diluent Buffer to the well(s) reserved for the standard blanks. Well(s) reserved for chromogen blank(s) should be left empty.
3. Pipette 100 µl of standards, controls, and diluted samples (typically >1:10 dilution for cell extract) to the appropriate microtiter wells. Tap gently on side of plate to thoroughly mix.
4. Cover wells with plate cover and incubate for 2 hours at room temperature.
5. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times.
6. Pipette 100 µl Streptavidin-conjugated HRP (ELISA kits) or Biotin-conjugated Detection Antibody (phosphoELISA™) solution into each well except the chromogen blank(s). Tap gently on the side of the plate to mix.
7. Cover wells with plate cover and incubate for 1 hour at room temperature.
8. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times.
9. Pipette 100 µl streptavidin-HRP (ELISA kits) or anti-rabbit IgG-HRP (phosphoELISA™ kits) solution to each well except the chromogen blank(s).
10. Cover wells with the plate cover and incubate for 30 minutes at room temperature.
11. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times.
12. Pipette 100 µl of Stabilized Chromogen to each well. The liquid in the wells will begin to turn blue.

13. Incubate for 30 minutes at room temperature and in the dark. Note: Do not cover the plate with aluminum foil or metalized mylar. The incubation time for chromogen substrate is often determined by the microtiter plate reader used. Many plate readers have the capacity to record a maximum optical density (OD) of 3.0. The OD values should be monitored and the substrate reaction stopped before the OD of the positive wells exceed the limits of the instrument. The OD values at 450 nm can only be read after the Stop Solution has been added to each well. If using a reader that records only to 3.0 OD, stopping the assay after 20 to 25 minutes is suggested.
14. Pipette 100 µl of Stop Solution to each well. Tap gently on the side of the plate to mix. The solution in the wells should change from blue to yellow.
15. Read the absorbance of each well at 450 nm having blanked the plate reader against a chromogen blank composed of 100 µl each of Stabilized Chromogen and Stop Solution. Read the plate within 2 hours after adding the Stop Solution.
16. Plot the absorbance of the standards against the standard concentration. (Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.) Draw the best smooth curve through these points to construct the standard curve. If using curve fitting software, the four parameter algorithm provides the best curve fit.
17. Read the protein concentrations for unknown samples and controls from the standard curve plotted in step 16. Multiply value(s) obtained for sample(s) by the appropriate dilution factor to correct for the dilution with Standard Diluent Buffer. (Samples producing signals higher than the highest standard should be further diluted in Standard Diluent Buffer and reanalyzed, multiplying the concentration by the appropriate dilution factor.)

Appendix B - Experiment 1 Data Tables

Table B-1: Experiment 1: Mouse Key

Exp. 1 Mouse Key			Diet			Week Dissected				
		Eliminated	Standard	Hight Fat (DIO)	Food Restricted (Weeks 8-9)	Week 0	Week 4	Week 8	Week 10	Week 13
Week 0 Baseline	B1					x				
	B2					x				
	B3					x				
	B4					x				
	B5					x				
	B6					x				
	B7					x				
Week 4 Standard Diet	C1		x				x			
	C2		x				x			
	C3		x				x			
	C4		x				x			
	C5		x				x			
	C6		x				x			
Week 8 Standard Diet	C7		x					x		
	C8	x								
	C9		x					x		
	C10		x					x		
	C11		x					x		
	C12		x					x		
Week 10 Standard Diet	C13	x								
	C14		x						x	
	C15		x						x	
	C16		x						x	
	C17		x						x	
	C18	x								
Week 13 Standard Diet	C19		x							x
	C20		x							x
	C21		x							x
	C22		x							x
	C23	x								
	C24		x							x
Week 4 DIO	T25			x			x			
	T26			x			x			
	T27			x			x			
	T28			x			x			
	T29			x			x			
	T30			x			x			
Week 8 DIO	T31	x								
	T32			x				x		
	T33			x				x		
	T34			x				x		
	T35			x				x		
	T36			x				x		
Week 10 DIO Food Restrict	T37	x								
	T38			x	x				x	
	T39			x	x				x	
	T40			x	x				x	
	T41			x	x				x	
	T42			x	x				x	
Week 13 DIO Food Restrict	T43			x	x					x
	T44			x	x					x
	T45	x								
	T46			x	x					x
	T47			x	x					x
	T48			x	x					x

Appendix B.1: Experiment 1 Body Weight Tables

Table B-2: Experiment 1: Weekly Body Weight (g)

Exp. 1 Weekly Body Weight (g)

		Initial	Sept 9-16 Week 1	Sept 16-23 Week 2	Sept 23-30 Week 3	Sept 30-Oct 7 Week 4	Oct 7-14 Week 5	Oct 7-15 Week 6	Oct 7-16 Week 7	Oct 7-17 Week 8	Oct 7-18 Week 9	Oct 7-19 Week 10	Oct 7-20 Week 11	Oct 7-21 Week 12	Oct 7-22 Week 13
Week 4 Standard Diet	C1	22.38	21.9	23.3	23.5	24	Dissected								
	C2	21.3	22.7	24.25	25.5	26.5									
	C3	21.5	18.5	25.3	28.6	28.7									
	C4	25.3	26.7	27.25	28.7	29.7									
	C5	24	19.3	27.35	29.6	29.9									
	C6	26.5	27.5	30.55	33.1	34.9									
Week 8 Standard Diet	C7	20.4	21.5	21.65	22.5	23.4	23.5	23.15	23.15	23.6	Dissected				
	C8	~~21.4~~	Animal Removed												
	C9	23.8	24.5	24.74	25.4	27.6	27.8	28	28.2	27					
	C10	22.7	20.4	25.67	28	28	29.1	31.9	32.4	24.7					
	C11	24.1	24.6	26.71	31.1	29	29.5	32.1	~~34.9~~	34					
	C12	22.5	24.5	27.25	29.4	30.5	30.5	32.7	33.8	32.5					
Week 10 Standard Diet	C13	~~27.7~~	~~27.3~~	~~30.15~~	~~31~~	~~33.5~~	~~33.2~~	~~33.7~~	~~35.1~~	~~35.8~~	~~35.1~~	~~32.7~~	Dissected		
	C14	23.5	23.3	27.69	28.3	29.5	29	30	32	32.3	31.5	30.4			
	C15	23.4	24.7	27.24	29.3	31.8	32.1	32.8	34.6	35.7	34.7	33.4			
	C16	23.4	24.6	26.96	29.8	31.5	30.6	30.9	32.5	32.3	32.7	31.6			
	C17	20.9	18.3	25.23	26	27.8	28.1	28.9	30.1	29.85	29.3	28.2			
	C18	~~21.4~~	~~24~~	~~19.96~~	~~24.9~~	~~27.2~~	~~21.7~~	~~25.6~~	~~28.1~~	~~29.2~~	~~29.2~~	~~28.4~~			
Week 13 Standard Diet	C19	19.7	18	23.14	24.4	25.1	26.9	27.7	28.2	28.3	27.9	30.1	29.48	29.6	30
	C20	23.8	24.4	26.14	27.1	28	27.7	28.3	28.9	30.1	30.1	31.6	31.08	31.7	31.9
	C21	24.3	26	29.29	29.8	31.7	31.5	33.1	33.9	34.8	34.8	34.8	34.69	35.4	36.7
	C22	24.7	26	30.54	32	32	32.8	34.4	35	36	34.9	36.1	35.5	38.6	36.4
	C23	~~20.8~~	~~21.4~~	~~23.63~~	~~23.6~~	~~18.7~~	~~23.8~~	~~26.6~~	~~26.6~~	~~26.4~~	~~27.2~~	~~27.4~~	~~28.9~~	~~29.4~~	~~21.5~~
	C24	22.1	23.5	27.1	26.9	28.4	28.3	29	29	29.8	30.3	30.3	32.11	32.3	31.9
Week 4 DIO	T25	21.7	23.8	28.91	31.1	34.2	Dissected								
	T26	20.2	21.4	23.15	24.5	26.3									
	T27	23.8	24.5	27.13	28.7	31.9									
	T28	21	22.1	22.33	24.1	24.4									
	T29	23.5	23.2	24.69	26	28.5									
	T30	25	25.9	27.32	28.9	32.1									
Week 8 DIO	T31	~~25~~	~~20.6~~	~~29.66~~	~~33.6~~	~~33.1~~	~~35.6~~	~~39~~	~~40.8~~	~~32.5~~	Dissected				
	T32	24.7	28.4	31.18	32.1	34.2	34.9	37.7	39.8	41					
	T33	22.7	24	25.73	27.7	29.1	29.3	31.9	34.1	35					
	T34	23.5	23.4	27.1	28.1	30.9	31.2	33.4	35.8	37.7					
	T35	21.7	22.1	23.63	24.1	26.2	27.1	29.6	31.2	29.8					
	T36	22.4	23.4	23.72	24.6	26.4	28.1	28	29.2	30.2					
Week 10 DIO Food Restrict	T37	~~19.9~~	~~16.7~~	~~22.02~~	~~24.8~~	~~25.6~~	~~26.6~~	~~28.8~~	~~30.5~~	~~31~~	~~28.4~~	~~27~~	Dissected		
	T38	22.6	23.9	26.33	29.5	31.7	33.8	35.8	38.6	39.8	37.8	37.5			
	T39	24.7	27.6	28.59	32	34.5	37	39.4	43.2	45.6	43.5	42.3			
	T40	23.6	26.3	26.98	30.1	31.9	34.9	36.9	38.2	39.8	38.5	37.8			
	T41	22	22.9	23.98	26.1	27.5	30	32.5	34.8	35.3	35	33.8			
	T42	21.4	22.3	23.18	25.1	25.2	27.8	29.6	32.6	33.5	33.4	32.1			
Week 13 DIO Food Restrict	T43	25.8	26.4	28.08	30.5	32.9	34.5	35.6	38.6	39.2	36.7	36.9	35.5	36.32	34.9
	T44	19.8	15.5	22.46	24.7	27.1	28	30.5	31.8	33.2	31.2	30.2	30.3	30.6	30.1
	T45	~~23.7~~	~~24.5~~	~~26.33~~	~~28.9~~	~~30.5~~	~~34.5~~	~~32.7~~	~~34.5~~	~~36~~	~~35.6~~	~~34.7~~	~~34.3~~	~~25.6~~	~~30.3~~
	T46	24.9	26.6	27.93	30	32.7	34.5	36.2	38.5	40.3	39.4	39.3	36.3	37.1	39.3
	T47	26.9	29.3	30.88	33	33.9	35.4	36.9	39.5	41.6	40.1	40.7	39.1	41.8	39.7
	T48	23.6	24.7	25.24	25.7	27.8	29.1	30.7	32.1	33.7	33.1	32.3	32	32.4	33.6

Table B-3: Experiment 1: Body Weight (g) Welch's 2-Tailed T-test
(Comparisons of 0.05 significance marked in green)

Exp. 1 Body Weight (g) T Test
Significance of 0.05 Highlighted in Green

	Week 0 Standard Diet	Week 0 DIO
	22.4	21.7
	21.3	20.2
	21.5	23.8
	25.3	21
	24.0	23.5
	26.5	25
	20.4	24.7
	23.8	22.7
	22.7	23.5
	24.1	21.7
	22.5	22.4
	23.5	22.6
	23.4	24.7
	23.4	23.6
	20.9	22
	19.7	21.4
	23.8	25.8
	24.3	19.8
	24.7	24.9
	22.1	26.9
		23.6
Sample Size	20.00	21.00
Mean	23.01	23.12
StDev	1.70	1.84
Sx	0.55	
Deg. Of Freedom	38.97	
t value	-0.190	
P(Means are equal)	85.03%	

	Week 4 Standard Diet	Week 4 DIO
	24.0	34.2
	26.5	26.3
	28.7	31.9
	29.7	24.4
	29.9	28.5
	34.9	32.1
	23.4	34.2
	27.6	29.1
	28.0	30.9
	29.0	26.2
	30.5	26.4
	29.5	31.7
	31.8	34.5
	31.5	31.9
	27.8	27.5
	25.1	25.2
	28.0	32.9
	31.7	27.1
	32.0	32.7
	28.4	33.9
		27.8
Sample Size	20.00	21.00
Mean	28.90	29.97
StDev	2.83	3.32
Sx	0.96	
Deg. Of Freedom	38.56	
t value	-1.114	
P(Means are equal)	27.22%	

	Week 8 Standard Diet	Week 8 DIO
	23.6	41.0
	27.0	35.0
	24.7	37.7
	34.0	29.8
	32.5	30.2
	32.3	39.8
	35.7	45.6
	32.3	39.8
	29.9	35.3
	28.3	33.5
	30.1	39.2
	34.8	33.2
	36.0	40.3
	29.8	41.6
		33.7
Sample Size	14.00	15.00
Mean	30.78	37.05
StDev	3.89	4.52
Sx	1.56	
Deg. Of Freedom	26.84	
t value	-4.010	
P(Means are equal)	0.05%	

	Week 10 Standard Diet	Week 10 DIO
	30.4	37.5
	33.4	42.3
	31.6	37.8
	28.2	33.8
	30.1	32.1
	31.6	36.9
	34.8	30.2
	36.1	39.3
	30.3	40.7
		32.3
Sample Size	9.00	10.00
Mean	31.83	36.29
StDev	2.50	4.02
Sx	1.52	
Deg. Of Freedom	15.24	
t value	-2.930	
P(Means are equal)	1.03%	

	Week 13 Standard Diet	Week 13 DIO
	30	34.9
	31.9	30.1
	36.7	39.3
	36.4	39.7
	31.9	33.6
Sample Size	5.00	5.00
Mean	33.38	35.52
StDev	3.00	4.04
Sx	2.25	
Deg. Of Freedom	7.38	
t value	-0.952	
P(Means are equal)	37.30%	

Appendix B.2: Experiment 1 Food and Energy Tables

Table B-4: Experiment 1: Standard Diet Given and Leftover (g)

Exp. 1 Standard Diet Given and Leftover (grams)

			Sept 9-16 Week 1	Sept 16-23 Week 2	Sept 23-30 Week 3	ept 30-Oct Week 4	Oct 7-14 Week 5	Oct 7-15 Week 6	Oct 7-16 Week 7	Oct 7-17 Week 8	Oct 7-18 Week 9	Oct 7-19 Week 10	Oct 7-20 Week 11	Oct 7-21 Week 12	Oct 7-22 Week 13
Week 4 Standard Diet	C1	Added	34.4	38.2	31.2	30.1	Dissected								
		Left	13.3	17.5	8.6	8.2									
	C2	Added	32.1	36.8	30.8	30.6									
		Left	10.6	13.3	5.1	7.1									
	C3	Added	33.4	34.7	53.8	30.9									
		Left	15.1	5.1	4.6	3.4									
	C4	Added	38.3	36.6	30.0	30.9									
		Left	9.7	12.6	2.9	6.6									
	C5	Added	37.3	35.7	30.7	30.3									
		Left	18.0	3.7	2.6	4.3									
	C6	Added	40.3	34.8	29.8	30.5									
		Left	15.9	6.4	0.0	2.5									
Week 8 Standard Diet	C7	Added	30.7	37.3	32.4	30.9	37.2	35.8	34.8	35.3	Dissected				
		Left	7.1	18.7	10.9	9.4	13.6	13.7	13.4	6.6					
	C9	Added	36.9	36.9	30.7	31.5	34.8	36.4	35.5	35.7					
		Left	13.1	13.1	6.1	6.4	9.1	14.8	13.1	9.2					
	C10	Added	35.0	34.1	31.1	31.8	34.0	34.8	36.1	34.6					
		Left	16.7	7.6	3.4	6.8	7.3	6.7	12.0	16.5					
	C11	Added	38.1	36.0	68.0	32.5	35.0	35.3	36.2	35.4					
		Left	13.0	10.3	11.8	7.1	7.9	6.7	8.5	6.3					
	C12	Added	34.1	34.6	32.3	32.6	37.0	35.9	36.6	35.7					
		Left	9.6	7.3	3.6	6.6	10.6	9.3	11.5	7.3					
Week 10 Standard Diet	C14	Added	35.8	34.3	29.4	30.8	34.8	34.5	36.0	35.0	35.7	35.2	Dissected		
		Left	14.4	6.2	3.7	7.0	12.2	11.6	11.8	7.5	12.9	0.0			
	C15	Added	35.1	34.7	31.0	30.0	36.9	34.3	36.1	47.2	35.2	35.3			
		Left	9.5	8.1	2.3	1.9	10.7	8.4	11.6	14.8	11.3	0.0			
	C16	Added	38.0	35.0	31.4	30.8	36.8	36.3	36.5	34.3	36.3	35.1			
		Left	12.3	10.3	1.8	2.9	12.2	10.6	10.5	4.4	10.1	0.0			
	C17	Added	31.9	35.1	29.8	30.5	37.6	35.1	36.4	35.4	37.8	35.0			
		Left	13.1	7.3	5.7	4.6	10.7	11.8	13.5	6.4	15.3	0.0			
Week 13 Standard Diet	C19	Added	32.7	36.7	30.5	32.1	35.4	34.5	36.8	35.3	34.6	34.6	36.7	37.2	37.6
		Left	14.2	11.4	1.5	8.7	10.6	11.4	15.0	8.0	11.3	9.6	12.9	19.3	14.2
	C20	Added	36.5	34.7	32.6	31.4	36.5	34.3	36.1	34.5	34.5	35.2	34.9	35.7	37.5
		Left	13.7	10.6	6.2	7.9	12.5	11.2	12.7	5.9	9.7	8.8	10.9	10.5	12.3
	C21	Added	37.3	35.3	29.6	31.9	36.4	35.7	36.0	35.8	34.7	34.8	34.4	36.8	36.5
		Left	13.6	8.5	2.7	5.9	12.1	9.7	11.2	5.9	10.0	8.6	8.6	11.4	11.6
	C22	Added	37.8	35.1	30.6	31.8	35.8	36.3	36.5	35.4	34.7	35.3	35.5	36.2	36.5
		Left	11.6	3.8	1.5	5.5	9.1	8.4	10.9	4.5	11.0	8.4	9.5	7.2	13.9
	C24	Added	35.7	35.9	31.2	30.0	32.7	35.3	35.0	35.1	35.3	35.0	36.5	36.5	36.0
		Left	13.9	8.8	4.1	4.4	7.8	10.7	12.6	4.9	11.1	9.4	10.5	12.3	12.7

Table B-5: Experiment 1: DIO Given and Leftover (g)

Exp. 1 High Fat Diet (DIO) Given and Leftover (grams)

			Sept 9-16 Week 1	Sept 16-23 Week 2	Sept 23-30 Week 3	ept 30-Oct Week 4	Oct 7-14 Week 5	Oct 7-15 Week 6	Oct 7-16 Week 7	Oct 7-17 Week 8	Oct 7-18 Week 9	Oct 7-19 Week 10	Oct 7-20 Week 11	Oct 7-21 Week 12	Oct 7-22 Week 13
Week 4 DIO	T25	Added	34.8	36.2	26.2	29.7	Dissected								
		Left	18.8	13.8	3.5	7.6									
	T26	Added	31.3	36.8	25.3	28.4									
		Left	15.5	19.5	7.1	8.8									
	T27	Added	38.1	35.6	26.4	28.1									
		Left	21.4	17.4	6.7	6.5									
	T28	Added	32.1	36.9	26.7	27.3									
		Left	15.5	21.3	10.3	11.8									
	T29	Added	37.5	35.4	27.0	30.8									
		Left	21.0	18.3	8.9	12.0									
	T30	Added	39.0	35.2	27.0	29.4									
		Left	20.7	16.2	7.5	9.1									
Week 8 DIO	T32	Added	37.8	34.5	25.3	30.2	32.3	31.4	30.0	29.5	Dissected				
		Left	14.8	12.6	5.4	9.8	11.0	10.7	9.4	5.0					
	T33	Added	35.7	35.7	26.8	28.3	30.8	31.5	31.8	30.1					
		Left	17.6	18.2	5.0	7.8	11.4	12.4	11.8	6.0					
	T34	Added	36.8	35.9	25.6	29.1	31.2	32.4	31.0	29.8					
		Left	23.1	14.6	7.1	8.4	13.2	14.0	11.4	5.4					
	T35	Added	32.5	35.7	26.7	29.0	32.1	31.2	31.3	29.9					
		Left	17.6	20.4	10.4	10.7	14.1	12.0	13.5	9.9					
	T36	Added	36.0	36.9	25.6	30.3	32.2	30.0	31.6	30.5					
		Left	20.5	20.6	8.4	12.2	14.3	15.0	15.8	6.7					
Week 10 DIO	T38	Added	35.5	35.9	25.8	30.2	32.1	30.4	30.1	30.2	23.5	32.9	Dissected		
		Left	18.9	16.3	5.5	10.0	12.2	11.4	11.1	5.4	0.0	0.0			
	T39	Added	39.6	34.8	26.2	29.6	32.1	31.7	29.5	41.0	26.5	37.1			
		Left	18.7	14.4	3.2	6.8	10.3	10.2	6.6	13.6	0.0	0.0			
	T40	Added	37.4	35.1	26.5	30.1	30.9	31.7	31.1	29.6	23.0	32.2			
		Left	19.5	13.4	4.1	6.7	7.7	9.0	10.3	3.7	0.0	0.0			
	T41	Added	33.9	36.4	26.5	30.7	30.9	30.4	30.9	29.7	21.0	29.4			
		Left	18.7	19.8	8.8	11.9	12.4	11.2	12.4	6.2	0.0	0.0			
	T42	Added	33.3	36.5	25.6	28.5	31.8	32.2	30.9	30.7	20.0	28.0			
		Left	16.8	19.1	8.2	10.3	11.5	13.1	10.9	5.6	0.0	0.0			
Week 13 DIO	T43	Added	41.2	36.4	26.9	31.0	31.5	31.8	30.0	30.2	23.5	28.2	40.9	39.9	40.8
		Left	21.6	14.2	6.0	9.2	10.8	11.5	9.5	7.3	0.0	0.0	18.1	13.4	16.5
	T44	Added	32.5	35.5	25.2	29.3	30.9	32.1	31.4	28.9	19.5	23.4	40.7	40.7	40.6
		Left	18.4	14.7	7.3	8.5	13.2	12.7	13.5	4.7	0.0	0.0	17.9	17.1	17.0
	T46	Added	39.8	34.8	26.4	30.8	31.9	31.1	32.0	30.1	23.5	28.2	40.8	41.0	42.6
		Left	20.6	14.0	6.9	9.7	11.4	11.9	12.2	5.7	0.0	0.0	20.2	13.6	16.3
	T47	Added	41.4	36.5	25.2	29.7	30.4	31.0	30.5	42.3	24.0	28.8	41.4	40.0	41.9
		Left	21.5	16.4	5.6	9.0	10.5	11.2	10.8	16.7	0.0	0.0	16.1	9.3	17.2
	T48	Added	37.2	34.7	26.2	29.8	29.6	30.7	30.1	30.5	19.5	23.4	40.2	41.2	41.4
		Left	20.2	18.5	10.4	10.8	12.0	12.7	12.9	7.3	0.0	0.0	15.8	15.4	15.9

Table B-6: Experiment 1: Weekly Food Consumption (g)

Exp. 1 Weekly Food Consumption (g)

		Sept 9-16 Week 1	Sept 16-23 Week 2	Sept 23-30 Week 3	Sept 30-Oct Week 4	Oct 7-14 Week 5	Oct 7-15 Week 6	Oct 7-16 Week 7	Oct 7-17 Week 8	Oct 7-18 Week 9	Oct 7-19 Week 10	Oct 7-20 Week 11	Oct 7-21 Week 12	Oct 7-22 Week 13
Week 4 Standard Diet	C1	21.1	20.7	22.6	21.9	Dissected								
	C2	21.5	23.5	25.7	23.5									
	C3	18.3	29.6	49.2	27.5									
	C4	28.6	24.0	27.1	24.3									
	C5	19.3	32.0	28.1	26.0									
	C6	24.4	28.4	29.8	28.0									
Week 8 Standard Diet	C7	23.6	18.6	21.5	21.5	23.6	22.1	21.4	28.7	Dissected				
	C9	23.8	23.8	24.6	25.1	25.7	21.6	22.4	26.5					
	C10	18.3	26.5	27.7	25.0	26.7	28.1	24.1	18.1					
	C11	25.1	25.7	56.2	25.4	27.1	28.6	27.7	29.1					
	C12	24.5	27.3	28.7	26.0	26.4	26.6	25.1	28.4					
Week 10 Standard Diet	C14	21.4	28.1	25.7	23.8	22.6	22.9	24.2	27.5	22.8	35.2			
	C15	25.6	26.6	28.7	28.1	26.2	25.9	24.5	32.4	23.9	35.3			
	C16	25.7	24.7	29.6	27.9	24.6	25.7	26.0	29.9	26.2	35.1			
	C17	18.8	27.8	24.1	25.9	26.9	23.3	22.9	29.0	22.5	35.0			
Week 13 Standard Diet	C19	18.5	25.3	29.0	23.4	24.8	23.1	21.8	27.3	23.3	25.0	23.8	17.8	23.4
	C20	22.8	24.1	26.4	23.5	24.0	23.1	23.4	28.6	24.8	26.4	24.0	25.3	25.2
	C21	23.7	26.8	26.9	26.0	24.3	26.0	24.8	29.9	24.7	26.2	25.8	25.4	24.9
	C22	26.2	31.3	29.1	26.3	26.7	27.9	25.6	30.9	23.7	26.9	26.0	29.0	22.6
	C24	21.8	27.1	27.1	25.6	24.9	24.6	22.4	30.2	24.2	25.6	26.0	24.1	23.3
Week 4 DIO	T25	16.0	22.4	22.7	22.1	Dissected								
	T26	15.8	17.3	18.2	19.6									
	T27	16.7	18.2	19.7	21.6									
	T28	16.6	15.6	16.4	15.5									
	T29	16.5	17.1	18.1	18.8									
	T30	18.3	19.1	19.5	20.3									
Week 8 DIO	T32	23.0	21.9	19.9	20.4	21.3	20.7	20.6	24.5	Dissected				
	T33	18.1	17.6	21.8	20.5	19.4	19.1	20.0	24.1					
	T34	13.7	21.3	18.5	20.7	18.0	18.4	19.6	24.4					
	T35	14.9	15.3	16.3	18.3	18.0	19.2	17.8	20.0					
	T36	15.5	16.3	17.2	18.1	17.9	15.0	15.8	23.8					
Week 10 DIO	T38	16.6	19.6	20.3	20.2	19.9	19.0	19.0	24.8	23.5	32.9	Dissected		
	T39	20.9	20.4	23.0	22.8	21.8	21.5	22.9	27.4	26.5	37.1			
	T40	17.9	21.7	22.4	23.4	23.2	22.7	20.8	25.9	23.0	32.2			
	T41	15.2	16.6	17.7	18.8	18.5	19.2	18.5	23.5	21.0	29.4			
	T42	16.5	17.4	17.4	18.2	20.3	19.1	20.0	25.1	20.0	28.0			
Week 13 DIO Food Restrict	T43	19.6	22.2	20.9	21.8	20.7	20.3	20.5	22.9	23.5	28.2	22.8	26.5	24.3
	T44	14.1	20.8	17.9	20.8	17.7	19.4	17.9	24.2	19.5	23.4	22.8	23.6	23.6
	T46	19.2	20.8	19.5	21.1	20.5	19.2	19.8	24.4	23.5	28.2	20.6	27.5	26.3
	T47	19.9	20.1	19.6	20.7	19.9	19.8	19.7	25.6	24.0	28.8	25.3	30.7	24.7
	T48	17.0	16.2	15.8	19.0	17.6	18.0	17.2	23.2	19.5	23.4	24.4	25.8	25.5

Table B-7: Experiment 1: Weekly Energy Consumption (kcal)

Exp. 1 Weekly Energy Consumption (kcal)

		Sept 9-16 Week 1	Sept 16-23 Week 2	Sept 23-30 Week 3	Sept 30-Oct Week 4	Oct 7-14 Week 5	Oct 7-15 Week 6	Oct 7-16 Week 7	Oct 7-17 Week 8	Oct 7-18 Week 9	Oct 7-19 Week 10	Oct 7-20 Week 11	Oct 7-21 Week 12	Oct 7-22 Week 13
Week 4 Standard Diet	C1	65.5	64.1	70.2	68.0	Dissected								
	C2	66.7	72.8	79.7	72.9									
	C3	56.7	91.7	152.5	85.2									
	C4	88.7	74.5	84.1	75.4									
	C5	59.8	99.2	87.1	80.7									
	C6	75.6	88.2	92.2	86.7									
Week 8 Standard Diet	C7	73.2	57.7	66.5	66.8	73.2	68.4	66.3	89.0	Dissected				
	C9	73.8	73.7	76.4	77.8	79.7	67.0	69.4	82.2					
	C10	56.7	82.1	85.8	77.4	82.8	87.1	74.7	56.1					
	C11	77.8	79.8	174.3	78.8	84.0	88.7	85.9	90.2					
	C12	76.0	84.5	89.1	80.7	81.8	82.5	77.8	88.0					
Week 10 Standard Diet	C14	66.3	87.1	79.8	73.7	70.1	71.0	75.0	85.3	70.7	109.1	Dissected		
	C15	79.4	82.4	89.0	87.0	81.2	80.3	76.0	100.4	74.1	109.4			
	C16	79.7	76.4	91.8	86.3	76.3	79.7	80.6	92.7	81.2	108.8			
	C17	58.3	86.1	74.7	80.3	83.4	72.2	71.0	89.9	69.8	108.5			
Week 13 Standard Diet	C19	57.4	78.5	89.8	72.6	76.9	71.6	67.6	84.6	72.2	77.5	73.7	55.3	72.6
	C20	70.7	74.6	81.8	72.8	74.4	71.6	72.5	88.7	76.9	81.8	74.3	78.3	78.1
	C21	73.5	83.0	83.5	80.5	75.3	80.6	76.9	92.7	76.6	81.2	80.1	78.7	77.2
	C22	81.2	96.9	90.1	81.7	82.8	86.5	79.4	95.8	73.5	83.4	80.7	89.9	70.1
	C24	67.6	84.1	84.0	79.4	77.2	76.3	69.4	93.6	75.0	79.4	80.7	74.8	72.2
Week 4 DIO	T25	81.6	114.4	115.5	112.9	Dissected								
	T26	80.6	88.3	92.6	100.1									
	T27	85.2	92.9	100.4	110.0									
	T28	84.7	79.7	83.4	78.8									
	T29	84.2	87.2	92.2	96.1									
	T30	93.3	97.2	99.3	103.4									
Week 8 DIO	T32	117.3	111.8	101.4	104.2	108.6	105.6	105.1	125.0	Dissected				
	T33	92.3	89.5	111.3	104.6	98.9	97.4	102.0	122.9					
	T34	69.9	108.5	94.2	105.5	91.8	93.8	100.0	124.4					
	T35	76.0	77.9	83.2	93.3	91.8	97.9	90.8	102.0					
	T36	79.1	83.0	87.5	92.5	91.3	76.5	80.6	121.4					
Week 10 DIO	T38	84.7	99.8	103.7	103.1	101.5	96.9	96.9	126.5	72.9	102.0	Dissected		
	T39	106.6	103.9	117.2	116.2	111.2	109.7	116.8	139.7	82.2	115.0			
	T40	91.3	110.6	114.1	119.1	118.3	115.8	106.1	132.1	71.3	99.8			
	T41	77.5	84.8	90.5	96.0	94.4	97.9	94.4	119.9	65.1	91.1			
	T42	84.2	88.7	88.7	92.6	103.5	97.4	102.0	128.0	62.0	86.8			
Week 13 DIO Food Restrict	T43	100.0	113.2	106.3	111.2	105.6	103.5	104.6	116.8	72.9	87.4	70.7	82.2	75.3
	T44	71.9	106.0	91.2	105.9	90.3	98.9	91.3	123.4	60.5	72.5	70.7	73.1	73.2
	T46	97.9	105.9	99.4	107.5	104.6	97.9	101.0	124.4	72.9	87.4	63.9	85.1	81.5
	T47	101.5	102.6	100.0	105.8	101.5	101.0	100.5	130.6	74.4	89.3	78.4	95.1	76.6
	T48	86.7	82.4	80.4	96.7	89.8	91.8	87.7	118.3	60.5	72.5	75.6	80.0	79.1

Table B-8: Experiment 1: Weekly Food Consumption (g) Welch's 2-Tailed T-test
(Comparisons of 0.05 significance marked in green)

Exp. 1 Food Consumed (g) T Test
Significance of 0.05 Highlighted in Green

Week 1 Standard Diet	Week 1 DIO
21.1	16
21.5	15.8
18.3	16.7
28.6	16.6
19.3	16.5
24.4	18.3
23.6	23
23.8	18.1
18.3	13.7
25.1	14.9
24.5	15.5
21.4	16.6
25.6	20.9
25.7	17.9
18.8	15.2
18.5	16.5
22.8	19.6
23.7	14.1
26.2	19.2
21.8	19.9
	17
Sample Size	20.00	21.00
Mean	22.65	17.24
StDev	2.98	2.31
Sx	0.84	
Deg. Of Freedom	35.78	
t value	6.482	
P(Means are equal)	0.00%	

Week 4 Standard Diet	Week 4 DIO
21.9	22.1
23.5	19.6
27.5	21.6
24.3	15.5
26.0	18.8
28.0	20.3
21.5	20.4
25.1	20.5
25.0	20.7
25.4	18.3
26.0	18.1
23.8	20.2
28.1	22.8
27.9	23.4
25.9	18.8
23.4	18.2
23.5	21.8
26.0	20.8
26.3	21.1
25.6	20.7
	19.0
Sample Size	20.00	21.00
Mean	25.24	20.12
StDev	1.89	1.83
Sx	0.58	
Deg. Of Freedom	38.71	
t value	8.796	
P(Means are equal)	0.00%	

Week 8 Standard Diet	Week 8 DIO
28.7	24.5
26.5	24.1
18.1	24.4
29.1	20.0
28.4	23.8
27.5	24.8
32.4	27.4
29.9	25.9
29.0	23.5
27.3	25.1
28.6	22.9
29.9	24.2
30.9	24.4
30.2	25.6
	23.2
Sample Size	14.00	15.00
Mean	28.32	24.25
StDev	3.31	1.63
Sx	0.98	
Deg. Of Freedom	18.68	
t value	4.149	
P(Means are equal)	0.06%	

Week 10 Standard Diet	Week 10 DIO
35.2	32.9
35.3	37.1
35.1	32.2
35	29.4
25	28
26.4	28.2
26.2	23.4
26.9	28.2
25.6	28.8
	23.4
Sample Size	9.00	10.00
Mean	30.08	29.16
StDev	4.84	4.16
Sx	2.08	
Deg. Of Freedom	15.92	
t value	0.441	
P(Means are equal)	66.57%	

Week 13 Standard Diet	Week 13 DIO
23.43	24.3
25.2	23.6
24.9	26.3
22.6	24.7
23.3	25.5
Sample Size	5.00	5.00
Mean	23.89	24.88
StDev	1.11	1.05
Sx	0.68	
Deg. Of Freedom	7.97	
t value	-1.452	
P(Means are equal)	18.97%	

Table B-9: Experiment 1: Weekly Energy Consumption (kcal) Welch's 2-Tailed T-test
(Comparisons of 0.05 significance marked in green)

Exp. 1 Energy Consumed (g) T Test
Significance of 0.05 Highlighted in Green

Week 1 Standard Diet	Week 1 DIO
65.5	81.6
66.7	80.58
56.7	85.17
88.7	84.66
59.8	84.15
75.6	93.33
73.2	117.3
73.8	92.31
56.7	69.87
77.8	75.99
76.0	79.05
66.3	84.66
79.4	106.59
79.7	91.29
58.3	77.52
57.4	84.15
70.7	99.96
73.5	71.91
81.2	97.92
67.6	101.49
	86.7
Sample Size: 20.00	21.00
Mean: 70.22	87.91
StDev: 9.24	11.76
Sx: 3.29	
Deg. Of Freedom: 37.68	
t value: -5.371	
P(Means are equal): 0.00%	

Week 4 Standard Diet	Week 4 DIO
68.0	112.9
72.9	100.1
85.2	110.0
75.4	78.8
80.7	96.1
86.7	103.4
66.8	104.2
77.8	104.6
77.4	105.5
78.8	93.3
80.7	92.5
73.7	103.1
87.0	116.2
86.3	119.1
80.3	96.0
72.6	92.6
72.8	111.2
80.5	105.9
81.7	107.5
79.4	105.8
	96.7
Sample Size: 20.00	21.00
Mean: 78.23	102.63
StDev: 5.87	9.31
Sx: 2.42	
Deg. Of Freedom: 33.94	
t value: -10.093	
P(Means are equal): 0.00%	

Week 8 Standard Diet	Week 8 DIO
89.0	125.0
82.2	122.9
56.1	124.4
90.2	102.0
88.0	121.4
85.3	126.5
100.4	139.7
92.7	132.1
89.9	119.9
84.6	128.0
88.7	116.8
92.7	123.4
95.8	124.4
93.6	130.6
	118.3
Sample Size: 14.00	15.00
Mean: 87.80	123.69
StDev: 10.27	8.33
Sx: 3.49	
Deg. Of Freedom: 25.09	
t value: -10.294	
P(Means are equal): 0.00%	

Week 10 Standard Diet	Week 10 DIO
109.12	101.99
109.43	115.01
108.81	99.82
108.5	91.14
77.5	86.8
81.84	87.42
81.22	72.54
83.39	87.42
79.36	89.28
	72.54
Sample Size: 9.00	10.00
Mean: 93.24	90.40
StDev: 15.01	12.91
Sx: 6.46	
Deg. Of Freedom: 15.92	
t value: 0.441	
P(Means are equal): 66.57%	

Week 13 Standard Diet	Week 13 DIO
72.633	75.33
78.12	73.16
77.19	81.53
70.06	76.57
72.23	79.05
Sample Size: 5.00	5.00
Mean: 74.05	77.13
StDev: 3.45	3.25
Sx: 2.12	
Deg. Of Freedom: 7.97	
t value: -1.452	
P(Means are equal): 18.97%	

Appendix B.3: Experiment 1 Body Composition Tables

Table B-10: Experiment 1: Dissected Tissue Weights (g)

Exp. 1 Dissected Tissue Weights (g)

		Liver (g)	Carcass (g)
Week 4 Standard Diet	C1	1.025	15.680
	C2	1.230	17.353
	C3	1.210	19.095
	C4	1.507	19.860
	C5	1.422	19.457
	C6	1.743	23.000
Week 8 Standard Diet	C7	1.069	15.360
	C9	1.139	17.980
	C10	0.960	17.970
	C11	1.637	23.400
	C12	1.435	23.170
Week 10 Standard Diet	C14	1.601	22.410
	C15	1.692	24.840
	C16	1.449	23.881
	C17	1.300	20.990
Week 13 Standard Diet	C19	1.400	21.400
	C20	1.346	22.880
	C21	1.701	25.580
	C22	1.681	25.800
	C24	1.435	22.990
Week 4 DIO	T25	1.266	24.460
	T26	1.037	17.677
	T27	1.335	21.971
	T28	1.052	16.410
	T29	1.216	19.727
	T30	1.291	21.864
Week 8 DIO	T32	1.474	31.380
	T33	1.203	26.580
	T34	1.245	33.801
	T35	0.923	22.086
	T36	0.995	20.820
Week 10 DIO	T38	1.710	27.480
	T39	1.790	31.562
	T40	1.680	25.580
	T41	1.428	24.590
	T42	1.264	22.495
Week 13 DIO Food Restrict	T43	1.547	24.859
	T44	1.106	21.703
	T46	1.765	27.544
	T47	1.576	27.921
	T48	1.441	23.495

Table B-11: Experiment 1: Soxhlet Fat Extraction (g)

Exp. 1 Standard Diet Soxhlet Fat Extraction (g)

		Tube	Tube Weight (g)	Tube & Sample (g)	Tube, Sample, & Gauze (g)	Extracted (g)
Week 0 Baseline	B1	1	18.55	19.46	20.62	20.23
		2	20.30	21.21	22.43	22.04
		3	14.48	15.38	16.05	15.67
	B2	1	15.48	16.59	17.44	16.96
		2	15.42	16.45	17.22	16.76
		3	18.65	19.52	20.08	19.70
	B3	1	25.46	26.54	27.59	27.22
		2	21.52	22.46	23.07	22.57
		3	17.02	18.00	19.14	18.81
	B4	1	20.82	21.85	22.87	22.55
		2	12.95	13.95	15.19	14.90
		3	14.82	15.74	16.96	16.69
	B5	1	13.02	14.27	15.59	15.16
		2	15.74	16.81	17.50	17.13
		3	16.82	17.45	18.41	18.09
	B6	1	16.99	17.93	19.09	18.73
		2	21.95	22.78	23.48	23.16
		3	18.86	19.79	20.86	20.51
	B7	1	16.11	16.93	18.14	17.87
		2	17.09	18.02	19.17	18.87
		3	16.78	17.89	18.78	18.43
Week 4 Standard Diet	C1	1	21.83	22.80	24.16	23.67
		2	16.48	17.43	18.20	17.75
		3	16.31	17.36	18.34	17.87
	C2	1	15.44	16.52	17.53	16.98
		2	17.53	18.57	20.81	20.24
		3	17.85	18.86	20.43	19.89
	C3	1	16.96	17.90	18.98	18.47
		2	24.30	25.33	26.03	25.47
		3	21.21	22.22	22.83	22.28
	C4	1	25.36	26.82	27.68	26.82
		2	20.04	21.18	22.58	21.88
		3	18.46	19.35	20.43	19.87
	C5	1	21.44	22.40	23.20	22.74
		2	14.78	15.70	16.89	16.43
		3	21.27	22.23	23.32	22.85
	C6	1	16.80	17.95	18.72	17.99
		2	18.86	19.82	20.98	20.35
		3	14.77	15.98	16.65	15.89
Week 8 Standard Diet	C7	1	16.10	17.14	18.35	18.00
		2	17.31	18.30	19.77	19.43
		3	15.47	16.51	17.21	16.85
	C9	1	20.96	21.84	22.91	22.54
		2	16.82	17.66	18.77	18.41
		3	15.41	16.51	17.73	17.27
	C10	1	21.94	22.99	24.13	23.68
		2	18.83	19.65	20.89	20.54
		3	14.47	15.70	16.45	15.93
	C11	1	17.08	18.27	19.09	18.44
		2	21.52	22.37	23.03	22.56
		3	17.46	18.42	19.44	18.91
	C12	1	18.84	19.68	21.00	20.51
		2	16.78	17.56	18.60	18.16
		3	13.02	14.17	14.86	14.21
Week 10 Standard Diet	C14	1	17.30	18.28	18.95	18.34
		2	17.08	18.34	18.94	18.17
		3	16.80	17.90	19.07	18.42
	C15	1	13.25	14.21	15.27	14.69
		2	17.02	18.09	19.49	18.84
		3	16.31	17.44	18.27	17.59
	C16	1	14.47	15.75	16.74	15.96
		2	13.01	14.09	15.45	14.79
		3	16.09	17.07	18.23	17.62
	C17	1	17.46	18.44	19.33	18.80
		2	21.94	22.89	24.27	23.70
		3	20.93	21.65	22.34	21.94
Week 13 Standard Diet	C19	1	20.04	20.99	21.88	21.32
		2	20.32	21.35	22.03	21.44
		3	16.42	17.63	18.46	17.76
	C20	1	14.79	15.62	16.79	16.28
		2	18.59	19.58	20.36	19.76
		3	24.22	25.30	25.99	25.33
	C21	1	16.85	17.94	19.09	18.40
		2	21.45	22.36	23.42	22.85
		3	21.78	22.75	24.11	23.48
	C22	1	14.79	15.98	16.97	16.21
		2	17.81	18.75	19.97	19.16
		3	25.39	26.45	27.84	27.15
	C24	1	14.81	15.75	16.42	15.87
		2	20.29	21.31	22.07	21.43
		3	15.90	16.86	17.61	16.98

Exp. 1 DIO Soxhlet Fat Extraction (g)

		Tube	Tube Weight (g)	Tube & Sample (g)	Tube, Sample, & Gauze (g)	Extracted (g)
Week 4 DIO	T25	1	20.27	21.82	22.56	21.48
		2	18.94	19.84	21.14	20.50
		3	21.61	22.62	23.83	23.11
	T26	1	12.71	13.67	14.88	14.35
		2	15.87	16.87	17.57	17.03
		3	13.27	14.28	15.28	14.73
	T27	1	15.62	17.02	18.06	17.15
		2	16.91	18.30	19.51	18.62
		3	16.45	17.51	18.75	18.04
	T28	1	16.56	17.53	18.59	18.22
		2	21.84	22.82	23.57	23.20
		3	17.53	18.98	20.08	19.53
	T29	1	14.80	15.94	16.92	16.28
		2	16.45	17.70	18.36	17.66
		3	17.61	18.74	20.20	19.56
	T30	1	14.10	15.23	15.93	15.24
		2	16.15	17.38	18.69	17.92
		3	20.54	21.92	23.05	22.18
Week 8 DIO	T32	1	18.27	15.91	16.58	15.76
		2	25.96	22.87	23.63	22.84
		3	18.96	17.35	18.35	17.44
	T33	1	16.26	14.30	15.11	14.40
		2	18.62	17.62	18.70	17.86
		3	20.20	18.51	19.60	18.92
	T34	1	17.35	15.15	16.30	15.51
		2	18.84	17.68	19.16	18.25
		3	20.62	19.16	20.49	19.67
	T35	1	19.85	18.07	19.06	18.29
		2	23.98	21.12	22.67	22.13
		3	23.51	21.57	22.79	22.12
	T36	1	18.08	17.57	18.71	18.11
		2	19.74	17.22	18.30	17.60
		3	25.44	22.79	23.86	23.43
Week 10 DIO Food Restrict	T38	1	13.25	14.11	15.25	14.62
		2	18.80	19.75	20.42	19.75
		3	13.00	14.07	14.82	14.05
	T39	1	20.89	21.77	22.82	22.16
		2	18.80	19.91	20.67	19.84
		3	18.82	20.01	21.00	20.15
	T40	1	16.43	17.40	18.61	18.01
		2	17.51	18.46	19.53	18.93
		3	16.76	17.68	18.36	17.79
	T41	1	18.93	20.13	20.79	19.99
		2	16.68	17.77	19.16	18.42
		3	14.78	15.88	16.57	15.84
	T42	1	17.62	19.01	20.36	19.50
		2	16.77	17.95	19.11	18.39
		3	21.52	22.55	23.87	23.22
Week 13 DIO Food Restrict	T43	1	16.57	17.78	18.84	18.08
		2	16.13	17.46	18.49	17.65
		3	15.62	16.66	18.04	17.37
	T44	1	20.54	21.74	22.33	21.61
		2	16.41	17.37	18.15	17.57
		3	21.80	22.98	23.65	22.95
	T46	1	21.81	23.38	24.04	22.74
		2	14.65	15.61	16.76	16.07
		3	21.38	22.40	23.54	22.80
	T47	1	14.12	15.17	16.15	15.45
		2	13.27	14.16	14.91	14.60
		3	18.93	19.94	20.70	20.03
	T48	1	17.54	18.49	19.83	19.19
		2	21.92	23.07	23.9	23.14
		3	17.01	18.27	19.15	18.31

Table B-12: Experiment 1: Body Composition Mass (g)

Exp. 1 Body Composition Mass (g)

		Percentage Fat	Percentage Lean	Dry Powder Weight (g)	Dry Fat Body mass (g) (%Fat x Dry Powder Weight)	Dry Lean Body Mass (g) (%Lean x Dry Powder Weight)	Wet Powder Weight (g)	Wet Lean Body Mass (g) (%Lean x Wet Powder Weight)	Average Lean Body Mass (g)	Standard Deviation	Standard Error
Week 4 Standard Diet	C1	47.44%	52.56%	6.74	3.20	3.54	15.4	8.09	8.25	0.80918025	0.33034645
	C2	53.00%	47.00%	7.47	3.96	3.51	16.5	7.75			
	C3	54.36%	45.64%	8.51	4.63	3.88	18.4	8.40			
	C4	61.08%	38.92%	9.67	5.91	3.76	19.06	7.42			
	C5	48.78%	51.22%	8.21	4.01	4.20	19.04	9.75			
	C6	63.95%	36.05%	11.61	7.42	4.19	22.36	8.06			
Week 8 Standard Diet	C7	34.34%	65.66%	6.31	2.17	4.14	23.84	15.65	10.18	3.2679997	1.4614939
	C9	42.55%	57.45%	8.04	3.42	4.62	17.65	10.14			
	C10	55.04%	44.96%	8.11	4.46	3.65	21.44	9.64			
	C11	55.04%	44.96%	11.73	6.46	5.27	16.02	7.20			
	C12	57.39%	42.61%	11.85	6.80	5.05	19.44	8.28			
Week 10 Standard Diet	C14	60.55%	39.45%	11.65	7.05	4.60	15.4	6.07	7.61	1.88936306	0.94468153
	C15	60.43%	39.57%	12.86	7.77	5.09	17.98	7.11			
	C16	61.32%	38.68%	12.49	7.66	4.83	17.84	6.90			
	C17	54.96%	45.04%	10.03	5.51	4.52	23.02	10.37			
Week 13 Standard Diet	C19	57.99%	42.01%	10.54	6.11	4.43	23.02	9.67	9.76	1.47602587	0.66009884
	C20	61.11%	38.89%	11.61	7.09	4.52	30.76	11.96			
	C21	63.67%	36.33%	13.16	8.38	4.78	26.09	9.48			
	C22	64.50%	35.50%	14.08	9.08	5.00	27.77	9.86			
	C24	61.95%	38.05%	11.2	6.94	4.26	20.55	7.82			
Week 4 DIO	T25	70.84%	29.16%	14.24	10.09	4.15	23.2	6.77	9.36	2.79603809	1.14147777
	T26	54.79%	45.21%	8.48	4.65	3.83	21.86	9.88			
	T27	65.34%	34.66%	11.79	7.70	4.09	24.61	8.53			
	T28	37.94%	62.06%	6.42	2.44	3.98	23.47	14.56			
	T29	56.23%	43.77%	9.48	5.33	4.15	20.81	9.11			
	T30	62.25%	37.75%	11.4	7.10	4.30	19.34	7.30			
Week 8 DIO	T32	75.52%	24.48%	19.54	14.76	4.78	27.24	6.67	8.13	1.5580116	0.69676397
	T33	70.04%	29.96%	15.66	10.97	4.69	31.13	9.33			
	T34	74.61%	25.39%	17.53	13.08	4.45	25.08	6.37			
	T35	65.09%	34.91%	12.21	7.95	4.26	24.1	8.41			
	T36	55.78%	44.22%	10.9	6.08	4.82	22.27	9.85			
Week 10 DIO	T38	71.33%	28.67%	15.89	11.33	4.56	22.7	6.51	7.16	1.79884528	0.80446807
	T39	73.81%	26.19%	18.62	13.74	4.88	24.89	6.52			
	T40	62.34%	37.66%	12.95	8.07	4.88	25.54	9.62			
	T41	67.29%	32.71%	13.27	8.93	4.34	15.08	4.93			
	T42	62.25%	37.75%	11.38	7.08	4.30	21.75	8.21			
Week 13 DIO Food Restrict	T43	63.46%	36.54%	12.69	8.05	4.64	24.38	8.91	8.15	1.06826121	0.47774093
	T44	59.91%	40.09%	10.69	6.40	4.29	21.2	8.50			
	T46	75.74%	24.26%	15.48	11.72	3.76	26.99	6.55			
	T47	66.50%	33.50%	14.66	9.75	4.91	27.35	9.16			
	T48	66.71%	33.29%	12.33	8.23	4.10	22.9	7.62			

Table B-13: Experiment 1: Body Composition Percentages

Exp. 1 Body Composition Percentages

		H20 Mass (g)	Percentage H20	Average H20 Percentage	Standard Deviation	Standard Error	Percentage Dry Fat	Average Dry Fat Percentage	Standard Deviation	Standard Error	Percentage Dry Lean	Average Dry Lean Percentage	Standard Deviation	Standard Error	Total Carcass Weight (g)	Average Total Carcass Weight (g)	Standard Deviation	Standard Error
Week 4 Standard Diet	C1	8.66	**56.23%**	53.16%	3.66%	1.50%	**20.76%**	25.86%	5.17%	2.11%	**23.00%**	20.99%	1.55%	0.63%	**15.40**	18.46	2.4130976	0.985143
	C2	9.03	**54.73%**				**24.00%**				**21.28%**				**16.50**			
	C3	9.89	**53.75%**				**25.14%**				**21.11%**				**18.40**			
	C4	9.39	**49.27%**				**30.99%**				**19.75%**				**19.06**			
	C5	10.83	**56.88%**				**21.04%**				**22.08%**				**19.04**			
	C6	10.75	**48.08%**				**33.21%**				**18.72%**				**22.36**			
Week 8 Standard Diet	C7	17.53	**73.53%**	51.20%	18.53%	8.29%	**9.09%**	24.92%	12.61%	5.64%	**17.38%**	23.89%	6.72%	3.01%	**23.84**	19.68	3.0812854	1.3779927
	C9	9.61	**54.45%**				**19.38%**				**26.17%**				**17.65**			
	C10	13.33	**62.17%**				**20.82%**				**17.01%**				**21.44**			
	C11	4.29	**26.78%**				**40.30%**				**32.92%**				**16.02**			
	C12	7.59	**39.04%**				**34.98%**				**25.97%**				**19.44**			
Week 10 Standard Diet	C14	3.75	**24.35%**	34.81%	14.61%	7.30%	**45.81%**	38.98%	10.10%	5.05%	**29.84%**	26.21%	4.54%	2.27%	**15.40**	18.56	3.2006249	1.6003125
	C15	5.12	**28.48%**				**43.22%**				**28.30%**				**17.98**			
	C16	5.35	**29.99%**				**42.93%**				**27.08%**				**17.84**			
	C17	12.99	**56.43%**				**23.95%**				**19.62%**				**23.02**			
Week 13 Standard Diet	C19	12.48	**54.21%**	52.17%	6.43%	2.88%	**26.58%**	29.64%	4.62%	2.06%	**19.23%**	18.19%	2.23%	1.00%	**23.02**	25.64	3.9914371	1.7850249
	C20	19.15	**62.26%**				**23.06%**				**14.68%**				**30.76**			
	C21	12.93	**49.56%**				**32.12%**				**18.32%**				**26.09**			
	C22	13.69	**49.30%**				**32.70%**				**18.00%**				**27.77**			
	C24	9.35	**45.50%**				**33.76%**				**20.74%**				**20.55**			
Week 4 DIO	T25	8.96	**38.62%**	53.34%	12.69%	5.18%	**43.48%**	28.12%	11.73%	4.79%	**17.90%**	18.53%	2.16%	0.88%	**23.20**	22.22	1.9311629	0.7883939
	T26	13.38	**61.21%**				**21.26%**				**17.54%**				**21.86**			
	T27	12.82	**52.09%**				**31.30%**				**16.61%**				**24.61**			
	T28	17.05	**72.65%**				**10.38%**				**16.97%**				**23.47**			
	T29	11.33	**54.44%**				**25.62%**				**19.94%**				**20.81**			
	T30	7.94	**41.05%**				**36.70%**				**22.25%**				**19.34**			
Week 8 DIO	T32	7.70	**28.27%**	41.69%	11.45%	5.12%	**54.17%**	40.37%	12.05%	5.39%	**17.56%**	17.94%	2.36%	1.05%	**27.24**	25.96	3.3991661	1.5201533
	T33	15.47	**49.69%**				**35.23%**				**15.07%**				**31.13**			
	T34	7.55	**30.10%**				**52.15%**				**17.75%**				**25.08**			
	T35	11.89	**49.34%**				**32.97%**				**17.69%**				**24.10**			
	T36	11.37	**51.06%**				**27.30%**				**21.65%**				**22.27**			
Week 10 DIO	T38	6.81	**30.00%**	32.83%	15.75%	7.04%	**49.93%**	45.71%	12.86%	5.75%	**20.07%**	21.46%	4.11%	1.84%	**22.70**	21.99	4.1630001	1.8617503
	T39	6.27	**25.19%**				**55.21%**				**19.59%**				**24.89**			
	T40	12.59	**49.30%**				**31.61%**				**19.10%**				**25.54**			
	T41	1.81	**12.00%**				**50.21%**				**28.78%**				**15.08**			
	T42	10.37	**47.68%**				**32.57%**				**19.75%**				**21.75**			
Week 13 DIO Food Restrict	T43	11.69	**47.95%**	46.55%	2.58%	1.15%	**33.03%**	35.65%	4.93%	2.20%	**19.02%**	17.81%	2.37%	1.06%	**24.38**	24.56	2.6346973	1.1782725
	T44	10.51	**49.58%**				**30.21%**				**20.21%**				**21.20**			
	T46	11.51	**42.65%**				**43.44%**				**13.91%**				**26.99**			
	T47	12.69	**46.40%**				**35.65%**				**17.96%**				**27.35**			
	T48	10.57	**46.16%**				**35.92%**				**17.93%**				**22.90**			

Table B-14: Experiment 1: Dry Fat Percentages Welch's 2-Tailed T-test
(Comparisons of 0.05 significance marked in green)

Exp. 1 Dry Fat Percentages T Test
Significance of 0.05 Highlighted in Green

	Week 4 Standard Diet	**Week 4 DIO**
	20.76%	43.48%
	24.00%	21.26%
	25.14%	31.30%
	30.99%	10.38%
	21.04%	25.62%
	33.21%	36.70%
Sample Size	6.00	6.00
Mean	26.87%	25.05%
StDev	5.06%	10.06%
Sx	0.05	
Deg. Of Freedom	7.38	
t value	0.397	
P(Means are equal)	70.34%	

	Week 8 Standard Diet	**Week 8 DIO**
	9.09%	54.17%
	19.38%	35.23%
	20.82%	52.15%
	40.30%	32.97%
	34.98%	27.30%
Sample Size	5.00	5.00
Mean	24.92%	40.37%
StDev	12.61%	12.05%
Sx	0.08	
Deg. Of Freedom	7.98	
t value	-1.981	
P(Means are equal)	8.81%	

	Week 10 Standard Diet	**Week 10 DIO**
	45.81%	49.93%
	43.22%	55.21%
	42.93%	31.61%
	23.95%	59.21%
		32.57%
Sample Size	4.00	5.00
Mean	38.98%	45.71%
StDev	10.10%	12.86%
Sx	0.08	
Deg. Of Freedom	7.00	
t value	-0.879	
P(Means are equal)	41.32%	

	Week 13 Standard Diet	**Week 13 DIO**
	26.55%	33.03%
	23.06%	30.21%
	32.12%	43.44%
	32.70%	35.65%
	33.76%	35.92%
Sample Size	5.00	5.00
Mean	29.64%	35.65%
StDev	4.62%	4.93%
Sx	0.03	
Deg. Of Freedom	7.97	
t value	-1.990	
P(Means are equal)	8.70%	

Appendix B.4: Experiment 1 Glucose Clearance Tables

Table B-15: Experiment 1: Week 0 Baseline Glucose Levels

Exp. 1 Glucose Concentration			Standard						
			Concentration [mg/1 gram solution]	Amount Given	Lean Body Mass (g)				
Week 0 Baseline			226.5	0.213	8.86				
			Concentration [mg/1 gram solution]	Amount Given					
			166.67	0.255					**Total Area**
B1	Before:	2.65	Decimal Time:	-0.1	0.5	1.0	1.5	2.0	
	After:	2.39	Glucose:	212.0	347.0	302.0	258.0	201.0	
	Injected:	0.26	Area Under Curve	144.4	158.2	140.0	113.8		556.4
			Standardized to Glucose Injected	111.5	122.2	108.1	87.9		429.6
			Standardized to G Concentration	151.5	166.0	146.9	119.4		583.9
			Standardized to Amount Given	126.6	138.6	122.7	99.7		487.6
	Lean Body Mass	7.78	Standardized to Lean Body Mass	144.2	158.0	139.8	113.6		555.7
B2	Before:	2.63	Decimal Time:	0.0	0.5	1.0	1.5	2.0	
	After:	2.38	Glucose:	197.0	356.0	358.0	286.0	203.0	
	Injected:	0.25	Area Under Curve	139.8	176.0	164.1	121.6		601.5
			Standardized to Glucose Injected	112.3	141.4	131.8	97.6		483.1
			Standardized to G Concentration	152.6	192.1	179.2	132.7		656.6
			Standardized to Amount Given	127.4	160.5	149.6	110.8		548.3
	Lean Body Mass	8.33	Standardized to Body Weight	135.6	170.8	159.2	117.9		583.5
B3	Before:	2.62	Decimal Time:	0.0	0.5	1.0	1.5	2.0	
	After:	2.37	Glucose:	171.0	310.0	300.0	294.0	232.0	
	Injected:	0.25	Area Under Curve	121.6	150.0	148.1	133.3		552.9
			Standardized to Glucose Injected	95.7	118.1	116.6	104.9		435.3
			Standardized to G Concentration	130.1	160.5	158.5	142.6		591.6
			Standardized to Amount Given	108.7	134.0	132.3	119.1		494.1
	Lean Body Mass	9.18	Standardized to Body Weight	104.8	129.3	127.7	114.9		476.7
B4	Before:	2.60	Decimal Time:	0.1	0.6	1.1	1.6	2.1	
	After:	2.35	Glucose:	201.0	216.0	235.0	295.0	250.0	
	Injected:	0.25	Area Under Curve	105.4	110.9	132.1	136.3		484.7
			Standardized to Glucose Injected	83.3	87.6	104.5	107.7		383.1
			Standardized to G Concentration	113.2	119.1	141.9	146.4		520.7
			Standardized to Amount Given	94.6	99.5	118.5	122.2		434.8
	Body Weight	11.40	Standardized to Body Weight	73.5	77.3	92.1	95.0		338.0
B5	Before:	2.65	Decimal Time:	0.1	0.6	1.1	1.6	2.1	
	After:	2.39	Glucose:	186.0	220.0	207.0	191.0	158.0	
	Injected:	0.26	Area Under Curve	102.6	105.6	98.7	87.9		394.8
			Standardized to Glucose Injected	77.7	80.0	74.8	66.6		299.1
			Standardized to G Concentration	105.7	108.7	101.6	90.5		406.5
			Standardized to Amount Given	88.2	90.8	84.9	75.6		339.5
	Lean Body Mass	8.88	Standardized to Body Weight	88.0	90.6	84.7	75.4		338.8
B6	Before:	2.59	Decimal Time:	0.1	0.6	1.1	1.6	2.1	
	After:	2.34	Glucose:	175.0	293.0	255.0	259.0	199.0	
	Injected:	0.26	Area Under Curve	118.8	136.2	127.0	115.5		497.5
			Standardized to Glucose Injected	93.2	106.9	99.6	90.6		390.2
			Standardized to G Concentration	126.6	145.2	135.4	123.1		530.3
			Standardized to Amount Given	105.8	121.3	113.0	102.8		442.8
	Lean Body Mass	8.72	Standardized to Body Weight	107.5	123.2	114.9	104.4		450.0
B7	Before:	2.60	Decimal Time:	0.2	0.7	1.2	1.6	2.2	
	After:	2.35	Glucose:	195.0	348.0	274.0	255.0	174.0	
	Injected:	0.25	Area Under Curve	135.7	155.1	131.4	118.1		540.2
			Standardized to Glucose Injected	108.5	124.1	105.1	94.5		432.2
			Standardized to G Concentration	147.5	168.6	142.8	128.4		587.3
			Standardized to Amount Given	123.2	140.8	119.3	107.2		490.5
	Lean Body Mass	10.62	Standardized to Body Weight	102.7	117.4	99.5	89.4		409.0
Mean Baseline	Before:	2.62	Decimal Time:	0.1	0.6	1.1	1.6	2.1	
	After:	2.37	Glucose:	199.0	340.4	287.0	249.0	193.1	
	Injected:	0.25	Area Under Curve	136.7	154.8	133.8	112.4		537.6
			Standardized to Glucose Injected	109.3	123.8	107.0	89.9		430.1
			Standardized to G Concentration	148.6	168.3	145.4	122.2		584.4
			Standardized to Amount Given	124.1	140.5	121.5	102.0		488.1
	Body Weight	8.92	Standardized to Body Weight	123.3	139.6	120.7	101.4		484.9

Table B-16: Experiment 1: Week 4 Standard Diet Glucose Levels

Exp. 1 Glucose Tolerance Week 4 Standard Diet			**Standard**						
			Concentration [mg/1 gram solution]	Amount Given	Lean Body Mass (g)				
			226.5	0.213	8.86				**Total Area**
C1	Before:	2.284	Decimal Time:	0.0	0.5	1.0	1.5	2.0	
	After:	2.501	Glucose:	198.0	359.0	297.0	205.0	161.0	
	Injected:	0.217	Area Under Curve	139.7	164.7	126.6	90.7		521.8
			Standardized to Glucose Injected	128.8	151.8	116.7	83.6		480.9
	Lean Body Mass	8.09	Standardized to Body Weight	141.0	166.2	127.8	91.6		526.5
C2	Before:	2.280	Decimal Time:	0.0	0.5	1.0	1.5	2.0	
	After:	2.496	Glucose:	174.0	258.0	237.0	229.0	191.0	
	Injected:	0.216	Area Under Curve	108.7	123.8	116.2	106.1		454.7
			Standardized to Glucose Injected	100.6	114.6	107.6	98.2		421.0
	Body Weight	7.75	Standardized to Body Weight	115.0	130.9	122.9	112.3		481.1
C3	Before:	2.265	Decimal Time:	0.1	0.6	1.1	1.6	2.0	
	After:	2.479	Glucose:	171.0	310.0	300.0	294.0	232.0	
	Injected:	0.214	Area Under Curve	120.7	152.9	149.6	128.7		551.9
			Standardized to Glucose Injected	112.8	142.9	139.8	120.2		515.8
	Body Weight	8.40	Standardized to Body Weight	119.0	150.8	147.5	126.9		544.2
C4	Before:	2.258	Decimal Time:	0.1	0.6	1.1	1.6	2.1	
	After:	2.470	Glucose:	201.0	216.0	235.0	295.0	250.0	
	Injected:	0.212	Area Under Curve	105.6	111.7	132.4	135.9		485.6
			Standardized to Glucose Injected	99.6	105.4	124.9	128.2		458.1
	Body Weight	7.42	Standardized to Body Weight	119.0	125.8	149.2	153.1		547.1
C5	Before:	2.261	Decimal Time:	0.1	0.6	1.1	1.6	2.1	
	After:	2.477	Glucose:	186.0	220.0	207.0	191.0	158.0	
	Injected:	0.216	Area Under Curve	102.7	105.6	100.3	86.8		395.5
			Standardized to Glucose Injected	95.1	97.8	92.9	80.3		366.2
	Body Weight	9.75	Standardized to Body Weight	86.4	88.9	84.4	73.0		332.7
C6	Before:	2.271	Decimal Time:	0.1	0.6	1.1	1.6	2.0	
	After:	2.467	Glucose:	175.0	293.0	255.0	259.0	199.0	
	Injected:	0.196	Area Under Curve	117.8	135.4	128.3	84.5		466.1
			Standardized to Glucose Injected	120.3	138.2	130.9	86.3		475.6
	Body Weight	8.06	Standardized to Body Weight	132.2	151.9	143.9	94.8		522.8
Wk 4 C Mean	Before:	2.27	Decimal Time:	0.0	0.5	1.0	1.5	2.0	
	After:	2.482	Glucose:	184.2	276.0	255.2	245.5	198.5	
	Injected:	0.212	Area Under Curve	115.9	132.3	125.6	110.6		484.3
			Standardized to Glucose Injected	109.3	124.8	118.5	104.3		456.9
	Body Weight	8.246	Standardized to Body Weight	117.5	134.1	127.3	112.1		490.9

Table B-17: Experiment 1: Week 4 DIO Glucose Levels

Exp. 1 Glucose Tolerance
Week 4
DIO

			Standard						
			Concentration [mg/1 gram solution]	Amount Given	Lean Body Mass (g)				
			226.5	0.213	8.86				**Total Area**
T25	Before:	2.264	Decimal Time:	0.2	0.7	1.2	1.7	2.2	
	After:	2.480	Glucose:	217.0	401.0	339.0	307.0	241.0	
	Injected:	0.216	Area Under Curve	154.6	188.4	159.4	135.8		638.2
			Standardized to Glucose Injected	143.1	174.4	147.6	125.7		590.9
	Body Weight	6.765	Standardized to Body Weight	187.5	228.5	193.4	164.7		773.9
T26	Before:	2.273	Decimal Time:	0.2	0.7	1.2	1.7	2.2	
	After:	2.483	Glucose:	190.0	352.0	347.0	269.0	205.0	
	Injected:	0.210	Area Under Curve	135.3	175.1	153.5	119.0		582.9
			Standardized to Glucose Injected	128.8	166.8	146.2	113.4		555.2
	Body Weight	9.882	Standardized to Body Weight	115.5	149.6	131.1	101.6		497.8
T27	Before:	2.296	Decimal Time:	0.2	0.7	1.2	1.7	2.2	
	After:	2.511	Glucose:	215.0	365.0	354.0	317.0	276.0	
	Injected:	0.215	Area Under Curve	146.5	179.3	166.4	148.7		641.0
			Standardized to Glucose Injected	136.3	166.7	154.8	138.4		596.3
	Body Weight	8.531	Standardized to Body Weight	141.6	173.2	160.8	143.7		619.3
T28	Before:	2.249	Decimal Time:	0.2	0.7	1.2	1.7	2.2	
	After:	2.464	Glucose:	174.0	317.0	241.0	202.0	176.0	
	Injected:	0.215	Area Under Curve	123.2	139.3	110.1	94.4		466.9
			Standardized to Glucose Injected	114.6	129.6	102.4	87.8		434.3
	Body Weight	14.565	Standardized to Body Weight	69.7	78.8	62.3	53.4		264.2
T29	Before:	2.260	Decimal Time:	0.2	0.7	1.2	1.7	2.2	
	After:	2.478	Glucose:	171.0	328.0	298.0	230.0	186.0	
	Injected:	0.218	Area Under Curve	124.6	157.3	131.3	103.4		516.6
			Standardized to Glucose Injected	114.3	144.3	120.5	94.8		473.9
	Body Weight	9.108	Standardized to Body Weight	111.2	140.4	117.2	92.3		461.1
T30	Before:	2.296	Decimal Time:	0.3	0.8	1.3	1.8	2.3	
	After:	2.506	Glucose:	203.0	322.0	298.0	229.0	209.0	
	Injected:	0.210	Area Under Curve	131.2	153.8	136.7	104.9		526.6
			Standardized to Glucose Injected	124.9	146.5	130.1	99.9		501.5
	Body Weight	7.300	Standardized to Body Weight	151.6	177.8	158.0	121.3		608.7
Wk 4 T Mean	Before:	2.273	Decimal Time:	0.0	0.5	1.0	1.5	2.0	
	After:	2.487	Glucose:	195.0	347.5	312.8	259.0	215.5	
	Injected:	0.214	Area Under Curve	135.2	166.2	143.0	117.6		562.1
			Standardized to Glucose Injected	126.4	155.3	133.7	109.9		525.3
	Body Weight	9.358	Standardized to Body Weight	119.7	147.0	126.6	104.1		497.4

Table B-18: Experiment 1: Week 8 Standard Diet Glucose Levels

Exp. 1 Glucose Concentration			Standard						
			Concentration [mg/1 gram solution]	Amount Given	Lean Body Mass (g)				
Week 8			226.5	0.213	8.86				
Standard Diet			Concentration [mg/1 gram solution]	Amount Given					
			222.78	0.215					**Total Area**
C7	Before:	2.49	Decimal Time:	0.0	0.5	1.0	1.5	2.0	
	After:	2.27	Glucose:	200.0	456.0	320.0	207.0	164.0	
	Injected:	0.22	Area Under Curve	165.4	193.5	131.0	93.5		583.4
			Standardized to Glucose Injected	150.3	175.9	119.1	85.0		530.3
			Standardized to G Concentration	152.8	178.8	121.1	86.4		539.2
			Standardized to Amount Given	151.6	177.3	120.1	85.7		534.8
	Lean Body Mass	15.65	Standardized to Body Weight	85.8	100.4	68.0	48.5		302.7
C9	Before:	2.48	Decimal Time:	0.0	0.5	1.0	1.5	2.0	
	After:	2.27	Glucose:	174.0	314.0	309.0	219.0	191.0	
	Injected:	0.21	Area Under Curve	122.7	156.7	131.1	102.8		513.4
			Standardized to Glucose Injected	118.0	150.7	126.1	98.9		493.7
			Standardized to G Concentration	120.0	153.2	128.2	100.5		501.9
			Standardized to Amount Given	119.0	151.9	127.1	99.7		497.8
	Lean Body Mass	10.14	Standardized to Body Weight	104.0	132.8	111.1	87.1		435.0
C10	Before:	2.47	Decimal Time:	0.1	0.6	1.1	1.6	2.1	
	After:	2.26	Glucose:	121.0	357.0	279.0	186.0	123.0	
	Injected:	0.21	Area Under Curve	119.7	158.6	116.7	76.3		471.3
			Standardized to Glucose Injected	114.5	151.7	111.7	73.1		451.0
			Standardized to G Concentration	116.5	154.3	113.5	74.3		458.5
			Standardized to Amount Given	115.5	153.0	112.6	73.7		454.8
	Lean Body Mass	12.31	Standardized to Body Weight	83.2	110.2	81.1	53.0		327.5
C11	Before:	2.47	Decimal Time:	0.1	0.6	1.1	1.6	2.1	
	After:	2.25	Glucose:	180.0	266.0	309.0	266.0	174.0	
	Injected:	0.21	Area Under Curve	114.2	140.3	143.7	109.3		507.5
			Standardized to Glucose Injected	107.3	131.8	134.9	102.6		476.5
			Standardized to G Concentration	109.0	134.0	137.2	104.3		484.5
			Standardized to Amount Given	108.1	132.8	136.0	103.5		480.5
	Lean Body Mass	7.20	Standardized to Body Weight	133.0	163.4	167.3	127.3		591.1
C12	Before:	2.47	Decimal Time:	0.1	0.6	1.1	1.6	2.1	
	After:	2.25	Glucose:	173.0	244.0	223.0	209.0	194.0	
	Injected:	0.21	Area Under Curve	105.2	116.4	108.8	100.1		430.5
			Standardized to Glucose Injected	98.4	108.7	101.7	93.5		402.3
			Standardized to G Concentration	100.0	110.6	103.4	95.1		409.0
			Standardized to Amount Given	99.2	109.7	102.5	94.3		405.6
	Lean Body Mass	8.28	Standardized to Body Weight	106.1	117.3	109.7	100.9		433.9
Wk 8 C Mean	Before:	2.47	Decimal Time:	0.1	0.6	1.1	1.6	2.1	
	After:	2.26	Glucose:	169.6	327.4	288.0	217.4	169.2	
	Injected:	0.21	Area Under Curve	125.5	153.0	126.4	96.4		501.2
			Standardized to Glucose Injected	117.9	143.8	118.8	90.6		471.1
			Standardized to G Concentration	119.9	146.2	120.7	92.1		478.9
			Standardized to Amount Given	118.9	145.0	119.7	91.3		475.0
	Lean Body Mass	10.72	Standardized to Body Weight	98.3	119.9	99.0	75.5		392.7

Table B-19: Experiment 1: Week 8 DIO Glucose Levels

Exp. 1 Glucose Concentration			Standard						
			Concentration [mg/1 gram solution]	Amount Given	Lean Body Mass (g)				
Week 8 DIO			226.5	0.213	8.86				
			Concentration [mg/1 gram solution]	Amount Given					
			222.78	0.215					Total Area
T32	Before:	2.48	Decimal Time:	0.2	0.7	1.2	1.7	2.2	
	After:	2.27	Glucose:	184.0	329.0	356.0	312.0	231.0	
	Injected:	0.22	Area Under Curve	129.5	171.5	163.8	136.5		601.4
			Standardized to Glucose Injected	119.9	158.8	151.7	126.4		556.9
			Standardized to G Concentration	121.9	161.5	154.2	128.5		566.2
			Standardized to Amount Given	120.9	160.2	153.0	127.4		561.5
	Lean Body Mass	6.668	Standardized to Body Weight	160.7	212.8	203.3	169.3		746.1
T33	Before:	2.45	Decimal Time:	0.2	0.7	1.2	1.7	2.2	
	After:	2.25	Glucose:	193.0	368.0	321.0	272.0	212.0	
	Injected:	0.20	Area Under Curve	140.5	172.7	147.8	120.9		581.9
			Standardized to Glucose Injected	140.5	172.7	147.8	120.9		581.9
			Standardized to G Concentration	142.8	175.6	150.2	123.0		591.6
			Standardized to Amount Given	141.7	174.2	149.0	121.9		586.7
	Lean Body Mass	9.327	Standardized to Body Weight	134.6	165.5	141.5	115.8		557.4
T34	Before:	2.46	Decimal Time:	0.2	0.7	1.2	1.7	2.2	
	After:	2.25	Glucose:	175.0	391.0	353.0	287.0	193.0	
	Injected:	0.21	Area Under Curve	140.2	186.5	162.6	119.3		608.7
			Standardized to Glucose Injected	135.5	180.2	157.1	115.3		588.1
			Standardized to G Concentration	137.8	183.2	159.7	117.2		597.9
			Standardized to Amount Given	136.6	181.7	158.4	116.3		593.0
	Lean Body Mass	8.182	Standardized to Body Weight	148.0	196.8	171.5	125.9		642.1
T35	Before:	2.46	Decimal Time:	0.2	0.7	1.2	1.7	2.2	
	After:	2.21	Glucose:	155.0	374.0	379.0	259.0	166.0	
	Injected:	0.25	Area Under Curve	132.5	189.9	157.4	106.6		586.4
			Standardized to Glucose Injected	106.8	153.2	126.9	86.0		472.9
			Standardized to G Concentration	108.6	155.7	129.0	87.4		480.8
			Standardized to Amount Given	107.7	154.4	128.0	86.7		476.8
	Lean Body Mass	8.414	Standardized to Body Weight	113.4	162.6	134.8	91.3		502.1
T36	Before:	2.47	Decimal Time:	0.3	0.8	1.3	1.8	2.3	
	After:	2.26	Glucose:	163.0	441.0	386.0	216.0	157.0	
	Injected:	0.21	Area Under Curve	151.3	209.4	148.8	93.0		602.5
			Standardized to Glucose Injected	143.4	198.5	141.1	88.1		571.1
			Standardized to G Concentration	145.8	201.8	143.4	89.6		580.6
			Standardized to Amount Given	144.6	200.1	142.2	88.9		575.8
	Lean Body Mass	11.290	Standardized to Body Weight	113.4	157.1	111.6	69.8		451.9
Wk 8 T Mean	Before:	2.47	Decimal Time:	0.2	0.7	1.2	1.7	2.2	
	After:	2.25	Glucose:	174.0	380.6	359.0	269.2	191.8	
	Injected:	0.22	Area Under Curve	138.8	185.9	156.1	115.3		596.1
			Standardized to Glucose Injected	128.3	171.8	144.3	106.5		550.9
			Standardized to G Concentration	130.4	174.7	146.7	108.3		560.1
			Standardized to Amount Given	129.4	173.3	145.5	107.4		555.5
	Lean Body Mass	8.78	Standardized to Body Weight	130.6	174.9	146.9	108.4		560.8

Table B-20: Experiment 1: Week 10 Standard Diet Glucose Levels

Exp. 1 Glucose Concentration			Standard						
			Concentration [mg/1 gram solution]	Amount Given	Lean Body Mass (g)				
Week 10			226.5	0.213	8.86				
Standard Diet			Concentration [mg/1 gram solution]	Amount Given					
			222.78	0.215					Total Area
C14	Before:	2.48	Decimal Time:	0.2	0.7	1.2	1.8	2.2	
	After:	2.27	Glucose:	182.0	226.0	174.0	247.0	241.0	
	Injected:	0.21	Area Under Curve	101.8	100.8	109.1	117.9		429.5
			Standardized to Glucose Injected	95.2	94.2	101.9	110.2		401.4
			Standardized to G Concentration	93.5	92.5	100.1	108.2		394.3
			Standardized to Amount Given	92.2	91.2	98.7	106.7		388.8
	Lean Body Mass	6.07	Standardized to Body Weight	134.4	133.1	144.0	155.6		567.1
C15	Before:	2.51	Decimal Time:	0.3	0.7	1.3	1.8	2.3	
	After:	2.30	Glucose:	170.0	302.0	298.0	269.0	200.0	
	Injected:	0.22	Area Under Curve	115.1	158.3	148.4	108.1		529.9
			Standardized to Glucose Injected	107.0	147.2	138.1	100.6		492.9
			Standardized to G Concentration	105.1	144.6	135.6	98.8		484.1
			Standardized to Amount Given	103.7	142.6	133.7	97.4		477.4
	Lean Body Mass	7.11	Standardized to Body Weight	129.1	177.6	166.6	121.3		594.5
C16	Before:	2.46	Decimal Time:	0.3	0.8	1.3	1.8	2.3	
	After:	2.24	Glucose:	162.0	330.0	257.0	212.0	183.0	
	Injected:	0.22	Area Under Curve	122.8	156.9	117.7	92.2		489.5
			Standardized to Glucose Injected	113.2	144.6	108.5	84.9		451.2
			Standardized to G Concentration	111.2	142.0	106.6	83.4		443.1
			Standardized to Amount Given	109.6	140.0	105.1	82.3		437.0
	Lean Body Mass	7.11	Standardized to Body Weight	136.5	174.4	130.9	102.5		544.2
C17	Before:	2.47	Decimal Time:	0.3	0.8	1.3	1.8	2.3	
	After:	2.25	Glucose:	159.0	245.0	251.0	224.0	176.0	
	Injected:	0.21	Area Under Curve	100.5	129.5	118.9	96.3		445.2
			Standardized to Glucose Injected	95.3	122.8	112.7	91.3		422.0
			Standardized to G Concentration	93.6	120.6	110.7	89.6		414.4
			Standardized to Amount Given	92.3	118.9	109.1	88.4		408.7
	Lean Body Mass	6.90	Standardized to Body Weight	118.5	152.7	140.2	113.5		524.8
Wk 10 C Mean	Before:	2.48	Decimal Time:	0.3	0.8	1.3	1.8	2.3	
	After:	2.27	Glucose:	168.3	275.8	245.0	238.0	200.0	
	Injected:	0.21	Area Under Curve	110.1	136.0	123.4	103.6		473.0
			Standardized to Glucose Injected	102.8	126.9	115.2	96.7		441.6
			Standardized to G Concentration	100.9	124.7	113.1	95.0		433.7
			Standardized to Amount Given	99.5	122.9	111.5	93.7		427.7
	Lean Body Mass	6.80	Standardized to Body Weight	129.7	160.2	145.3	122.0		557.2

Table B-21: Experiment 1: Week 10 DIO Glucose Levels

Exp. 1 Glucose Concentration			Standard						
			Concentration [mg/1 gram solution]	Amount Given	Lean Body Mass (g)				
Week 10 DIO			226.5	0.213	8.86				
			Concentration [mg/1 gram solution]	Amount Given					
			222.78	0.215					Total Area
T38	Before:	2.48	Decimal Time:	0.3	0.9	1.3	1.9	2.3	
	After:	2.27	Glucose:	120.0	286.0	262.0	196.0	178.0	
	Injected:	0.22	Area Under Curve	105.2	132.1	116.2	90.0		443.4
			Standardized to Glucose Injected	97.8	122.8	108.1	83.7		412.5
			Standardized to G Concentration	96.1	120.7	106.1	82.2		405.1
			Standardized to Amount Given	94.8	119.0	104.7	81.1		399.5
	Lean Body Mass	6.508	Standardized to Body Weight	129.0	162.0	142.5	110.4		543.9
T39	Before:	2.47	Decimal Time:	0.4	0.9	1.4	1.9	2.4	
	After:	2.26	Glucose:	169.0	275.0	246.0	247.0	195.0	
	Injected:	0.22	Area Under Curve	115.4	125.0	124.6	109.2		474.1
			Standardized to Glucose Injected	107.3	116.2	115.9	101.6		441.0
			Standardized to G Concentration	105.4	114.2	113.8	99.8		433.2
			Standardized to Amount Given	104.0	112.6	112.2	98.4		427.2
	Lean Body Mass	6.519	Standardized to Body Weight	141.3	153.0	152.5	133.7		580.5
T40	Before:	2.48	Decimal Time:	0.4	0.9	1.4	1.9	2.4	
	After:	2.26	Glucose:	281.0	316.0	240.0	169.0	166.0	
	Injected:	0.22	Area Under Curve	151.2	136.5	103.8	83.4		475.0
			Standardized to Glucose Injected	139.4	125.8	95.7	76.9		437.8
			Standardized to G Concentration	136.9	123.6	94.0	75.5		430.0
			Standardized to Amount Given	135.0	121.9	92.7	74.5		424.1
	Lean Body Mass	9.618	Standardized to Body Weight	124.4	112.3	85.4	68.6		390.6
T41	Before:	2.48	Decimal Time:	0.4	0.9	1.4	1.9	2.4	
	After:	2.25	Glucose:	176.0	444.0	353.0	278.0	204.0	
	Injected:	0.22	Area Under Curve	156.3	197.8	157.3	120.8		632.2
			Standardized to Glucose Injected	141.4	179.0	142.4	109.4		572.2
			Standardized to G Concentration	138.9	175.8	139.8	107.4		562.0
			Standardized to Amount Given	137.0	173.4	137.9	105.9		554.2
	Lean Body Mass	4.933	Standardized to Body Weight	246.1	311.5	247.7	190.3		995.5
T42	Before:	2.53	Decimal Time:	0.4	0.9	1.4	2.1	2.4	
	After:	2.31	Glucose:	168.0	212.0	197.0	195.0	154.0	
	Injected:	0.22	Area Under Curve	96.4	100.5	131.3	57.4		385.6
			Standardized to Glucose Injected	88.0	91.8	119.9	52.5		352.2
			Standardized to G Concentration	86.4	90.1	117.8	51.5		345.9
			Standardized to Amount Given	85.2	88.9	116.2	50.8		341.1
	Lean Body Mass	8.210	Standardized to Body Weight	92.0	95.9	125.4	54.8		368.1
Wk 10 T Mean	Before:	2.49	Decimal Time:	0.4	0.9	1.4	1.9	2.4	
	After:	2.27	Glucose:	182.8	306.6	259.6	217.0	179.4	
	Injected:	0.22	Area Under Curve	125.1	138.2	128.2	91.3		482.7
			Standardized to Glucose Injected	115.1	127.1	117.9	84.0		444.1
			Standardized to G Concentration	113.0	124.8	115.8	82.5		436.2
			Standardized to Amount Given	111.4	123.1	114.2	81.4		430.1
	Lean Body Mass	7.16	Standardized to Body Weight	138.0	152.4	141.4	100.7		532.5

Table B-22: Experiment 1: Week 13 Standard Diet Glucose Levels

Exp. 1 Glucose Concentration			Standard						
			Concentration [mg/1 gram solution]	Amount Given	Lean Body Mass (g)				
Week 13			226.5	0.213	8.86				
Standard Diet			Concentration [mg/1 gram solution]	Amount Given					
			230.61	0.217					Total Area
C19	Before:	2.49	Decimal Time:	0.0	0.5	1.0	1.5	2.0	
	After:	2.27	Glucose:	168.0	269.0	227.0	213.0	195.0	
	Injected:	0.23	Area Under Curve	109.1	124.7	109.3	104.2		447.2
			Standardized to Glucose Injected	96.6	110.3	96.7	92.2		395.8
			Standardized to G Concentration	94.9	108.4	95.0	90.5		388.7
			Standardized to Amount Given	93.0	106.3	93.1	88.8		381.3
	Lean Body Mass	9.67	Standardized to Body Weight	85.2	97.4	85.4	81.4		349.3
C20	Before:	2.48	Decimal Time:	0.0	0.5	1.0	1.5	2.0	
	After:	2.26	Glucose:	185.0	342.0	398.0	282.0	207.0	
	Injected:	0.23	Area Under Curve	131.7	186.3	168.7	122.4		609.1
			Standardized to Glucose Injected	116.0	164.2	148.6	107.8		536.6
			Standardized to G Concentration	113.9	161.2	146.0	105.9		527.1
			Standardized to Amount Given	111.8	158.2	143.2	103.9		516.9
	Lean Body Mass	11.96	Standardized to Body Weight	82.8	117.1	106.0	76.9		382.9
C21	Before:	2.52	Decimal Time:	0.1	0.6	1.1	1.6	2.1	
	After:	2.29	Glucose:	163.0	254.0	203.0	192.0	181.0	
	Injected:	0.23	Area Under Curve	104.3	114.3	98.7	93.2		410.4
			Standardized to Glucose Injected	91.0	99.8	86.2	81.4		358.4
			Standardized to G Concentration	89.4	98.0	84.7	79.9		352.0
			Standardized to Amount Given	87.7	96.1	83.0	78.4		345.3
	Lean Body Mass	9.48	Standardized to Body Weight	82.0	89.9	77.6	73.3		322.8
C22	Before:	2.52	Decimal Time:	0.1	0.6	1.1	1.6	2.1	
	After:	2.30	Glucose:	184.0	244.0	237.0	215.0	175.0	
	Injected:	0.22	Area Under Curve	107.0	120.7	112.6	97.4		437.7
			Standardized to Glucose Injected	95.5	107.8	100.5	87.0		390.8
			Standardized to G Concentration	93.8	105.9	98.7	85.5		383.9
			Standardized to Amount Given	92.0	103.8	96.8	83.8		376.5
	Lean Body Mass	7.90	Standardized to Body Weight	103.2	116.4	108.6	94.0		422.2
C24	Before:	2.49	Decimal Time:	0.1	0.6	1.1	1.6	2.1	
	After:	2.27	Glucose:	178.0	280.0	268.0	243.0	241.0	
	Injected:	0.22	Area Under Curve	115.3	137.1	126.8	123.1		502.2
			Standardized to Glucose Injected	102.9	122.4	113.2	109.9		448.4
			Standardized to G Concentration	101.1	120.2	111.2	107.9		440.4
			Standardized to Amount Given	99.1	117.9	109.0	105.9		431.9
	Lean Body Mass	7.82	Standardized to Body Weight	112.3	133.6	123.5	120.0		489.4
Wk 13 C Mean	Before:	2.47	Decimal Time:	0.1	0.6	1.1	1.6	2.1	
	After:	2.27	Glucose:	175.6	277.8	266.6	229.0	199.8	
	Injected:	0.21	Area Under Curve	113.4	136.6	123.2	108.0		481.3
			Standardized to Glucose Injected	110.1	132.6	119.6	104.9		467.3
			Standardized to G Concentration	108.1	130.3	117.5	103.0		458.9
			Standardized to Amount Given	106.1	127.8	115.2	101.0		450.1
	Lean Body Mass	9.37	Standardized to Body Weight	100.3	120.9	109.0	95.6		425.8

Table B-23: Experiment 1: Week 13 DIO Glucose Levels

Exp. 1 Glucose Concentration			Standard						
			Concentration [mg/1 gram solution]	Amount Given	Lean Body Mass (g)				
Week 13 DIO			226.5	0.213	8.86				
			Concentration [mg/1 gram solution]	Amount Given					
			230.61	0.217					Total Area
T43	Before:	2.48	Decimal Time:	0.1	0.6	1.1	1.6	2.1	
	After:	2.27	Glucose:	161.0	231.0	210.0	213.0	173.0	
	Injected:	0.21	Area Under Curve	97.6	110.4	106.1	97.0		411.1
			Standardized to Glucose Injected	94.3	106.7	102.5	93.7		397.2
			Standardized to G Concentration	92.6	104.8	100.7	92.0		390.1
			Standardized to Amount Given	90.8	102.8	98.8	90.3		382.6
	Lean Body Mass	8.908	Standardized to Body Weight	90.3	102.2	98.2	89.8		380.6
T44	Before:	2.46	Decimal Time:	0.2	0.7	1.2	1.7	2.2	
	After:	2.25	Glucose:	181.0	371.0	302.0	231.0	186.0	
	Injected:	0.21	Area Under Curve	137.8	167.7	134.8	105.3		545.6
			Standardized to Glucose Injected	130.7	158.9	127.8	99.8		517.2
			Standardized to G Concentration	128.3	156.1	125.5	98.0		508.0
			Standardized to Amount Given	125.9	153.1	123.1	96.1		498.2
	Lean Body Mass	8.498	Standardized to Body Weight	131.2	159.6	128.3	100.2		519.4
T46	Before:	2.47	Decimal Time:	0.2	0.7	1.2	1.7	2.2	
	After:	2.26	Glucose:	132.0	184.0	164.0	161.0	137.0	
	Injected:	0.21	Area Under Curve	79.1	87.1	81.3	74.6		322.1
			Standardized to Glucose Injected	75.7	83.3	77.8	71.4		308.2
			Standardized to G Concentration	74.3	81.9	76.4	70.1		302.7
			Standardized to Amount Given	72.9	80.3	74.9	68.8		296.9
	Lean Body Mass	6.547	Standardized to Body Weight	98.7	108.7	101.4	93.0		401.8
T47	Before:	2.50	Decimal Time:	0.2	0.7	1.2	1.7	2.2	
	After:	2.29	Glucose:	215.0	285.0	214.0	203.0	172.0	
	Injected:	0.21	Area Under Curve	124.9	126.4	102.7	97.4		451.5
			Standardized to Glucose Injected	119.0	120.4	97.9	92.8		430.0
			Standardized to G Concentration	116.9	118.2	96.1	91.2		422.4
			Standardized to Amount Given	114.6	116.0	94.3	89.4		414.3
	Lean Body Mass	12.049	Standardized to Body Weight	84.3	85.3	69.3	65.7		304.6
T48	Before:	2.47	Decimal Time:	0.2	0.7	1.2	1.7	2.3	
	After:	2.27	Glucose:	162.0	274.0	219.0	189.0	154.0	
	Injected:	0.20	Area Under Curve	110.4	124.1	100.0	88.6		423.1
			Standardized to Glucose Injected	108.8	122.3	98.5	87.3		416.8
			Standardized to G Concentration	106.8	120.1	96.7	85.7		409.4
			Standardized to Amount Given	104.8	117.8	94.9	84.1		401.6
	Lean Body Mass	7.624	Standardized to Body Weight	121.8	136.9	110.3	97.7		466.7
Wk 13 T Mean	Before:	2.48	Decimal Time:	0.2	0.7	1.2	1.7	2.2	
	After:	2.27	Glucose:	170.2	269.0	221.8	199.4	164.4	
	Injected:	0.21	Area Under Curve	110.0	123.2	104.9	92.6		430.6
			Standardized to Glucose Injected	105.8	118.4	100.9	89.0		414.1
			Standardized to G Concentration	103.9	116.3	99.1	87.4		406.7
			Standardized to Amount Given	101.9	114.1	97.2	85.7		398.9
	Lean Body Mass	8.73	Standardized to Body Weight	103.5	115.9	98.7	87.1		405.0

Table B-24: Experiment 1: Glucose Levels Welch's 2-Tailed T-test
(Comparisons of 0.05 significance marked in green)

Exp. 1 Glucose Tolerance T Test
Significance of 0.05 Highlighted in Green

	Week 4 Standard Diet	**Week 4 DIO**
	521.80	638.20
	454.69	582.93
	551.86	640.97
	485.57	466.89
	395.46	516.60
	466.07	526.56
Sample Size	6.00	6.00
Mean	470.73	546.79
StDev	56.45	66.86
Sx	35.72	
Deg. Of Freedom	9.73	
t value	-2.129	
P(Means are equal)	6.21%	

	Week 8 Standard Diet	**Week 8 DIO**
	583.37	601.42
	513.41	581.90
	471.31	608.67
	507.48	586.37
	430.45	602.46
Sample Size	5.00	5.00
Mean	501.20	596.16
StDev	56.65	11.43
Sx	25.85	
Deg. Of Freedom	4.33	
t value	-3.674	
P(Means are equal)	2.13%	

	Week 10 Standard Diet	**Week 10 DIO**
	429.52	443.39
	529.87	474.11
	489.53	475.03
	445.17	632.25
		385.62
Sample Size	4.00	5.00
Mean	473.52	482.08
StDev	45.36	91.47
Sx	46.77	
Deg. Of Freedom	6.07	
t value	-0.183	
P(Means are equal)	86.08%	

	Week 13 Standard Diet	**Week 13 DIO**
	447.24	411.08
	609.08	545.63
	410.39	322.06
	437.72	451.54
	502.18	423.10
Sample Size	5.00	5.00
Mean	481.32	430.68
StDev	78.82	80.45
Sx	50.37	
Deg. Of Freedom	8.00	
t value	1.005	
P(Means are equal)	34.82%	

Appendix C - Experiment 2 Data Tables

Table C-1: Experiment 2: Mouse Key

Exp. 2 Mouse Key

		Diet		Food Intake		Week Dissected			Injected (Week 10)	
	Eliminated	**Standard**	**Hight Fat (DIO)**	**Ad Libidum**	**Food Restricted**	**Week 0**	**Week 8**	**Week 10**	**Saline**	**AICAR**
B1						x				
B2						x				
B3						x				
B4						x				
B5						x				
C1		x		x			x			
C2		x		x			x			
C3		x		x			x			
C4		x		x			x			
C5		x		x			x			
C7		x		x				x	x	
C8		x		x				x	x	
C9		x		x				x	x	
C10		x		x				x	x	
C11		x		x				x	x	
C12		x		x				x		x
C13	x									
C14		x		x				x		x
C15		x		x				x		x
C16		x		x				x		x
C17	x									
C18	x									
T19			x	x			x			
T20			x	x			x			
T21			x	x			x			
T22			x	x			x			
T23			x	x			x			
T24			x	x				x	x	
T25			x	x				x	x	
T26			x	x				x	x	
T27			x	x				x	x	
T28			x	x				x	x	
T29			x	x				x	x	
T30			x	x				x		x
T31			x	x				x		x
T32	x		x	x				x		x
T33			x	x				x		x
T34			x	x				x		x
T35			x	x				x		x
T36	x		x	x				x		x
T37			x		x			x	x	
T38			x		x			x	x	
T39			x		x			x	x	
T40			x		x			x	x	
T41			x		x			x	x	

Appendix C.1: Experiment 2 Body Weight Tables

Table C-2: Experiment 2: Weekly Body Weight (g)

		Exp. 2 Weekly Body Weight										
		3/2/12	3/9/12	3/16/12	3/23/12	3/30/12	4/6/12	4/13/12	4/20/12	4/27/12	5/4/12	5/11/12
		initial	week 1	week 2	week 3	week 4	week 5	week 6	week 7	week 8	week 9	week 10
Week 0 Baselne	B1	22.6	Dissected									
	B2	22.1										
	B3	21.6										
	B4	22.8										
	B5	22.2										
Week 8 Standard Diet Ad Lib	C1	19.6	21.2	22.5	23.5	24.4	24.7	24.9	25.6	26.1	Dissected	
	C2	23.2	24.8	26.1	26.9	27.9	29.7	31.9	32.1	32.2		
	C3	22.5	24.1	25.3	26.6	26.2	26.8	28.0	28.3	31.3		
	C4	23.5	24.6	24.5	25.0	25.1	26.5	26.9	27.2	27.4		
	C5	21.7	22.6	23.5	24.8	24.6	25.3	25.6	26.2	26.8		
Week 10 Standard Diet Ad Lib Saline	C7	22.3	23.7	26.1	26.0	26.3	26.2	27.6	27.0	28.8	28.9	28.5
	C8	24.0	25.0	26.1	26.3	26.6	28.2	27.2	27.5	28.7	29.2	28.7
	C9	22.7	23.5	24.4	25.6	25.5	25.9	26.3	27.9	27.1	20.7	27.7
	C10	19.6	20.8	20.7	21.4	22.8	22.7	23.4	24.9	25.5	25.3	23.9
	C11	22.7	23.2	24.9	27.6	27.9	30.7	31.0	32.0	32.9	32.9	33.9
Week 10 Standard Diet Ad Lib AICAR	C12	20.6	21.8	21.3	22.6	22.9	23.6	24.2	25.5	26.0	24.9	27.6
	C13	~~21.5~~	~~23.8~~	~~24.6~~	Animal Removed							
	C14	22.1	23.9	23.7	24.7	25.0	25.8	25.9	27.1	27.3	20.5	24.7
	C15	22.3	22.4	23.3	24.0	25.1	26.4	26.8	27.1	28.3	28.4	27.5
	C16	20.9	22.3	22.1	23.6	24.0	24.7	24.8	26.8	26.8	27.4	27.6
	C17	~~21.6~~	~~21.4~~	~~14.9~~	Animal Removed							
	C18	~~20.6~~	~~21.4~~	~~21.8~~	~~23.0~~	~~23.1~~	~~22.9~~	~~23.8~~	~~25.5~~	~~26.2~~	Animal Removed	
Week 8 DIO Ad Lib	T19	21.6	18.8	22.8	25.4	24.4	29.1	31.2	33.5	36.2	Dissected	
	T20	21.0	24.6	26.9	27.4	32.1	35.6	39.0	41.5	43.4		
	T21	21.7	26.4	28.6	32.1	36.9	41.2	43.9	45.8	47.5		
	T22	22.9	25.5	27.8	29.9	34.2	36.7	39.2	40.7	32.1		
	T23	20.7	23.4	25.1	25.8	28.6	29.5	31.8	33.6	35.2		
Week 10 DIO Ad Lib Saline	T24	19.7	23.3	25.1	26.0	30.4	33.0	34.9	37.8	40.0	41.5	40.8
	T25	22.2	24.6	27.4	28.1	33.8	37.6	40.0	42.6	44.7	45.8	43.6
	T26	21.2	24.5	27.9	30.2	34.3	36.9	40.4	42.8	46.2	38.8	42.8
	T27	21.2	23.7	18.5	24.2	28.8	31.4	33.8	35.3	38.4	38.9	38.9
	T28	16.6	23.2	25.8	26.3	30.1	32.8	34.8	37.2	40.0	31.0	36.7
	T29	20.2	15.5	23.7	26.3	30.9	34.8	38.0	40.6	42.6	42.9	42.9
Week 10 DIO Ad Lib AICAR	T30	22.1	23.2	25.4	26.4	29.7	32.4	34.3	35.0	36.5	34.5	34.8
	T31	21.5	25.8	26.5	30.3	34.0	36.6	38.2	40.8	43.3	41.2	35.0
	T32	~~22.0~~	~~24.9~~	~~26.8~~	~~28.7~~	~~32.5~~	~~35.8~~	~~37.4~~	~~40.6~~	~~38.0~~	Animal Removed	
	T33	19.8	23.9	26.1	28.7	33.3	25.1	34.2	37.4	29.0	35.8	38.4
	T34	18.9	22.2	24.6	25.8	19.5	30.3	34.1	36.9	39.6	37.3	37.0
	T35	20.8	25.7	28.3	30.4	35.1	38.1	40.0	42.2	44.9	42.3	40.5
	T36	~~21.7~~	~~20.6~~	~~21.6~~	~~22.2~~	~~24.8~~	~~26.5~~	~~18.8~~	~~25.7~~	Animal Removed		
Week 10 DIO Food Restrict Saline	T37	15.5	22.5	25.0	27.0	29.4	32.3	34.6	36.9	39.2	37.6	37.9
	T38	21.8	24.4	26.4	28.9	32.5	34.8	37.4	40.1	41.9	39.5	39.5
	T39	21.5	23.6	27.2	29.3	32.8	36.8	38.6	41.7	44.6	43.5	43.2
	T40	21.0	23.4	24.5	26.3	29.4	32.3	34.9	37.7	40.2	38.9	39.3
	T41	21.0	24.2	25.4	27.5	30.9	32.8	36.1	38.0	41.3	35.4	35.0

Table C-3: Experiment 2: Body Weight (g) Welch's 2-Tailed T-test
(Comparisons of 0.05 significance marked in green)

Exp 2. Body Weight (g) T Test
Significance of 0.05 Highlighted in Green

	Week 8 Standard Diet Ad Lib	Week 8 DIO Ad Lib
	26.09	36.18
	32.20	43.41
	31.33	47.54
	27.40	32.10
	26.82	35.20
	28.75	40.00
	28.73	44.67
	27.07	46.15
	25.53	38.40
	32.93	40.00
	26.02	42.60
	27.32	36.50
	28.32	43.32
	26.76	29.00
		39.60
		44.90
		39.20
		41.87
		44.62
		40.22
		41.28
Sample Size	14.00	21.00
Mean	28.23	40.32
StDev	2.35	4.64
Sx	1.19	
Deg. Of Freedom	31.23	
t value	-10.143	
P(Means are equal)	0.00%	

	Week 10 Standard Diet Ad Lib Saline	Week 10 Standard Diet Ad Lib AICAR
	28.45	27.60
	28.66	24.70
	27.74	27.54
	23.90	27.64
	33.85	
Sample Size	5.00	4.00
Mean	28.52	26.87
StDev	3.55	1.45
Sx	1.74	
Deg. Of Freedom	5.52	
t value	0.946	
P(Means are equal)	38.76%	

	Week 10 DIO Ad Lib Saline	Week 10 DIO Ad Lib AICAR
	40.76	34.79
	43.59	35.00
	42.77	38.35
	38.92	36.95
	36.74	40.54
	42.92	
Sample Size	6.00	5.00
Mean	40.95	37.13
StDev	2.68	2.41
Sx	1.54	
Deg. Of Freedom	8.92	
t value	2.489	
P(Means are equal)	3.76%	

	Week 10 Standard Diet Ad Lib Saline	Week 10 DIO Ad Lib Saline
	28.45	40.76
	28.66	43.59
	27.74	42.77
	23.90	38.92
	33.85	36.74
		42.92
Sample Size	5.00	6.00
Mean	28.52	40.95
StDev	3.55	2.68
Sx	1.93	
Deg. Of Freedom	7.38	
t value	-6.444	
P(Means are equal)	0.04%	

	Week 10 Standard Diet Ad Lib AICAR	Week 10 DIO Ad Lib AICAR
	27.60	34.79
	24.70	35.00
	27.54	38.35
	27.64	36.95
		40.54
Sample Size	4.00	5.00
Mean	26.87	37.13
StDev	1.45	2.41
Sx	1.30	
Deg. Of Freedom	6.63	
t value	-7.909	
P(Means are equal)	0.02%	

	Week 10 DIO Ad Lib Saline	Week 10 DIO Food Restrict
	40.76	37.85
	43.59	39.50
	42.77	43.19
	38.92	39.33
	36.74	35.00
	42.92	
Sample Size	6.00	5.00
Mean	40.95	38.97
StDev	2.68	2.97
Sx	1.72	
Deg. Of Freedom	8.25	
t value	1.148	
P(Means are equal)	28.42%	

	Week 10 DIO Ad Lib AICAR	Week 10 DIO Food Restrict
	34.79	37.85
	35.00	39.50
	38.35	43.19
	36.95	39.33
	40.54	35.00
Sample Size	5.00	5.00
Mean	37.13	38.97
StDev	2.41	2.97
Sx	1.71	
Deg. Of Freedom	7.67	
t value	-1.082	
P(Means are equal)	31.53%	

Appendix C.2: Experiment 2 Food and Energy Tables

Table C-4: Experiment 2: Standard Diet Food Given and Leftover (g)

Exp. 2 Standard Diet Given and Leftover (grams)

			3/9/12 week 1	3/16/12 week 2	3/23/12 week 3	3/30/12 week 4	4/6/12 week 5	4/13/12 week 6	4/20/12 week 7	4/27/12 week 8	5/4/12 week 9	5/11/12 week 10
Week 8 Standard Diet Ad Lib	C1	given	30.0	30.9	29.6	36.0	32.3	34.4	32.6	30.1		
		added	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	Dissected	
		left	5.2	6.1	6.1	11.9	10.0	11.7	9.4	7.9		
	C2	given	30.1	30.8	30.4	34.8	33.5	35.3	32.3	32.5		
		added	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	Dissected	
		left	4.7	4.7	6.0	9.3	6.9	7.4	6.7	7.9		
	C3	given	30.0	30.2	29.9	34.6	33.9	35.2	30.2	32.6		
		added	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	Dissected	
		left	4.9	6.5	7.0	12.4	10.9	11.9	6.9	7.3		
	C4	given	29.9	29.8	30.4	34.9	34.8	35.1	31.1	29.8		
		added	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	Dissected	
		left	6.8	6.8	10.0	12.9	11.7	11.5	9.0	7.2		
	C5	given	30.2	31.2	30.5	35.3	32.5	34.1	31.7	31.8		
		added	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	Dissected	
		left	8.5	8.2	7.8	12.8	10.5	11.9	8.8	8.5		
Week 10 Standard Diet Ad Lib Saline	C7	given	29.9	30.1	31.5	35.6	33.9	34.6	30.2	29.6	30.2	30.7
		added	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		left	8.2	5.1	8.9	12.1	11.0	10.7	5.7	2.5	6.6	8.7
	C8	given	30.1	31.8	31.4	35.4	35.4	35.8	29.4	32.7	30.1	31.6
		added	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		left	5.8	6.6	7.9	12.8	11.5	14.9	5.6	7.9	7.7	8.6
	C9	given	30.2	30.5	29.2	35.4	35.6	35.3	31.1	29.6	29.7	29.3
		added	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	11.5
		left	5.8	5.7	5.8	13.1	13.3	11.7	5.9	7.3	15.0	11.7
	C10	given	30.0	30.5	30.7	35.2	35.1	35.1	32.0	32.1	30.6	31.8
		added	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		left	9.5	13.0	12.1	13.7	15.3	15.7	9.6	9.8	10.3	14.1
	C11	given	29.7	30.7	30.7	35.4	34.4	35.9	30.7	30.4	29.7	31.3
		added	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		left	7.1	6.0	6.5	10.0	7.2	12.1	3.6	5.4	3.9	5.8
Week 10 Standard Diet Ad Lib AICAR	C12	given	29.7	30.6	30.2	35.6	36.0	35.5	30.9	29.1	31.5	30.7
		added	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		left	12.2	8.8	9.6	15.1	14.7	13.8	9.4	7.7	12.4	10.3
	C13	given	~~29.6~~	~~30.0~~	~~31.0~~							
		added	~~0.0~~	~~0.0~~	~~0.0~~			Animal Removed				
		left	~~4.9~~	~~5.7~~								
	C14	given	30.9	31.3	31.7	35.5	35.0	35.9	29.8	32.2	29.8	29.9
		added	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	11.6
		left	7.2	8.8	9.8	13.0	13.4	13.5	6.2	8.4	17.2	12.2
	C15	given	29.5	30.8	31.2	35.1	35.4	34.5	31.4	32.3	30.6	30.4
		added	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		left	4.5	3.2	6.0	8.4	9.4	8.6	6.0	5.6	7.2	6.7
	C16	given	30.1	30.6	31.6	34.7	34.8	34.5	32.0	31.1	30.8	30.2
		added	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		left	7.7	10.0	10.6	13.0	13.0	13.2	9.6	9.3	10.0	8.4
	C17	given	~~30.1~~	~~31.3~~	~~30.0~~							
		added	~~0.0~~	~~0.0~~	~~0.0~~			Animal Removed				
		left	~~9.6~~	~~18.4~~								
	C18	given	~~30.1~~	~~29.8~~	~~31.6~~	~~35.3~~	~~33.1~~	~~35.7~~	~~31.7~~	~~31.0~~	~~30.9~~	Animal Removed
		added	~~0.0~~	~~0.0~~	~~0.0~~	~~0.0~~	~~0.0~~	~~0.0~~	~~0.0~~	~~0.0~~	~~0.0~~	
		left	~~8.3~~	~~7.1~~	~~10.3~~	~~13.4~~	~~11.3~~	~~12.9~~	~~8.5~~	~~7.8~~		

Table C-5: Experiment 2: DIO Food Given and Leftover (g)

Exp. 2 High Fat Diet (DIO) Given and Leftover (grams)

			3/9/12	3/16/12	3/23/12	3/30/12	4/6/12	4/13/12	4/20/12	4/27/12	5/4/12	5/11/12
			week 1	week 2	week 3	week 4	week 5	week 6	week 7	week 8	week 9	week 10
Week 8 DIO Ad Lib	T19	given	24.1	30.4	24.4	34.9	33.4	35.3	35.9	36.2		
		added	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	Dissected	
		left	12.0	8.7	5.4	14.8	13.7	15.6	13.8	15.2		
	T20	given	23.8	31.4	25.2	35.6	34.7	34.6	35.3	35.0		
		added	6.2	0.0	0.0	0.0	42.2	0.0	0.0	0.0	Dissected	
		left	8.5	10.9	5.1	1.9	28.8	10.0	11.4	12.2		
	T21	given	22.8	30.3	25.3	35.9	34.4	34.8	35.5	36.7		
		added	6.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	Dissected	
		left	8.1	11.2	4.6	13.5	12.4	14.0	15.5	17.8		
	T22	given	23.8	30.9	25.8	34.9	35.1	35.2	33.7	36.7		
		added	6.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	Dissected	
		left	10.3	10.5	5.3	12.2	14.7	14.7	14.2	29.0		
	T23	given	23.3	30.8	25.8	35.9	34.9	35.5	35.0	34.9		
		added	6.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	Dissected	
		left	11.4	12.7	8.0	15.3	16.5	16.7	15.9	16.4		
Week 10 DIO Ad Lib Saline	T24	given	24.4	29.5	25.4	35.9	36.9	35.5	36.1	35.7	35.6	34.9
		added	6.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		left	11.1	11.0	7.0	11.9	14.0	12.9	12.3	11.3	10.2	15.3
	T25	given	23.0	29.8	25.3	35.3	35.2	35.6	35.4	37.0	35.1	36.3
		added	5.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	15.6	0.0
		left	8.8	9.1	8.6	6.8	3.9	4.8	3.7	5.6	10.2	19.7
	T26	given	23.0	29.9	25.1	35.5	33.7	35.0	34.8	36.0	35.2	34.6
		added	6.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		left	10.1	8.9	5.7	12.7	13.5	14.5	14.3	14.9	22.2	12.8
	T27	given	23.6	31.8	25.9	36.0	36.3	35.3	34.7	35.5	35.0	35.0
		added	6.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		left	9.9	16.8	5.4	13.1	14.1	14.0	14.0	13.1	14.5	16.6
	T28	given	22.9	29.9	25.8	35.1	35.2	35.3	34.8	36.7	34.8	34.7
		added	5.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		left	7.9	11.0	7.5	13.1	14.6	14.9	14.2	15.2	25.3	12.1
	T29	given	23.4	30.6	25.4	34.9	34.3	36.2	34.7	36.8	35.6	36.2
		added	0.0	0.0	0.0	0.0	14.8	0.0	0.0	0.0	0.0	0.0
		left	11.1	9.5	6.4	6.5	12.6	8.2	10.1	13.1	14.3	19.0
Week 10 DIO Ad Lib AICAR	T30	given	23.1	29.8	26.0	35.4	34.2	35.7	35.7	35.4	35.7	34.8
		added	6.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		left	12.6	11.7	8.7	15.2	13.8	16.2	18.6	18.1	23.0	20.2
	T31	given	23.3	30.7	25.7	35.0	35.1	34.7	35.1	34.5	35.9	34.8
		added	6.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		left	8.4	12.7	4.2	13.4	14.4	15.5	15.0	14.9	22.2	28.1
	T32	given	~~22.6~~	~~30.8~~	~~24.9~~	~~35.5~~	~~36.0~~	~~35.1~~	~~35.7~~	~~36.0~~	~~34.3~~	Animal Removed
		added	~~5.9~~	~~0.0~~	~~0.0~~	~~23.4~~	~~44.2~~	~~9.9~~	~~0.0~~	~~0.0~~	~~0.0~~	
		left	~~10.2~~	~~12.4~~	~~6.8~~	~~5.7~~	~~6.8~~	~~14.8~~	~~14.3~~	~~17.8~~		
	T33	given	23.5	30.4	25.5	35.7	34.6	34.5	35.0	34.8	34.8	36.0
		added	6.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		left	12.1	11.8	5.9	12.1	25.1	8.9	11.8	25.4	12.2	14.4
	T34	given	23.9	29.7	25.0	35.5	35.6	35.5	35.6	34.8	35.5	36.1
		added	6.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		left	11.0	10.5	6.6	22.0	8.3	11.7	12.6	13.4	20.2	19.6
	T35	given	23.6	29.3	25.6	35.6	35.0	35.8	35.3	35.6	35.0	35.8
		added	7.0	0.0	0.0	23.5	15.4	0.0	0.0	0.0	0.0	0.0
		left	9.1	8.5	5.1	4.2	9.3	12.2	11.5	16.9	20.8	20.6
	T36	given	~~23.7~~	~~29.5~~	~~25.8~~	~~35.0~~	~~36.0~~	~~35.7~~	~~35.2~~	~~35.3~~	Animal Removed	
		added	~~6.5~~	~~0.0~~	~~0.0~~	~~0.0~~	~~0.0~~	~~0.0~~	~~0.0~~	~~0.0~~		
		left	~~14.0~~	~~13.9~~	~~11.6~~	~~17.2~~	~~18.3~~	~~28.8~~	~~14.0~~			
Week 10 DIO Food Restrict Saline	T37	given	24.5	31.8	25.0	36.1	36.7	34.7	35.1	36.4	29.8	29.9
		added	7.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		left	11.0	12.1	5.3	14.9	14.2	11.1	12.1	14.0	5.8	1.0
	T38	given	23.5	29.4	25.1	35.5	35.9	35.9	36.6	37.0	28.8	28.9
		added	6.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		left	10.2	10.2	4.2	11.9	14.5	14.1	14.2	15.4	9.0	2.2
	T39	given	22.9	29.7	25.6	35.8	36.9	34.1	35.6	35.9	31.0	30.8
		added	7.1	0.0	0.0	22.1	20.8	9.0	0.0	0.0	0.0	0.0
		left	9.2	9.0	5.4	5.9	7.1	10.1	7.2	12.2	5.3	1.9
	T40	given	24.2	29.7	25.1	35.5	34.9	34.6	35.7	35.8	28.4	28.1
		added	5.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		left	11.2	11.8	6.4	14.0	14.0	14.0	14.4	14.7	5.4	2.8
	T41	given	24.1	29.9	26.0	34.9	35.5	35.5	35.8	36.0	29.2	28.9
		added	6.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		left	10.7	12.4	7.2	14.5	15.2	14.4	14.7	14.1	12.2	3.6

Table C-6: Experiment 2: Food Consumption of Restricted Group (g)

Restricted Food Consumption														
		4/28/12	4/29/12	4/30/12	5/1/12	5/2/12	5/3/12	5/4/12	5/5/12	5/6/12	5/7/12	5/8/12	5/9/12	5/10/12	5/11/12
T37	given	4.3	4.4	4.2	4.2	4.2	4.4	4.2	4.4	4.2	4.2	4.3	4.3	4.3	4.3
	added	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	left	1.8	0.6	3.2	0.0	0.0	0.0	0.2	0.2	0.3	0.1	0.0	0.0	0.4	0.1
T38	given	4.2	4.2	4.1	4.1	4.2	4.1	4.1	4.1	4.1	4.2	4.1	4.2	4.1	4.2
	added	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	left	2.1	1.4	0.0	0.1	3.4	1.4	0.7	0.2	0.3	0.1	0.0	0.0	1.4	0.1
T39	given	4.3	4.4	4.4	4.5	4.4	4.5	4.4	4.4	4.4	4.4	4.4	4.4	4.4	4.4
	added	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	left	0.4	2.1	0.2	0.4	1.3	0.6	0.4	0.5	0.1	0.2	0.5	0.1	0.2	0.3
T40	given	4.0	4.2	4.0	4.0	4.0	4.1	4.1	4.2	4.0	4.0	4.0	4.0	3.9	4.0
	added	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	left	1.4	0.8	0.2	0.2	0.9	1.3	0.7	0.9	0.5	0.2	0.2	0.0	0.6	0.4
T41	given	4.1	4.2	4.2	4.2	4.2	4.2	4.1	4.2	4.1	4.1	4.1	4.1	4.1	4.1
	added	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	left	3.0	2.8	1.7	1.2	1.8	0.6	1.1	0.3	1.1	0.1	0.8	0.3	1.0	0.2

Table C-7: Experiment 2: Food Consumption (g)

		Food Consumed (g)									
		3/9/12	3/16/12	3/23/12	3/30/12	4/6/12	4/13/12	4/20/12	4/27/12	5/4/12	5/11/12
		1	2	3	4	5	6	7	8	9	10
Week 8 Standard Diet Ad Lib	C1	24.80	24.80	23.44	24.15	22.30	22.66	23.26	22.20		
	C2	25.40	26.10	24.36	25.53	26.51	27.88	25.58	24.64		
	C3	25.10	23.70	22.83	22.29	22.99	23.31	23.37	25.33	Dissected	
	C4	23.10	23.00	20.42	22.03	23.12	23.61	22.10	22.62		
	C5	21.70	23.00	22.75	22.44	22.02	22.29	22.91	23.33		
Week 10 Standard Diet Ad Lib Saline	C7	21.70	25.00	22.59	23.51	22.86	23.93	24.46	27.13	23.57	21.99
	C8	24.30	25.20	23.53	22.65	23.85	20.86	23.77	24.73	22.44	23.03
	C9	24.40	24.80	23.45	22.25	22.27	23.62	25.14	22.31	14.75	29.08
	C10	20.50	17.50	18.56	21.56	19.83	19.40	22.44	22.27	20.35	17.63
	C11	22.60	24.70	24.25	25.38	27.20	23.74	27.10	25.01	25.84	25.48
Week 10 Standard Diet Ad Lib AICAR	C12	17.50	21.80	20.54	20.46	21.29	21.70	21.48	21.43	19.15	20.41
	C14	23.70	22.50	21.89	22.52	21.61	22.35	23.59	23.80	12.63	29.27
	C15	25.00	27.60	25.29	26.68	25.93	25.92	25.40	26.76	23.38	23.70
	C16	22.40	20.60	20.96	21.74	21.78	21.26	22.37	21.84	20.85	21.80
Week 8 DIO Ad Lib	T19	12.10	21.70	19.02	20.08	19.68	19.70	22.06	21.04		
	T20	21.50	20.50	20.10	33.73	48.09	24.63	23.84	22.81		
	T21	21.20	19.10	20.66	22.41	22.01	20.81	19.94	18.98	Dissected	
	T22	20.30	20.40	20.47	22.68	20.35	20.42	19.59	7.71		
	T23	18.60	18.10	17.76	20.51	18.34	18.84	19.13	18.54		
Week 10 DIO Ad Lib Saline	T24	20.00	18.50	18.45	24.01	22.97	22.59	23.80	24.34	25.33	19.64
	T25	20.00	20.70	16.71	28.44	31.26	30.75	31.75	31.44	40.55	16.56
	T26	19.00	21.00	19.38	22.74	20.22	20.52	20.45	21.07	12.96	21.78
	T27	19.90	15.00	20.48	22.83	22.19	21.32	20.69	22.40	20.48	18.44
	T28	20.70	18.90	18.28	21.98	20.63	20.43	20.59	21.49	9.45	22.64
	T29	12.30	21.11	19.06	28.47	36.54	27.96	24.60	23.73	21.28	17.15
Week 10 DIO Ad Lib AICAR	T30	16.70	18.11	17.28	20.18	20.43	19.48	17.09	17.33	12.77	14.67
	T31	21.20	18.01	21.49	21.62	20.74	19.15	20.14	19.63	13.64	6.77
	T33	18.20	18.60	19.62	23.60	9.54	25.62	23.21	9.38	22.60	21.53
	T34	19.80	19.20	18.47	13.42	27.29	23.83	23.00	21.38	15.34	16.49
	T35	21.50	20.80	20.54	54.96	41.03	23.57	23.80	18.67	14.19	15.22
Week 10 DIO Food Restrict Saline	T37	20.60	19.70	19.77	21.19	22.53	23.63	23.06	22.34	24.01	28.95
	T38	19.70	19.20	20.96	23.57	21.37	21.79	22.31	21.54	19.82	26.75
	T39	20.80	20.70	20.18	51.95	50.68	33.06	28.41	23.69	25.72	28.94
	T40	18.90	17.90	18.70	21.55	20.89	20.54	21.34	21.12	22.99	25.30
	T41	19.50	17.50	18.80	20.33	20.36	21.07	21.03	21.88	16.94	25.31

Table C-8: Experiment 2: Energy Intake (kcal)

Energy Intake (kcal)

		3/9/12 week 1	3/16/12 week 2	3/23/12 week 3	3/30/12 week 4	4/6/12 week 5	4/13/12 week 6	4/20/12 week 7	4/27/12 week 8	5/4/12 week 9	5/11/12 week 10
Week 8 Standard Diet Ad Lib	C1	76.88	76.88	72.66	74.87	69.13	70.25	72.11	68.82		
	C2	78.74	80.91	75.52	79.14	82.18	86.43	79.30	76.38		
	C3	77.81	73.47	70.77	69.10	71.27	72.26	72.45	78.52	Dissected	
	C4	71.61	71.30	63.30	68.29	71.67	73.19	68.51	70.12		
	C5	67.27	71.30	70.53	69.56	68.26	69.10	71.02	72.32		
Week 10 Standard Diet Ad Lib Saline	C7	67.27	77.50	70.03	72.88	70.87	74.18	75.83	84.10	73.07	68.17
	C8	75.33	78.12	72.94	70.22	73.94	64.67	73.69	76.66	69.56	71.39
	C9	75.64	76.88	72.70	68.98	69.04	73.22	77.93	69.16	45.73	90.15
	C10	63.55	54.25	57.54	66.84	61.47	60.14	69.56	69.04	63.09	54.65
	C11	70.06	76.57	75.18	78.68	84.32	73.59	84.01	77.53	80.10	78.99
Week 10 Standard Diet Ad Lib AICAR	C12	54.25	67.58	63.67	63.43	66.00	67.27	66.59	66.43	59.37	63.27
	C14	73.47	69.75	67.86	69.81	66.99	69.29	73.13	73.78	39.15	90.74
	C15	77.50	85.56	78.40	82.71	80.38	80.35	78.74	82.96	72.48	73.47
	C16	69.44	63.86	64.98	67.39	67.52	65.91	69.35	67.70	64.64	67.58
Week 8 DIO Ad Lib	T19	61.71	110.67	97.00	102.41	100.37	100.47	112.51	107.30		
	T20	109.65	104.55	102.51	172.02	245.26	125.61	121.58	116.33		
	T21	108.12	97.41	105.37	114.29	112.25	106.13	101.69	96.80	Dissected	
	T22	103.53	104.04	104.40	115.67	103.79	104.14	99.91	39.32		
	T23	94.86	92.31	90.58	104.60	93.53	96.08	97.56	94.55		
Week 10 DIO Ad Lib Saline	T24	102.00	94.35	94.10	122.45	117.15	115.21	121.38	124.13	129.18	100.16
	T25	102.00	105.57	85.22	145.04	159.43	156.83	161.93	160.34	206.81	84.46
	T26	96.90	107.10	98.84	115.97	103.12	104.65	104.30	107.46	66.10	111.08
	T27	101.49	76.50	104.45	116.43	113.17	108.73	105.52	114.24	104.45	94.04
	T28	105.57	96.39	93.23	112.10	105.21	104.19	105.01	109.60	48.20	115.46
	T29	62.73	107.66	97.21	145.20	186.35	142.60	125.46	121.02	108.53	87.47
Week 10 DIO Ad Lib AICAR	T30	85.17	92.36	88.13	102.92	104.19	99.35	87.16	88.38	65.13	74.82
	T31	108.12	91.85	109.60	110.26	105.77	97.67	102.71	100.11	69.56	34.53
	T33	92.82	94.86	100.06	120.36	48.65	130.66	118.37	47.84	115.26	109.80
	T34	100.98	97.92	94.20	68.44	139.18	121.53	117.30	109.04	78.23	84.10
	T35	109.65	106.08	104.75	280.30	209.25	120.21	121.38	95.22	72.37	77.62
Week 10 DIO Food Restrict Saline	T37	105.06	100.47	100.83	108.07	114.90	120.51	117.61	113.93	74.44	89.75
	T38	100.47	97.92	106.90	120.21	108.99	111.13	113.78	109.85	61.44	82.93
	T39	106.08	105.57	102.92	264.95	258.47	168.61	144.89	120.82	79.73	89.71
	T40	96.39	91.29	95.37	109.91	106.54	104.75	108.83	107.71	71.27	78.43
	T41	99.45	89.25	95.88	103.68	103.84	107.46	107.25	111.59	52.51	78.46

Table C-9: Experiment 2: Food Consumed (g) Welch's 2-Tailed T-test
(Comparisons of 0.05 significance marked in green)

Exp 2. Food Consumed (g) T Test
Significance of 0.05 Highlighted in Green

	Week 8 Standard Diet Ad Lib	Week 8 DIO Ad Lib
	22.20	21.04
	24.64	22.81
	25.33	18.98
	22.62	7.71
	23.33	18.54
	27.13	24.34
	24.73	31.44
	22.31	21.07
	22.27	22.40
	25.01	21.49
	21.43	23.73
	23.80	17.33
	26.76	19.63
	21.84	9.38
		21.38
		18.67
		22.34
		21.54
		23.69
		21.12
		21.88
Sample Size	14.00	21.00
Mean	23.81	20.50
StDev	1.83	4.90
Sx	1.18	
Deg. Of Freedom	27.38	
t value	2.818	
P(Means are equal)	0.89%	

	Week 10 Standard Diet Ad Lib Saline	Week 10 Standard Diet Ad Lib AICAR
	21.99	20.41
	23.03	29.27
	29.08	23.70
	17.63	21.80
	25.48	
Sample Size	5.00	4.00
Mean	23.44	23.80
StDev	4.24	3.89
Sx	2.72	
Deg. Of Freedom	6.80	
t value	-0.130	
P(Means are equal)	90.09%	

	Week 10 DIO Ad Lib Saline	Week 10 DIO Ad Lib AICAR
	19.64	14.67
	16.56	6.77
	21.78	21.53
	18.44	16.49
	22.64	15.22
	17.15	
Sample Size	6.00	5.00
Mean	19.37	14.94
StDev	2.46	5.31
Sx	2.58	
Deg. Of Freedom	5.42	
t value	1.719	
P(Means are equal)	14.62%	

	Week 10 Standard Diet Ad Lib Saline	Week 10 DIO Ad Lib Saline
	21.99	19.64
	23.03	16.56
	29.08	21.78
	17.63	18.44
	25.48	22.64
		17.15
Sample Size	5.00	6.00
Mean	23.44	19.37
StDev	4.24	2.46
Sx	2.15	
Deg. Of Freedom	6.17	
t value	1.898	
P(Means are equal)	10.65%	

	Week 10 Standard Diet Ad Lib AICAR	Week 10 DIO Ad Lib AICAR
	20.41	14.67
	29.27	6.77
	23.70	21.53
	21.80	16.49
		15.22
Sample Size	4.00	5.00
Mean	23.80	14.94
StDev	3.89	5.31
Sx	3.07	
Deg. Of Freedom	6.98	
t value	2.886	
P(Means are equal)	2.78%	

	Week 10 DIO Ad Lib Saline	Week 10 DIO Food Restrict
	19.64	28.95
	16.56	26.75
	21.78	28.94
	18.44	25.30
	22.64	25.31
	17.15	
Sample Size	6.00	5.00
Mean	19.37	27.05
StDev	2.46	1.83
Sx	1.29	
Deg. Of Freedom	8.92	
t value	-5.932	
P(Means are equal)	0.03%	

	Week 10 DIO Ad Lib AICAR	Week 10 DIO Food Restrict
	14.67	28.95
	6.77	26.75
	21.53	28.94
	16.49	25.30
	15.22	25.31
Sample Size	5.00	5.00
Mean	14.94	27.05
StDev	5.31	1.83
Sx	2.51	
Deg. Of Freedom	4.94	
t value	-4.825	
P(Means are equal)	0.85%	

Table C-10: Experiment 2: Energy Intake (kcal) Welch's 2-Tailed T-test
(Comparisons of 0.05 significance marked in green)

Exp 2. Energy Consumed (kcal) T Test
Significance of 0.05 Highlighted in Green

Week 8 Standard Diet Ad Lib	Week 8 DIO Ad Lib
68.82	107.30
76.38	116.33
78.52	96.80
70.12	39.32
72.32	94.55
84.10	124.13
76.66	160.34
69.16	107.46
69.04	114.24
77.53	109.60
66.43	121.02
73.78	88.38
82.96	100.11
67.70	47.84
	109.04
	95.22
	113.93
	109.85
	120.82
	107.71
	111.59

Sample Size	14.00	21.00
Mean	73.82	104.55
StDev	5.67	25.00
Sx	5.66	
Deg. Of Freedom	22.99	
t value	-5.427	
P(Means are equal)	0.00%	

Week 10 Standard Diet Ad Lib Saline	Week 10 Standard Diet Ad Lib AICAR
68.17	63.27
71.39	90.74
90.15	73.47
54.65	67.58
78.99	

Sample Size	5.00	4.00
Mean	72.67	73.76
StDev	13.15	12.06
Sx	8.42	
Deg. Of Freedom	6.80	
t value	-0.130	
P(Means are equal)	90.09%	

Week 10 DIO Ad Lib Saline	Week 10 DIO Ad Lib AICAR
100.16	74.82
84.46	34.53
111.08	109.80
94.04	84.10
115.46	77.62
87.47	

Sample Size	6.00	5.00
Mean	98.78	76.17
StDev	12.55	27.07
Sx	13.15	
Deg. Of Freedom	5.42	
t value	1.719	
P(Means are equal)	14.62%	

Week 10 Standard Diet Ad Lib Saline	Week 10 DIO Ad Lib Saline
68.17	100.16
71.39	84.46
90.15	111.08
54.65	94.04
78.99	115.46
	87.47

Sample Size	5.00	6.00
Mean	72.67	98.78
StDev	13.15	12.55
Sx	7.80	
Deg. Of Freedom	8.47	
t value	-3.347	
P(Means are equal)	1.01%	

Week 10 Standard Diet Ad Lib AICAR	Week 10 DIO Ad Lib AICAR
63.27	74.82
90.74	34.53
73.47	109.80
67.58	84.10
	77.62

Sample Size	4.00	5.00
Mean	73.76	76.17
StDev	12.06	27.07
Sx	13.53	
Deg. Of Freedom	5.76	
t value	-0.178	
P(Means are equal)	86.56%	

Week 10 DIO Ad Lib Saline	Week 10 DIO Food Restrict
100.16	89.75
84.46	82.93
111.08	89.71
94.04	78.43
115.46	78.46
87.47	

Sample Size	6.00	5.00
Mean	98.78	83.86
StDev	12.55	5.67
Sx	5.71	
Deg. Of Freedom	7.21	
t value	2.611	
P(Means are equal)	3.48%	

Week 10 DIO Ad Lib AICAR	Week 10 DIO Food Restrict
74.82	89.75
34.53	82.93
109.80	89.71
84.10	78.43
77.62	78.46

Sample Size	5.00	5.00
Mean	76.17	83.86
StDev	27.07	5.67
Sx	12.37	
Deg. Of Freedom	4.35	
t value	-0.621	
P(Means are equal)	56.83%	

Appendix C.3: Experiment 2 Body Composition Tables

Table C-11: Experiment 2: Dissected Tissue Weights (g)

Exp. 2 Dissected Tissue Weights

		Fat (g)	Liver (g)	Carcass (g)
Week 0 Baselne	B1	0.57	1.50	13.96
	B2	0.61	1.29	13.88
	B3	0.68	1.05	12.97
	B4	0.62	1.51	13.86
	B5	0.59	1.34	13.54
Week 8 Standard Diet Ad Lib	C1	0.85	1.09	16.18
	C2	1.56	1.22	21.13
	C3	1.50	1.46	19.14
	C4	1.05	1.11	17.25
	C5	1.07	1.04	16.19
Week 10 Standard Diet Ad Lib Saline	C7	1.29	1.19	17.90
	C8	1.20	1.22	18.29
	C9	1.00	1.30	16.89
	C10	1.04	0.99	14.40
	C11	1.87	1.58	20.90
Week 10 Standard Diet Ad Lib AICAR	C12	0.72	1.12	15.75
	C14	0.87	1.32	17.11
	C15	0.81	0.91	17.46
	C16	1.29	1.26	17.30
Week 8 DIO Ad Lib	T19	2.26	1.27	22.25
	T20	4.29	1.19	27.98
	T21	4.62	1.97	31.05
	T22	2.44	1.00	22.03
	T23	2.56	1.18	23.15
Week 10 DIO Ad Lib Saline	T24	3.22	1.03	25.16
	T25	3.41	1.49	28.69
	T26	3.93	1.43	27.55
	T27	3.18	1.22	25.48
	T28	2.49	1.36	23.15
	T29	3.85	1.31	27.79
Week 10 DIO Ad Lib AICAR	T30	2.53	1.28	21.17
	T31	3.16	1.11	22.46
	T33	2.81	1.49	24.05
	T34	2.58	1.30	23.52
	T35	3.16	1.51	26.48
Week 10 DIO Food Restrict Saline	T37	2.61	1.13	23.45
	T38	2.51	1.49	26.16
	T39	3.03	1.39	27.34
	T40	3.33	1.30	25.14
	T41	3.07	1.31	22.12

Table C-12: Experiment 2: Soxhlet Fat Extraction (g)

Exp. 2 Standard Diet Soxhlet Fat Extraction (g)

		Tube	Tube Weight (g)	Tube & Sample (g)	Tube, Sample, & Gauze (g)	Extracted (g)
Week 0 Baselne	B1	1	17.00	17.94	19.14	18.88
		2	18.82	19.72	20.52	20.28
		3	17.61	18.43	19.44	19.22
	B2	1	18.93	19.84	20.79	20.54
		2	21.51	22.57	23.43	23.15
		3	15.38	16.40	17.41	17.14
	B3	1	21.78	22.72	23.57	23.31
		2	16.40	17.65	18.53	18.18
		3	17.29	18.32	19.61	19.30
	B4	1	16.02	17.08	18.23	17.99
		2	16.70	17.55	18.40	18.19
		3	16.29	17.15	18.03	17.85
	B5	1	13.24	14.36	15.10	14.81
		2	16.43	17.57	18.44	18.14
		3	16.75	17.83	18.70	18.43
Week 8 Standard Diet Ad Lib	C1	1	14.63	15.85	16.68	16.30
		2	20.88	21.96	22.82	22.50
		3	18.79	19.74	20.66	20.38
	C2	1	17.05	17.90	18.77	18.28
		2	17.50	18.83	19.64	18.91
		3	13.22	14.20	15.54	14.97
	C3	1	14.79	15.64	16.90	16.46
		2	16.76	17.66	18.48	18.03
		3	15.98	17.27	18.10	17.45
	C4	1	12.83	13.79	14.54	14.18
		2	15.47	16.59	17.38	16.95
		3	14.28	15.67	16.51	16.18
	C5	1	14.77	15.77	16.58	16.33
		2	16.24	17.15	18.04	17.80
		3	17.53	18.48	19.30	19.06
Week 10 Standard Diet Ad Lib Saline	C7	1	18.77	19.68	20.55	20.15
		2	16.97	18.14	19.11	18.63
		3	17.84	18.67	19.82	19.46
	C8	1	16.67	17.50	18.37	18.01
		2	20.04	20.90	21.76	21.39
		3	25.39	26.33	27.13	26.72
	C9	1	16.85	17.52	18.32	18.05
		2	16.92	17.68	18.76	18.48
		3	20.32	21.30	22.49	22.12
	C10	1	21.03	22.37	23.20	22.58
		2	22.66	23.70	25.00	24.49
		3	22.88	23.58	24.75	24.41
	C11	1	21.40	22.60	23.70	22.98
		2	19.91	20.88	21.80	21.26
		3	20.43	21.89	22.65	21.86
Week 10 Standard Diet Ad Lib AICAR	C12	1	21.03	22.07	23.17	22.78
		2	16.51	17.64	18.52	18.12
		3	15.93	16.93	17.92	17.56
	C14	1	16.12	16.98	18.24	17.89
		2	17.07	17.89	18.62	18.34
		3	22.11	23.28	24.15	23.75
	C15	1	16.82	17.83	18.68	18.38
		2	21.89	22.94	23.89	23.57
		3	19.87	20.99	21.82	21.48
	C16	1	16.98	17.83	18.97	18.55
		2	16.66	17.46	18.26	17.87
		3	17.06	18.03	19.07	18.60

Exp. 2 High Fat Diet (DIO) Soxhlet Fat Extraction (g)

		Tube	Tube Weight (g)	Tube & Sample (g)	Tube, Sample, & Gauze (g)	Extracted (g)
Week 8 DIO Ad Lib	T19	1	16.56	17.45	18.33	17.76
		2	17.58	18.74	19.86	19.11
		3	16.86	17.98	19.11	18.39
	T20	1	15.58	16.64	17.46	16.73
		2	22.39	23.54	24.36	23.56
		3	20.53	21.67	22.55	21.77
	T21	1	19.13	20.03	20.86	20.18
		2	14.42	15.40	16.28	15.53
		3	15.82	17.17	18.26	17.22
	T22	1	14.44	15.46	16.26	15.63
		2	20.26	21.20	22.24	21.64
		3	16.55	17.39	18.23	17.68
	T23	1	21.77	22.55	23.68	23.15
		2	18.38	19.43	20.62	20.06
		3	18.13	19.37	20.33	19.37
Week 10 DIO Ad Lib Saline	T24	1	24.23	25.26	26.17	25.48
		2	17.27	18.13	19.11	18.54
		3	21.91	22.83	23.70	23.09
	T25	1	16.41	17.35	18.18	17.49
		2	17.08	18.16	19.03	18.24
		3	16.98	18.10	18.92	18.08
	T26	1	17.09	17.93	19.37	18.75
		2	16.98	18.34	19.15	18.14
		3	16.52	17.49	18.50	17.79
	T27	1	18.93	20.31	21.10	20.19
		2	22.14	22.36	24.44	23.58
		3	23.54	24.70	25.50	24.73
	T28	1	22.61	23.85	24.88	24.09
		2	13.86	14.68	15.72	15.20
		3	16.12	17.30	18.54	17.80
	T29	1	21.44	22.47	23.40	22.75
		2	15.45	16.50	17.40	16.65
		3	15.94	16.90	17.75	16.97
Week 10 DIO Ad Lib AICAR	T30	1	13.23	14.44	15.40	14.71
		2	14.81	15.77	16.62	16.05
		3	18.60	19.80	20.66	19.93
	T31	1	21.80	22.74	23.48	22.81
		2	17.30	18.23	19.20	18.59
		3	18.82	19.71	20.57	19.95
	T33	1	16.04	16.86	17.67	17.14
		2	16.71	17.77	18.76	18.08
		3	16.76	17.54	18.64	18.12
	T34	1	19.12	19.96	20.83	20.27
		2	14.81	15.99	17.05	16.27
		3	14.43	15.36	16.09	15.50
	T35	1	16.75	17.91	19.18	18.35
		2	16.43	17.37	18.35	17.69
		3	17.50	18.44	19.76	19.09
Week 10 DIO Food Restrict Saline	T37	1	20.53	21.42	22.24	21.74
		2	18.93	19.98	20.77	20.17
		3	15.81	16.68	17.51	17.21
	T38	1	16.23	17.28	18.55	17.85
		2	14.40	15.37	16.19	15.55
		3	14.78	15.70	16.83	16.21
	T39	1	15.37	16.42	17.17	16.48
		2	16.40	17.62	18.41	17.60
		3	17.50	18.57	19.44	18.81
	T40	1	17.57	18.61	19.72	19.00
		2	22.36	23.33	24.12	23.47
		3	17.54	18.60	19.72	19.00
	T41	1	17.05	18.09	18.95	18.26
		2	21.51	22.42	23.25	22.64
		3	13.21	14.20	15.06	14.41

Table C-13: Experiment 2: Body Composition Mass (g)

Exp. 2 Body Composition Mass (g)

		Percentage Fat	Percentage Lean	Dry Powder Weight (g)	Dry Fat Body mass (g) (%Fat x Dry Powder Weight)	Dry Lean Body Mass (g) (%Lean x Dry Powder Weight)	Wet Powder Weight (g)	Wet Lean Body Mass (g) (%Lean x Wet Powder Weight)	Average Lean Body Mass (g)	Standard Deviation	Standard Error
Week 0 Baseline	B1	27.1%	72.9%	4.95	1.339	3.611	13.89	10.13	9.27	1.07	0.48
	B2	26.8%	73.2%	4.03	1.079	2.951	11.07	8.10			
	B3	26.8%	73.2%	4.66	1.248	3.412	12.63	9.25			
	B4	22.8%	77.2%	3.53	0.803	2.727	10.78	8.33			
	B5	25.7%	74.3%	5.08	1.307	3.773	14.20	10.55			
Week 8 Standard Diet Ad Lib	C1	30.1%	69.9%	6.23	1.874	4.356	15.33	10.72	10.94	1.29	0.58
	C2	56.9%	43.1%	10.88	6.191	4.689	22.08	9.52			
	C3	50.7%	49.3%	9.28	4.707	4.573	20.28	9.99			
	C4	33.2%	66.8%	7.07	2.348	4.722	17.67	11.80			
	C5	25.5%	74.5%	5.97	1.525	4.445	17.00	12.66			
Week 10 Standard Diet Ad Lib Saline	C7	42.7%	57.3%	7.65	3.270	4.380	18.35	10.51	10.02	1.44	0.64
	C8	43.3%	56.7%	8.28	3.583	4.697	19.72	11.19			
	C9	38.2%	61.8%	7.15	2.734	4.416	18.08	11.17			
	C10	47.7%	52.3%	6.55	3.124	3.426	14.88	7.78			
	C11	56.6%	43.4%	10.19	5.767	4.423	21.78	9.45			
Week 10 Standard Diet Ad Lib AICAR	C12	36.3%	63.7%	6.24	2.265	3.975	15.57	9.92	10.68	1.42	0.71
	C14	36.3%	63.7%	6.85	2.490	4.360	17.58	11.19			
	C15	30.2%	69.8%	6.42	1.937	4.483	17.76	12.40			
	C16	48.9%	51.1%	7.94	3.880	4.060	17.98	9.19			
Week 8 DIO Ad Lib	T19	64.3%	35.7%	12.98	8.350	4.630	23.65	8.44	8.55	0.70	0.31
	T20	69.0%	31.0%	17.88	12.329	5.551	31.42	9.76			
	T21	76.4%	23.6%	22.55	17.222	5.328	34.66	8.19			
	T22	63.7%	36.3%	12.4	7.898	4.502	23.01	8.35			
	T23	66.2%	33.8%	13.43	8.895	4.535	23.70	8.00			
Week 10 DIO Ad Lib Saline	T24	66.8%	33.2%	15.1	10.093	5.007	27.48	9.11	8.68	0.56	0.23
	T25	73.9%	26.1%	18.97	14.020	4.950	30.57	7.98			
	T26	73.8%	26.2%	18.94	13.972	4.968	30.55	8.01			
	T27	66.5%	33.5%	15.8	10.514	5.286	27.53	9.21			
	T28	63.5%	36.5%	13.08	8.306	4.774	24.85	9.07			
	T29	71.9%	28.1%	19.27	13.847	5.423	30.88	8.69			
Week 10 DIO Ad Lib AICAR	T30	59.0%	41.0%	11.46	6.762	4.698	23.28	9.54	8.63	0.72	0.32
	T31	69.1%	30.9%	15.08	10.415	4.665	24.77	7.66			
	T33	65.0%	35.0%	14.07	9.147	4.923	25.78	9.02			
	T34	65.6%	34.4%	13.4	8.791	4.609	25.18	8.66			
	T35	71.2%	28.8%	16.75	11.926	4.824	28.66	8.25			
Week 10 DIO Food Restrict Saline	T37	56.8%	43.2%	12.28	6.979	5.301	25.46	10.99	9.47	1.08	0.48
	T38	66.5%	33.5%	15.41	10.250	5.160	28.16	9.43			
	T39	66.2%	33.8%	16.34	10.812	5.528	29.50	9.98			
	T40	68.0%	32.0%	15.52	10.556	4.964	27.34	8.74			
	T41	66.3%	33.7%	13.82	9.159	4.661	24.35	8.21			

Table C-14: Experiment 2: Body Composition Percentages

Exp. 2 Body Composition Percentages

		H20 Mass (g)	Percentage H20	Average Water Percentage	Standard Deviation	Standard Error	Percentage Dry Fat	Average Dry Fat Percentage	Standard Deviation	Standard Error	Percentage Dry Lean	Average Dry Lean Percentage	Standard Deviation	Standard Error	Total Carcass Weight (g)	Average Total Carcass Weight (g)	Standard Deviation	Standard Error
Week 0 Baseline	B1	8.94	**64.36%**	64.51%	0.016	0.007	**9.64%**	9.19%	0.010	0.004	**26.00%**	26.30%	0.007	0.003	**13.89**	12.51	1.569	0.701
	B2	7.04	**63.60%**				**9.75%**				**26.65%**				**11.07**			
	B3	7.97	**63.10%**				**9.88%**				**27.01%**				**12.63**			
	B4	7.25	**67.25%**				**7.45%**				**25.29%**				**10.78**			
	B5	9.12	**64.23%**				**9.21%**				**26.57%**				**14.20**			
Week 8 Standard Diet Ad Lib	C1	9.10	**59.36%**	57.84%	0.055	0.025	**12.23%**	17.15%	0.081	0.036	**28.41%**	25.01%	0.030	0.013	**15.33**	18.47	2.691	1.204
	C2	11.20	**50.72%**				**28.04%**				**21.24%**				**22.08**			
	C3	11.00	**54.24%**				**23.21%**				**22.55%**				**20.28**			
	C4	10.60	**59.99%**				**13.29%**				**26.72%**				**17.67**			
	C5	11.03	**64.88%**				**8.97%**				**26.15%**				**17.00**			
Week 10 Standard Diet Ad Lib Saline	C7	10.70	**58.31%**	57.19%	0.027	0.012	**17.82%**	19.72%	0.043	0.019	**23.87%**	23.09%	0.016	0.007	**18.35**	18.56	2.526	1.130
	C8	11.44	**58.01%**				**18.17%**				**23.82%**				**19.72**			
	C9	10.93	**60.45%**				**15.12%**				**24.42%**				**18.08**			
	C10	8.33	**55.98%**				**21.00%**				**23.02%**				**14.88**			
	C11	11.59	**53.21%**				**26.48%**				**20.31%**				**21.78**			
Week 10 Standard Diet Ad Lib AfCAR	C12	9.33	**59.92%**	60.16%	0.033	0.017	**14.55%**	15.30%	0.045	0.022	**25.53%**	24.54%	0.013	0.007	**15.57**	17.22	1.114	0.557
	C14	10.73	**61.04%**				**14.16%**				**24.80%**				**17.58**			
	C15	11.34	**63.85%**				**10.91%**				**25.24%**				**17.76**			
	C16	10.04	**55.84%**				**21.58%**				**22.58%**				**17.98**			
Week 8 DIO Ad Lib	T19	10.67	**45.12%**	42.52%	0.044	0.020	**35.31%**	39.22%	0.062	0.028	**19.58%**	18.26%	0.018	0.008	**23.65**	27.29	5.381	2.407
	T20	13.54	**43.09%**				**39.24%**				**17.67%**				**31.42**			
	T21	12.11	**34.94%**				**49.69%**				**15.37%**				**34.66**			
	T22	10.61	**46.11%**				**34.32%**				**19.57%**				**23.01**			
	T23	10.27	**43.33%**				**37.53%**				**19.13%**				**23.70**			
Week 10 DIO Ad Lib Saline	T24	12.38	**45.85%**	41.43%	0.042	0.017	**36.73%**	40.80%	0.054	0.022	**18.22%**	17.77%	0.014	0.006	**27.48**	28.64	2.422	0.989
	T25	11.60	**37.95%**				**45.86%**				**16.19%**				**30.57**			
	T26	11.61	**38.00%**				**45.73%**				**16.26%**				**30.55**			
	T27	11.73	**42.61%**				**38.19%**				**19.20%**				**27.53**			
	T28	11.77	**47.36%**				**33.42%**				**19.21%**				**24.85**			
	T29	11.61	**37.60%**				**44.84%**				**17.56%**				**30.88**			
Week 10 DIO Ad Lib AfCAR	T30	11.82	**50.77%**	44.73%	0.045	0.020	**29.05%**	36.62%	0.054	0.024	**20.18%**	18.65%	0.012	0.005	**23.28**	25.53	1.976	0.884
	T31	9.69	**39.12%**				**42.05%**				**18.83%**				**24.77**			
	T33	11.71	**45.42%**				**35.48%**				**19.10%**				**25.78**			
	T34	11.78	**46.78%**				**34.91%**				**18.31%**				**25.18**			
	T35	11.91	**41.56%**				**41.61%**				**16.83%**				**28.66**			
Week 10 DIO Food Restrict Saline	T37	13.18	**51.77%**	45.63%	0.035	0.016	**27.41%**	35.34%	0.045	0.020	**20.82%**	19.04%	0.011	0.005	**25.46**	26.96	2.067	0.925
	T38	12.75	**45.28%**				**36.40%**				**18.32%**				**28.16**			
	T39	13.16	**44.61%**				**36.65%**				**18.74%**				**29.50**			
	T40	11.82	**43.23%**				**38.61%**				**18.16%**				**27.34**			
	T41	10.53	**43.24%**				**37.61%**				**19.14%**				**24.35**			

Table C-15: Experiment 2: Fat Percentages Welch's 2-Tailed T-test
(Comparisons of 0.05 significance marked in green)

Exp 2. Fat Percentages T Test
Significance of 0.05 Highlighted in Green

	Week 10 Standard Diet Ad Lib Saline	Week 10 Standard Diet Ad Lib AICAR
	0.18	0.15
	0.18	0.14
	0.15	0.11
	0.21	0.22
	0.26	
Sample Size	5.00	4.00
Mean	0.20	0.15
StDev	0.04	0.04
Sx	0.03	
Deg. Of Freedom	6.43	
t value	1.492	
P(Means are equal)	18.64%	

	Week 10 DIO Ad Lib Saline	Week 10 DIO Ad Lib AICAR
	0.37	0.29
	0.46	0.42
	0.46	0.35
	0.38	0.35
	0.33	0.42
	0.45	
Sample Size	6.00	5.00
Mean	0.41	0.37
StDev	0.05	0.05
Sx	0.03	
Deg. Of Freedom	8.63	
t value	1.283	
P(Means are equal)	23.53%	

	Week 10 Standard Diet Ad Lib Saline	Week 10 DIO Ad Lib Saline
	0.18	0.37
	0.18	0.46
	0.15	0.46
	0.21	0.38
	0.26	0.33
		0.45
Sample Size	5.00	6.00
Mean	0.20	0.41
StDev	0.04	0.05
Sx	0.03	
Deg. Of Freedom	9.00	
t value	-7.220	
P(Means are equal)	0.01%	

	Week 10 Standard Diet Ad Lib AICAR	Week 10 DIO Ad Lib AICAR
	0.15	0.29
	0.14	0.42
	0.11	0.35
	0.22	0.35
		0.42
Sample Size	4.00	5.00
Mean	0.15	0.37
StDev	0.04	0.05
Sx	0.03	
Deg. Of Freedom	6.96	
t value	-6.474	
P(Means are equal)	0.06%	

	Week 8 Standard Diet Ad Lib	Week 8 DIO Ad Lib
	0.12	0.35
	0.28	0.39
	0.23	0.50
	0.13	0.34
	0.09	0.38
Sample Size	5.00	5.00
Mean	0.17	0.39
StDev	0.08	0.06
Sx	0.05	
Deg. Of Freedom	7.47	
t value	-4.857	
P(Means are equal)	0.18%	

	Week 10 DIO Ad Lib Saline	Week 10 DIO Food Restrict
	0.37	0.27
	0.46	0.36
	0.46	0.37
	0.38	0.39
	0.33	0.38
	0.45	
Sample Size	6.00	5.00
Mean	0.41	0.35
StDev	0.05	0.05
Sx	0.03	
Deg. Of Freedom	8.99	
t value	1.832	
P(Means are equal)	10.42%	

	Week 10 DIO Ad Lib AICAR	Week 10 DIO Food Restrict
	0.29	0.27
	0.42	0.36
	0.35	0.37
	0.35	0.39
	0.42	0.38
Sample Size	5.00	5.00
Mean	0.37	0.35
StDev	0.05	0.05
Sx	0.03	
Deg. Of Freedom	7.77	
t value	0.408	
P(Means are equal)	69.52%	

Appendix C.4: Experiment 2 Glucose Clearance Tables

Table C-16: Experiment 2: Week 8 Standard Diet Glucose Levels

Exp. 2 Glucose Concentration

Week 8

Standard Diet Ad Lib

			Standard						
		Lean Body Mass (g)	Concentration (mg/1 gram solution)	Amount Given					
		10.94	220.63	0.214					**Total Area**
C1	Before:	2.30	Decimal Time:	0.000	0.501	0.999	1.499	1.994	
	After:	2.48	Glucose:	162	289	281	222	181	
	Injected:	0.18	Area Under Curve	112.9	142.1	125.8	99.6		480.4
			Standardized to Glucose Injected	125.4	157.9	139.8	110.6		533.7
			Standardized to G Concentration	125.4	157.9	139.8	110.6		533.7
			Standardized to Amount Given	125.4	157.9	139.8	110.6		533.7
	Lean Body Mass	10.72	Standardized to Lean Body Mass	128.0	161.1	142.7	112.9		544.7
C2	Before:	2.29	Decimal Time:	0.018	0.518	1.026	1.523	2.467	
	After:	2.50	Glucose:	197	356	338	349	250	
	Injected:	0.21	Area Under Curve	138.3	176.2	170.7	282.6		767.8
			Standardized to Glucose Injected	131.7	167.8	162.6	269.2		731.2
			Standardized to G Concentration	131.7	167.8	162.6	269.2		731.2
			Standardized to Amount Given	131.7	167.8	162.6	269.2		731.2
	Lean Body Mass	9.52	Standardized to LBM	151.3	192.9	186.8	309.3		840.4
C3	Before:	2.30	Decimal Time:	0.041	0.545	1.129	1.549	2.042	
	After:	2.50	Glucose:	151	244	319	283	282	
	Injected:	0.20	Area Under Curve	99.5	164.4	126.6	139.2		529.7
			Standardized to Glucose Injected	99.5	164.4	126.6	139.2		529.7
			Standardized to G Concentration	99.5	164.4	126.6	139.2		529.7
			Standardized to Amount Given	99.5	164.4	126.6	139.2		529.7
	Lean Body Mass	9.99	Standardized to LBM	108.8	180.0	138.5	152.3		579.7
C4	Before:	2.27	Decimal Time:	0.064	0.566	1.080	1.572	2.100	
	After:	2.50	Glucose:	160	278	263	194	164	
	Injected:	0.23	Area Under Curve	110.0	139.0	112.3	94.5		455.9
			Standardized to Glucose Injected	95.6	120.9	97.7	82.2		396.4
			Standardized to G Concentration	95.6	120.9	97.7	82.2		396.4
			Standardized to Amount Given	95.6	120.9	97.7	82.2		396.4
	Lean Body Mass	11.80	Standardized to LBM	88.6	112.0	90.5	76.2		367.4
C5	Before:	2.27	Decimal Time:	0.095	0.598	1.109	1.607	2.101	
	After:	2.50	Glucose:	204	282	286	210	188	
	Injected:	0.23	Area Under Curve	122.2	145.1	123.5	98.2		489.0
			Standardized to Glucose Injected	106.3	126.2	107.4	85.4		425.2
			Standardized to G Concentration	106.3	126.2	107.4	85.4		425.2
			Standardized to Amount Given	106.3	126.2	107.4	85.4		425.2
	Lean Body Mass	12.66	Standardized to LBM	91.9	109.0	92.8	73.8		367.5
Wk 8 C Mean	Before:	2.29	Decimal Time:	0.0	0.5	1.0	1.5	2.097	
	After:	2.50	Glucose:	174.8	289.8	297.4	251.6	213	
	Injected:	0.21	Area Under Curve	116.6	153.6	132.2	137.1		539.5
			Standardized to Glucose Injected	111.0	146.3	125.9	130.6		513.8
			Standardized to G Concentration	111.0	146.3	125.9	130.6		513.8
			Standardized to Amount Given	111.0	146.3	125.9	130.6		513.8
	Lean Body Mass	10.94	Standardized to LBM	111.0	146.3	125.9	130.6		513.8

Table C-17: Experiment 2: Week 8 DIO Glucose Levels

Exp. 2 Glucose Concentration

			Standard						
		Lean Body Mass (g)	Concentration (mg/1 gram solution)	Amount Given					
Week 8		10.94	220.63	0.214					
DIO Ad Lib			Concentration (mg/1 gram solution)	Amount Given					
			293.37	0.214					**Total Area**
T19	Before:	2.26	Decimal Time:	0.124	0.624	1.141	1.638	2.137	
	After:	2.49	Glucose:	199	415	420	373	362	
	Injected:	0.23	Area Under Curve	153.6	215.8	197.0	183.2		749.7
			Standardized to Glucose Injected	133.6	187.7	171.3	159.3		651.9
			Standardized to G Concentration	100.4	141.1	128.9	119.8		490.3
			Standardized to Amount Given	100.4	141.1	128.9	119.8		490.3
	Lean Body Mass	8.44	Standardized to LBM	130.2	183.0	167.1	155.4		635.6
T20	Before:	2.25	Decimal Time:	0.146	0.648	1.161	1.659	2.153	
	After:	2.47	Glucose:	206	491	470	494	411	
	Injected:	0.22	Area Under Curve	174.9	246.1	240.5	223.4		884.9
			Standardized to Glucose Injected	159.0	223.7	218.6	203.1		804.4
			Standardized to G Concentration	119.6	168.3	164.4	152.7		605.0
			Standardized to Amount Given	119.6	168.3	164.4	152.7		605.0
	Lean Body Mass	9.76	Standardized to LBM	134.1	188.7	184.3	171.2		678.3
T21	Before:	2.28	Decimal Time:	0.173	0.671	1.179	1.681	2.188	
	After:	2.49	Glucose:	255	572	540	492	428	
	Injected:	0.21	Area Under Curve	205.8	282.6	259.0	232.9		980.4
			Standardized to Glucose Injected	196.0	269.2	246.7	221.8		933.7
			Standardized to G Concentration	147.4	202.4	185.5	166.8		702.2
			Standardized to Amount Given	147.4	202.4	185.5	166.8		702.2
	Lean Body Mass	8.19	Standardized to LBM	196.9	270.4	247.8	222.9		937.9
T22	Before:	2.26	Decimal Time:	0.193	0.691	1.199	1.701	2.204	
	After:	2.48	Glucose:	149	507	539	505	360	
	Injected:	0.22	Area Under Curve	163.3	265.4	262.5	217.5		908.6
			Standardized to Glucose Injected	148.4	241.3	238.6	197.7		826.0
			Standardized to G Concentration	111.6	181.5	179.4	148.7		621.2
			Standardized to Amount Given	111.6	181.5	179.4	148.7		621.2
	Lean Body Mass	8.35	Standardized to LBM	146.1	237.6	234.9	194.6		813.2
T23	Before:	2.26	Decimal Time:	0.211	0.712	1.217	1.719	2.226	
	After:	2.47	Glucose:	209	519	374	444	322	
	Injected:	0.21	Area Under Curve	182.2	225.4	205.4	194.2		807.1
			Standardized to Glucose Injected	173.5	214.6	195.6	184.9		768.7
			Standardized to G Concentration	130.5	161.4	147.1	139.1		578.1
			Standardized to Amount Given	130.5	161.4	147.1	139.1		578.1
	Lean Body Mass	8.00	Standardized to LBM	178.4	220.6	201.1	190.1		790.1
Wk 8 T Mean	Before:	2.26	Decimal Time:	0.0	0.5	1.0	1.5	2.0	
	After:	2.48	Glucose:	203.6	500.8	468.6	461.6	376.6	
	Injected:	0.22	Area Under Curve	176.0	247.2	232.8	210.2		866.2
			Standardized to Glucose Injected	161.5	226.8	213.6	192.9		794.7
			Standardized to G Concentration	121.4	170.5	160.6	145.1		597.6
			Standardized to Amount Given	121.4	170.5	160.6	145.1		597.6
	Lean Body Mass	8.55	Standardized to LBM	155.4	218.2	205.5	185.6		764.8

Table C-18: Experiment 2: Week 10 Standard Diet Saline Glucose Levels

Exp. 2 Glucose Concentration

	Standard		
	Lean Body Mass (g)	Concentration (mg/1 gram solution)	Amount Given
Week 10	10.94	220.63	0.214
Standard Diet Ad Lib Saline		Concentration (mg/1 gram solution)	Amount Given
		221.94	0.244

									Total Area
C7	Before:	2.289	Decimal Time:	0.000	0.500	1.008	1.509	2.026	
	After:	2.562	Glucose:	152	342	252	181	178	
	Injected:	0.27	Area Under Curve	123.5	151.0	108.3	92.9		475.7
			Standardized to Glucose Injected	90.5	110.6	79.3	68.1		348.5
			Standardized to G Concentration	89.9	110.0	78.9	67.7		346.4
			Standardized to Amount Given	78.8	96.3	69.1	59.2		303.4
	Lean Body Mass	10.51	Standardized to LBM	82.0	100.2	71.9	61.7		315.8
C8	Before:	2.292	Decimal Time:	0.031	0.531	1.032	1.532	2.048	
	After:	2.577	Glucose:	168	255	229	196	194	
	Injected:	0.29	Area Under Curve	105.8	121.3	106.4	100.6		434.0
			Standardized to Glucose Injected	74.2	85.1	74.6	70.6		304.5
			Standardized to G Concentration	73.8	84.6	74.2	70.2		302.7
			Standardized to Amount Given	64.6	74.1	65.0	61.5		265.1
	Lean Body Mass	11.19	Standardized to LBM	63.2	72.4	63.5	60.1		259.3
C9	Before:	2.243	Decimal Time:	0.048	0.548	1.049	1.557	2.056	
	After:	2.519	Glucose:	223	436	348	345	203	
	Injected:	0.28	Area Under Curve	164.8	196.8	175.8	136.8		674.0
			Standardized to Glucose Injected	119.4	142.6	127.4	99.1		488.4
			Standardized to G Concentration	118.7	141.7	126.6	98.5		485.5
			Standardized to Amount Given	103.9	124.1	110.9	86.3		425.2
	Lean Body Mass	11.17	Standardized to LBM	101.8	121.6	108.6	84.5		416.5
C10	Before:	2.277	Decimal Time:	0.063	0.563	1.075	1.578	2.083	
	After:	2.557	Glucose:	149	252	209	231	232	
	Injected:	0.28	Area Under Curve	100.3	118.1	110.7	116.8		445.9
			Standardized to Glucose Injected	71.6	84.4	79.1	83.5		318.5
			Standardized to G Concentration	71.2	83.9	78.6	83.0		316.6
			Standardized to Amount Given	62.3	73.5	68.8	72.7		277.3
	Lean Body Mass	7.78	Standardized to LBM	87.6	103.2	96.7	102.1		389.7
C11	Before:	2.260	Decimal Time:	0.088	0.588	1.108	1.586	2.123	
	After:	2.542	Glucose:	181	222	196	221	201	
	Injected:	0.28	Area Under Curve	100.8	108.6	99.7	113.2		422.3
			Standardized to Glucose Injected	71.5	77.0	70.7	80.3		299.5
			Standardized to G Concentration	71.0	76.6	70.3	79.8		297.7
			Standardized to Amount Given	62.2	67.1	61.6	69.9		260.7
	Lean Body Mass	9.45	Standardized to LBM	72.0	77.6	71.2	80.8		301.6
Wk 10 C Saline Mean	Before:	2.27	Decimal Time:	0.0	0.5	1.0	1.5	2.0	
	After:	2.55	Glucose:	174.6	301.4	246.8	234.8	201.6	
	Injected:	0.28	Area Under Curve	119.0	139.4	119.9	112.3		490.6
			Standardized to Glucose Injected	85.2	99.9	85.9	80.5		351.5
			Standardized to G Concentration	84.7	99.3	85.4	80.0		349.4
			Standardized to Amount Given	74.2	87.0	74.8	70.0		306.0
	Lean Body Mass	10.02	Standardized to LBM	81.0	94.9	81.6	76.5		334.0

Table C-19: Experiment 2: Week 10 Standard Diet AICAR Glucose Levels

Exp. 2 Glucose Concentration

			Standard						
		Lean Body Mass (g)	Concentration (mg/1 gram solution)	Amount Given					
Week 10		10.94	220.63	0.214					
Standard Diet Ad Lib AICAR			Concentration (mg/1 gram solution)	Amount Given					
			221.94	0.244					**Total Area**
C12	Before:	2.245	Decimal Time:	0.104	0.606	1.125	1.607	2.134	
	After:	2.543	Glucose:	174	219	194	195	165	
	Injected:	0.30	Area Under Curve	98.6	107.3	93.8	94.8		394.4
			Standardized to Glucose Injected	66.2	72.0	62.9	63.6		264.7
			Standardized to G Concentration	65.8	71.6	62.6	63.2		263.2
			Standardized to Amount Given	57.6	62.7	54.8	55.4		230.5
	Lean Body Mass	9.92	Standardized to LBM	63.6	69.1	60.4	61.1		254.2
C14	Before:	2.283	Decimal Time:	0.117	0.621	1.139	1.632	2.149	
	After:	2.563	Glucose:	184	399	324	323	204	
	Injected:	0.28	Area Under Curve	147.0	187.3	159.5	136.4		630.1
			Standardized to Glucose Injected	105.0	133.8	113.9	97.4		450.1
			Standardized to G Concentration	104.4	133.0	113.3	96.8		447.4
			Standardized to Amount Given	91.4	116.5	99.2	84.8		391.8
	Lean Body Mass	11.19	Standardized to LBM	89.3	113.8	96.9	82.9		383.0
C15	Before:	2.281	Decimal Time:	0.132	0.634	1.149	1.648	2.166	
	After:	2.564	Glucose:	120	207	187	159	148	
	Injected:	0.28	Area Under Curve	82.1	101.6	86.2	79.5		349.4
			Standardized to Glucose Injected	58.0	71.8	60.9	56.2		246.9
			Standardized to G Concentration	57.7	71.4	60.6	55.9		245.5
			Standardized to Amount Given	50.5	62.5	53.0	48.9		215.0
	Lean Body Mass	12.40	Standardized to LBM	44.6	55.1	46.8	43.2		189.6
C16	Before:	2.292	Decimal Time:	0.144	0.646	1.163	1.664	2.173	
	After:	2.568	Glucose:	155	185	163	202	197	
	Injected:	0.28	Area Under Curve	85.3	90.0	91.5	101.4		368.2
			Standardized to Glucose Injected	61.8	65.2	66.3	73.5		266.8
			Standardized to G Concentration	61.5	64.9	65.9	73.1		265.3
			Standardized to Amount Given	53.8	56.8	57.7	64.0		232.3
	Lean Body Mass	9.19	Standardized to LBM	64.0	67.6	68.6	76.1		276.4
Wk 10 C AICAR Mean	Before:	2.28	Decimal Time:	0.0	0.5	1.0	1.5	2.0	
	After:	2.56	Glucose:	158.3	252.5	217.0	219.8	178.5	
	Injected:	0.28	Area Under Curve	103.2	121.5	107.8	103.1		435.6
			Standardized to Glucose Injected	72.6	85.5	75.9	72.5		306.5
			Standardized to G Concentration	72.2	85.0	75.4	72.1		304.7
			Standardized to Amount Given	63.2	74.4	66.0	63.1		266.8
	Lean Body Mass	10.68	Standardized to LBM	64.8	76.3	67.7	64.7		273.4

Table C-20: Experiment 2: Week 10 DIO Ad libitum Saline Glucose Levels

Exp. 2 Glucose Concentration

	Standard		
	Lean Body Mass (g)	Concentration (mg/1 gram solution)	Amount Given
Week 10	10.94	220.63	0.214
DIO Ad Lib Saline		Concentration (mg/1 gram solution)	Amount Given
		285.90	0.244

									Total Area
T24	Before:	2.294	Decimal Time:	0.000	0.503	1.008	1.512	2.003	
	After:	2.510	Glucose:	174	508	449	427	300	
	Injected:	0.22	Area Under Curve	171.4	241.5	221.1	178.5		812.5
			Standardized to Glucose Injected	158.7	223.6	204.7	165.3		752.4
			Standardized to G Concentration	122.5	172.6	158.0	127.6		580.6
			Standardized to Amount Given	107.3	151.1	138.3	111.7		508.5
	Lean Body Mass	9.111	Standardized to LBM	128.8	181.4	166.1	134.1		610.4
T25	Before:	2.253	Decimal Time:	0.031	0.530	1.028	1.531	2.028	
	After:	2.475	Glucose:	194	442	314	296	274	
	Injected:	0.22	Area Under Curve	158.6	188.2	153.3	141.7		641.8
			Standardized to Glucose Injected	142.8	169.5	138.2	127.7		578.2
			Standardized to G Concentration	110.2	130.8	106.6	98.5		446.2
			Standardized to Amount Given	96.5	114.6	93.4	86.3		390.7
	Lean Body Mass	7.977	Standardized to LBM	132.4	157.1	128.0	118.3		535.7
T26	Before:	2.253	Decimal Time:	0.058	0.560	1.055	1.548	2.059	
	After:	2.475	Glucose:	204	388	504	405	351	
	Injected:	0.22	Area Under Curve	148.5	220.6	224.0	193.5		786.6
			Standardized to Glucose Injected	133.8	198.8	201.8	174.3		708.7
			Standardized to G Concentration	103.2	153.4	155.7	134.5		546.9
			Standardized to Amount Given	90.4	134.3	136.4	117.8		478.9
	Lean Body Mass	8.014	Standardized to LBM	123.4	183.3	186.1	160.8		653.7
T27	Before:	2.281	Decimal Time:	0.088	0.592	1.094	1.578	2.086	
	After:	2.502	Glucose:	196	437	366	270	200	
	Injected:	0.22	Area Under Curve	159.6	201.9	153.8	119.4		634.6
			Standardized to Glucose Injected	144.4	182.7	139.2	108.0		574.3
			Standardized to G Concentration	111.4	141.0	107.4	83.4		443.2
			Standardized to Amount Given	97.6	123.5	94.1	73.0		388.1
	Lean Body Mass	9.210	Standardized to LBM	115.9	146.6	111.7	86.7		460.9
T28	Before:	2.293	Decimal Time:	0.118	0.625	1.124	1.606	2.117	
	After:	2.515	Glucose:	231	526	396	342	270	
	Injected:	0.22	Area Under Curve	191.9	230.2	177.7	156.4		756.3
			Standardized to Glucose Injected	172.9	207.4	160.1	140.9		681.3
			Standardized to G Concentration	133.4	160.1	123.6	108.7		525.8
			Standardized to Amount Given	116.8	140.2	108.2	95.2		460.4
	Lean Body Mass	9.070	Standardized to LBM	140.9	169.0	130.5	114.8		555.2
T29	Before:	2.255	Decimal Time:	0.140	0.646	1.152	1.633	2.137	
	After:	2.473	Glucose:	201	494	333	286	223	
	Injected:	0.22	Area Under Curve	175.7	209.4	148.8	128.4		662.3
			Standardized to Glucose Injected	161.2	192.1	136.5	117.8		607.6
			Standardized to G Concentration	124.4	148.2	105.4	90.9		468.9
			Standardized to Amount Given	108.9	129.8	92.3	79.6		410.6
	Lean Body Mass	8.690	Standardized to LBM	137.1	163.4	116.1	100.2		516.8
Wk 10 T Saline Mean	Before:	2.27	Decimal Time:	0.0	0.5	1.0	1.5	2.0	
	After:	2.49	Glucose:	200.0	465.8	393.7	337.7	269.7	
	Injected:	0.22	Area Under Curve	167.6	215.3	179.6	153.0		715.5
			Standardized to Glucose Injected	152.2	195.6	163.1	139.0		649.9
			Standardized to G Concentration	117.5	150.9	125.9	107.3		501.5
			Standardized to Amount Given	102.9	132.2	110.2	94.0		439.2
	Lean Body Mass	8.59	Standardized to LBM	130.9	168.2	140.3	119.6		559.1

Table C-21: Experiment 2: Week 10 DIO Ad libitum AICAR Glucose Levels

Exp. 2 Glucose Concentration

			Standard						
		Lean Body Mass (g)	Concentration (mg/1 gram solution)	Amount Given					
Week 10		10.94	220.63	0.214					
DIO Ad Lib AICAR			Concentration (mg/1 gram solution)	Amount Given					
			285.90	0.244					**Total Area**
T30	Before:	2.260	Decimal Time:	0.169	0.667	1.181	1.655	2.162	
	After:	2.480	Glucose:	204	570	520	548	565	
	Injected:	0.22	Area Under Curve	192.4	280.1	253.4	282.3		1008.1
			Standardized to Glucose Injected	174.9	254.6	230.3	256.6		916.5
			Standardized to G Concentration	135.0	196.5	177.7	198.0		707.2
			Standardized to Amount Given	118.2	172.1	155.7	173.4		619.4
	Lean Body Mass	9.54	Standardized to LBM	135.5	197.2	178.4	198.8		709.9
T31	Before:	2.250	Decimal Time:	0.189	0.693	1.203	1.683	2.184	
	After:	2.468	Glucose:	133	250	200	200	203	
	Injected:	0.22	Area Under Curve	96.3	114.8	96.1	100.9		408.1
			Standardized to Glucose Injected	88.4	105.3	88.2	92.5		374.4
			Standardized to G Concentration	68.2	81.3	68.0	71.4		288.9
			Standardized to Amount Given	59.7	71.2	59.6	62.5		253.0
	Lean Body Mass	7.66	Standardized to LBM	85.3	101.6	85.1	89.3		361.2
T33	Before:	2.262	Decimal Time:	0.219	0.729	1.226	1.721	2.216	
	After:	2.480	Glucose:	228	465	466	392	352	
	Injected:	0.22	Area Under Curve	176.8	231.2	212.6	184.0		804.6
			Standardized to Glucose Injected	162.2	212.1	195.0	168.8		738.2
			Standardized to G Concentration	125.2	163.7	150.5	130.3		569.7
			Standardized to Amount Given	109.6	143.3	131.8	114.1		498.9
	Lean Body Mass	9.02	Standardized to LBM	132.9	173.8	159.8	138.4		604.9
T34	Before:	2.279	Decimal Time:	0.251	0.754	1.261	1.755	2.244	
	After:	2.496	Glucose:	218	438	351	335	314	
	Injected:	0.22	Area Under Curve	165.0	199.7	169.5	158.8		693.0
			Standardized to Glucose Injected	152.1	184.0	156.2	146.4		638.7
			Standardized to G Concentration	117.4	142.0	120.6	113.0		492.9
			Standardized to Amount Given	102.8	124.4	105.6	98.9		431.6
	Lean Body Mass	8.66	Standardized to LBM	129.8	157.1	133.3	124.9		545.1
T35	Before:	2.262	Decimal Time:	0.275	0.773	1.283	1.776	2.272	
	After:	2.480	Glucose:	222	429	322	289	254	
	Injected:	0.22	Area Under Curve	162.0	191.7	150.5	134.6		638.9
			Standardized to Glucose Injected	148.6	175.9	138.1	123.5		586.1
			Standardized to G Concentration	114.7	135.7	106.6	95.3		452.3
			Standardized to Amount Given	100.5	118.9	93.3	83.5		396.1
	Lean Body Mass	8.25	Standardized to LBM	133.1	157.5	123.7	110.6		524.9
Wk 10 T AICAR Mean	Before:	2.26	Decimal Time:	0.0	0.5	1.0	1.5	2.0	
	After:	2.48	Glucose:	201.0	430.4	371.8	352.8	337.6	
	Injected:	0.22	Area Under Curve	158.6	203.6	176.6	171.8		710.5
			Standardized to Glucose Injected	145.3	186.6	161.9	157.4		651.2
			Standardized to G Concentration	112.2	144.0	124.9	121.5		502.6
			Standardized to Amount Given	98.2	126.1	109.4	106.4		440.1
	Lean Body Mass	8.63	Standardized to LBM	124.5	159.8	138.7	134.9		557.9

Table C-22: Experiment 2: Week 10 DIO Food Restricted Glucose Level

Exp. 2 Glucose Concentration

		Standard							
		Lean Body Mass (g)	Concentration (mg/1 gram solution)	Amount Given					
Week 10		10.94	220.63	0.214					
DIO Food Restrict Saline			Concentration (mg/1 gram solution)	Amount Given					
			285.90	0.244					**Total Area**
	Before:	2.255	Decimal Time:	0.304	0.811	1.309	1.798	2.300	
	After:	2.479	Glucose:	191	432	371	268	186	
	Injected:	0.22	Area Under Curve	157.9	200.1	156.2	114.0		628.2
T37			Standardized to Glucose Injected	141.0	178.6	139.5	101.8		560.9
			Standardized to G Concentration	108.8	137.9	107.6	78.6		432.8
			Standardized to Amount Given	95.3	120.7	94.3	68.8		379.1
	Lean Body Mass	10.99	Standardized to LBM	94.8	120.1	93.8	68.5		377.2
	Before:	2.262	Decimal Time:	0.325	0.833	1.332	1.819	2.329	
	After:	2.479	Glucose:	174	407	409	365	288	
	Injected:	0.22	Area Under Curve	147.7	203.5	188.4	166.4		706.1
T38			Standardized to Glucose Injected	136.1	187.6	173.7	153.4		650.8
			Standardized to G Concentration	105.0	144.8	134.0	118.4		502.2
			Standardized to Amount Given	92.0	126.8	117.4	103.7		439.8
	Lean Body Mass	9.43	Standardized to LBM	106.7	147.1	136.1	120.2		510.1
	Before:	2.262	Decimal Time:	0.344	0.851	1.355	1.847	2.350	
	After:	2.481	Glucose:	191	402	321	306	218	
	Injected:	0.22	Area Under Curve	150.4	182.3	154.3	131.7		618.7
T39			Standardized to Glucose Injected	136.7	165.7	140.3	119.8		562.4
			Standardized to G Concentration	105.5	127.9	108.3	92.4		434.0
			Standardized to Amount Given	92.4	112.0	94.8	80.9		380.1
	Lean Body Mass	9.98	Standardized to LBM	101.3	122.7	103.9	88.7		416.6
	Before:	2.266	Decimal Time:	0.366	0.869	1.377	1.866	2.371	
	After:	2.488	Glucose:	197	456	285	249	192	
	Injected:	0.22	Area Under Curve	164.2	188.2	130.7	111.4		594.5
T40			Standardized to Glucose Injected	149.3	171.1	118.8	101.2		540.5
			Standardized to G Concentration	115.2	132.1	91.7	78.1		417.1
			Standardized to Amount Given	100.9	115.6	80.3	68.4		365.3
	Lean Body Mass	8.74	Standardized to LBM	126.2	144.6	100.4	85.6		456.8
	Before:	2.285	Decimal Time:	0.387	0.886	1.404	1.886	2.397	
	After:	2.504	Glucose:	196	329	336	284	262	
	Injected:	0.22	Area Under Curve	130.9	172.3	149.2	139.6		592.1
T41			Standardized to Glucose Injected	119.0	156.7	135.7	126.9		538.2
			Standardized to G Concentration	91.8	120.9	104.7	97.9		415.4
			Standardized to Amount Given	80.4	105.9	91.7	85.8		363.8
	Lean Body Mass	8.21	Standardized to LBM	107.1	141.0	122.1	114.2		484.4
	Before:	2.27	Decimal Time:	0.0	0.5	1.0	1.5	2.0	
Wk 10 T	After:	2.49	Glucose:	189.8	405.2	344.4	294.4	229.2	
Saline	Injected:	0.22	Area Under Curve	150.2	189.5	155.8	132.5		628.0
Food			Standardized to Glucose Injected	136.5	172.3	141.6	120.5		570.9
Restrict			Standardized to G Concentration	105.4	132.9	109.3	93.0		440.6
Mean			Standardized to Amount Given	92.3	116.4	95.7	81.4		385.8
	Lean Body Mass	9.47	Standardized to LBM	106.6	134.4	110.5	94.0		445.5

Table C-23: Experiment 2: Glucose Clearance Welch's 2-Tailed T-test
(Comparisons of 0.05 significance marked in green)

Exp 2. Glucose Tolerance (mg glucose / dl blood) T Test
Significance of 0.05 Highlighted in Green

	Week 10 Standard Diet Ad Lib Saline	Week 10 Standard Diet Ad Lib AICAR
	475.68	394.44
	433.97	630.11
	674.04	349.41
	445.90	368.24
	422.28	
Sample Size	5.00	4.00
Mean	490.37	435.55
StDev	104.57	131.01
Sx	80.49	
Deg. Of Freedom	5.72	
t value	0.681	
P(Means are equal)	52.60%	

	Week 10 DIO Ad Lib Saline	Week 10 DIO Ad Lib AICAR
	812.54	1008.12
	641.77	408.12
	786.62	804.64
	634.62	692.99
	756.26	638.90
	662.27	
Sample Size	6.00	5.00
Mean	715.68	710.55
StDev	78.67	220.40
Sx	103.67	
Deg. Of Freedom	4.85	
t value	0.049	
P(Means are equal)	96.29%	

	Week 10 Standard Diet Ad Lib Saline	Week 10 DIO Ad Lib Saline
	475.68	812.54
	433.97	641.77
	674.04	786.62
	445.90	634.62
	422.28	756.26
		662.27
Sample Size	5.00	6.00
Mean	490.37	715.68
StDev	104.57	78.67
Sx	56.73	
Deg. Of Freedom	7.35	
t value	-3.971	
P(Means are equal)	0.54%	

	Week 10 Standard Diet Ad Lib AICAR	Week 10 DIO Ad Lib AICAR
	394.44	1008.12
	630.11	408.12
	349.41	804.64
	368.24	692.99
		638.90
Sample Size	4.00	5.00
Mean	435.55	710.55
StDev	131.01	220.40
Sx	118.35	
Deg. Of Freedom	6.60	
t value	-2.324	
P(Means are equal)	5.91%	

	Week 8 Standard Diet Ad Lib	Week 8 DIO Ad Lib
	480.37	749.69
	767.76	884.87
	529.70	980.41
	455.86	908.60
	489.01	807.13
Sample Size	5.00	5.00
Mean	544.54	866.14
StDev	127.59	89.80
Sx	69.77	
Deg. Of Freedom	7.18	
t value	-4.609	
P(Means are equal)	0.25%	

	Week 10 DIO Ad Lib Saline	Week 10 DIO Food Restrict
	812.54	628.20
	641.77	706.09
	786.62	618.69
	634.62	594.52
	756.26	592.07
	662.27	
Sample Size	6.00	5.00
Mean	715.68	627.91
StDev	78.67	46.36
Sx	38.23	
Deg. Of Freedom	8.25	
t value	2.296	
P(Means are equal)	5.08%	

	Week 10 DIO Ad Lib AICAR	Week 10 DIO Food Restrict
	1008.12	628.20
	408.12	706.09
	804.64	618.69
	692.99	594.52
	638.90	592.07
Sample Size	5.00	5.00
Mean	710.55	627.91
StDev	220.40	46.36
Sx	100.72	
Deg. Of Freedom	4.35	
t value	0.820	
P(Means are equal)	45.80%	

Glossary

Adenosine monophosphate (AMP): The nucleotide adenosine bonded to one phosphate group. Also, the result of removing two phosphate groups from adenosine triphosphate. Functionally, AMP contains a significantly lower amount of energy than ATP, as it is produced when energy is released by removing phosphate groups from ATP.

Adenosine triphosphate (ATP): The nucleotide adenosine bonded to a triphosphate group. ATP is the unit of energy in most cell processes, as phosphate groups are removed from ATP, thus releasing energy.

AICAR: AICAR, or 5-amino-1-β-Dffff-ribofuranosyl-imidazole-4-carboxamide, is a drug analog of AMP that stimulates AMPK activity.

Allosteric: The change in shape and activity of an enzyme resulting from a molecular binding of a regulatory substance to a site outside of the enzyme's active site.

AMP-activated protein kinase (AMPK): An energy sensor on both the cellular and organismal levels. AMPK works by responding to changes in the ratio of AMP to ATP. In this system, AMP represents 'used energy,' while ATP represents 'available energy,' so an increase in this ratio indicates an energy deficient state, while a decrease indicates an energy rich state. AMPK responds by controlling catabolic and anabolic processes in the cell and body.

Anabolism: A set of metabolic pathways that use energy to build large molecules from smaller subunits, such as monosaccharides, fatty acids, amino acids, and nucleotides.

Catabolism: A set of metabolic pathways that break down large molecules, such as polysaccharides, proteins, lipids, and nucleic acids, and release energy.

Citric Acid Cycle (= Krebs Cycle, = Tricarboxylic acid (TCA) Cycle): The Citric Acid Cycle is an aerobic cellular process in which acetyl CoA and oxygen is used to produce ATP and carbon dioxide.

Derivatization: A technique used to transform a chemical compound into a product of similar chemical structure.

Electron Transport Chain: An ETC couples electron transfer from an electron donor to an electron acceptor with the transfer of H+ (proton) across a membrane. This results in an electrochemical proton gradient, which produces energy in the form of ATP.

$FADH_2$: $FADH_2$ is the reduced form of FAD (flavin adenine dinucleotide). It carries high-energy electrons used for oxidative phosphorylation, sending its two high-energy electrons through the electron transport chain.

Fatty acid: A fatty acid is a carboxylic acid with a long hydrocarbon chain. The most common fatty acids have a chain of 12 – 18 carbon atoms. They can occur in either the esterified form as a triglyceride or in the nonesterified form as nonesterified fatty acid (NEFA).

Gluconeogenesis (GNG): Gluconeogenesis is a metabolic process in which glucose is generated from non-carbohydrate substrates, such as from fats or protein. GNG occurs in the liver when an animal's blood glucose levels drop too low.

Glycolysis: The metabolic pathway that converts glucose into pyruvate. The energy released in the process is used to form ATP and NADH.

Heterotrimeric: A macromolecule composed of three subunits of which at least one differs from the others.

High density lipoprotein (HDL): High density lipoprotein is one of five classes of lipoproteins, which are protein molecules that transport water-insoluble molecules such as cholesterol in the bloodstream. High density lipoprotein is considered as "healthy" because it can remove cholesterol from arteries which prevent plaque buildup.

Homogenization: Reducing a substance to extremely small particles and distributing it uniformly throughout a liquid.

Hyperinsulinemia: Hyperinsulinemia is a clinical condition in which insulin levels in the blood are elevated above the normal range. While hyperinsulinemia often occurs in Type II diabetes, the two words are not interchangeable.

Hyperphagia: Hyperphagia is abnormally increased appetite that results in overeating.

Hypertension: Hypertension is a medical condition in which blood pressure is chronically elevated.

Hysteresis: Hysteresis is the lagging period of time between a cause and its effect.

Insulin resistance: Insulin resistance is a symptom of metabolic syndrome in which the body's tissues do not respond effectively to insulin. Thus, cellular uptake of glucose is reduced and blood glucose and fat levels are elevated.

Lipogenesis: Lipogenesis is a metabolic process in which fatty acids are produced from non-lipid substrates, such as glucose. The term lipogenesis is used to encompass both fatty acid synthesis and triglyceride synthesis. Lipogenesis occurs in the liver and is secreted into the bloodstream.

Lipotoxicity: Lipotoxicity is the pathological damage when there is elevated fat level in

the blood or tissues, particularly the liver. The accumulation of lipids in the tissues ultimately leads to cell dysfunction and death.

Low density lipoprotein (LDL): Low density lipoprotein is one of five classes of lipoproteins, which are protein molecules that transport water-insoluble molecules such as cholesterol in the bloodstream. Studies have shown that an elevated level of low density lipoproteins promotes cardiovascular problems.

Metabolic syndrome (Insulin resistance syndrome): Metabolic syndrome is a combination of many risk factors that increase the risk for a heart attack, stroke, and Type II diabetes. The two identified causes of metabolic syndrome is insulin resistance and central obesity.

Metabolites: To fit the definition of a metabolite, a dietary ingredient must bear a semblance to a "living" molecule both in structure and function. The candidate molecule(s) must interact favorably with the host's metabolic machinery. Enhancing the level of the candidate molecule must not present a toxic challenge to the host or otherwise cause an untold situation that would differ from the elevation of the host's molecule. The candidate molecule must have a known metabolic fate that does not violate the principles of metabolic turnover. The candidate molecule must behave in accordance with the principles of metabolic turnover. It must show timely degradation and excretion. It must not leave a lasting imprint on the metabolic systems of the host or cause the host system to adapt to a new position of homeostasis or need. In essence, it must not be addictive. Molecules fitting these criteria, or supplement ingredients that give rise to said molecules, would be considered capable of meeting the host need for optimal health, growth and development.

Metabolomics (Metabonomics): Quantitiative and qualitative analysis of the complete set of metabolites present in a biological system.

Metabolome: The complete set of all metabolites formed by the cell in association with its metabolism. The metabolome comprises the endometabolome (all the intracellular metabolites) and the exometabolome (all the metabolites that are excreted into the growth medium or extracellular fluid).

NADH: NADH is the reduced form of NAD+ (nicotinamide adenine dinucleotide). It is used as a reducing agent by transferring and donating electrons.

Pleiotropic: In terms of AMPK, affecting multiple tissues and systems within an organism.

Set Point Theory: The body has an internal regulator that controls how much fat the body metabolizes. This regulator works as a thermostat within the body directing energy storage and consumption, it differs amongst people (20).

Triglyceride (TG) = Triacylglycerol (TAG): A triglyceride is an ester composed of a glycerol backbone and three fatty acids.

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