This thesis titled

Optimizing Low-Carbohydrate Diets to Promote Weight Loss in Mice

by

YUFENG ZHAI

has been approved for

the School of Human and Consumer Sciences

and the College of Health and Human Services by

________________________________________

Darlene E. Berryman

Associate Professor of Human and Consumer Sciences

________________________________________

Gary S. Neiman

Dean, College of Health and Human Services
ABSTRACT

ZHAI, YUFENG., M.S., August 2009, Food and Nutrition

Optimizing Low-Carbohydrate Diets to Promote Weight Loss in Mice (134 pp.)

Director of Thesis: Darlene E. Berryman

Studies have shown the benefits of low-carbohydrate diets on reducing weight in obese subjects. Establishing a mouse model to further evaluate the safety and efficacy of these diets is warranted due to controversial issues about the safety and efficacy of low-carbohydrate diets. In the present study, 40 diet-induced obese mice were split into 5 groups and placed on 1 of 5 diets: a HF diet (HF-HF), a standard chow (SC), three 2% carbohydrate diets with 5% protein (HF-LC5), 10% protein (HF-LC10), and 15% protein (HF-LC15), respectively. 10 mice were fed standard chow (SC-SC) as a non-obese control for whole study. The results showed that the body weights of mice in the HF-LC5, HF-LC10, HF-LC15 and HF-SC groups decreased with time. The most significant decrease in body weight was noted for the HF-LC5 (33%) and HF-SC (23%) mice. Accordingly, the percentage of fat mass decreased in the HF-LC5, HF-LC10, HF-LC15 and HF-SC groups with the most dramatic decrease in fat mass noted for the HF-LC5 and HF-SC groups. Similar energy consumption of low-carbohydrate diet groups to SC-SC group and small percentage of total weight loss in fluid mass suggested limited calorie intake and body water loss did not account for the majority of body weight loss with low-carbohydrate diets. The low-carbohydrate diet groups had increased levels of HDL and urinary ketones, as well as decreased levels of plasma insulin, IGF-1 blood glucose,
triglyceride, total cholesterol, and LDL. These results indicate that a diet with 5% protein and 2% carbohydrate can effectively promote weight loss in obese C57BL/6J mice. Future studies with this specially-formulated low-carbohydrate diet should be useful in assessing the long-term safety and effectiveness of low-carbohydrate diets for weight loss using mouse experimental systems.

Approved: _____________________________________________________________

Darlene E. Berryman

Associate Professor of Human and Consumer Sciences
ACKNOWLEDGMENTS

I would like to thank first and foremost my advisor, Darlene Berryman for the many opportunities to continue my research and education that she has given me. I would also like to thank Dr. Edward List and everyone else on the second floor of the Edison Biotechnology Institute, whom gave me lots of help and assistance with my thesis. I also want to thank committee members, Dr. David Holben and Dr. Shigeru Okada, who spent lots of time on my proposal and thesis.

I would like to also thank all of my friends and family members who have continued to be a system of support.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abstract</td>
<td>3</td>
</tr>
<tr>
<td>Acknowledgments</td>
<td>5</td>
</tr>
<tr>
<td>List of Tables</td>
<td>10</td>
</tr>
<tr>
<td>List of Figures</td>
<td>11</td>
</tr>
<tr>
<td><strong>CHAPTER 1: INTRODUCTION</strong></td>
<td>12</td>
</tr>
<tr>
<td>Statement of the Problem</td>
<td>16</td>
</tr>
<tr>
<td>Research Questions</td>
<td>17</td>
</tr>
<tr>
<td>Purpose of the Study</td>
<td>18</td>
</tr>
<tr>
<td>Limitations/Delimitations</td>
<td>19</td>
</tr>
<tr>
<td>Definition of Terms</td>
<td>20</td>
</tr>
<tr>
<td><strong>CHAPTER 2: REVIEW OF LITERATURE</strong></td>
<td>22</td>
</tr>
<tr>
<td>Obesity</td>
<td>22</td>
</tr>
<tr>
<td>The Diet Plans for Obesity</td>
<td>24</td>
</tr>
<tr>
<td>Low-Fat, High-Carbohydrate, Calorie-Restricted Diet Plan</td>
<td>25</td>
</tr>
<tr>
<td>Zone Diet Plan</td>
<td>28</td>
</tr>
<tr>
<td>Ornish Diet Plan</td>
<td>30</td>
</tr>
<tr>
<td>Low-Carbohydrate, High-Fat Diet Plan</td>
<td>31</td>
</tr>
<tr>
<td>Clinical Trials Testing Low-Carbohydrate Diets</td>
<td>32</td>
</tr>
<tr>
<td>Several Clinical Trials Show No Long-Term Effectiveness for Weight Loss</td>
<td>39</td>
</tr>
</tbody>
</table>
Weight Loss Caused by Low-Carbohydrate Diets Led to Improved Glucose Homeostasis ................................................................................................................ 102

IGF-1 Levels was Only Decreased in LC5 Groups .................................................... 103

Adiponectin Production Changed With LC5 and SC Diets ................................. 104

Lipid Profile Improved With Low-Carbohydrate Diets in Mice ......................... 106

Comparing to Other Published Studies in Mice ................................................. 109

Future Studies ..................................................................................................... 113

Conclusions ........................................................................................................ 114

References ......................................................................................................... 118
LIST OF TABLES

Table 1: Summary of Popular Diet Plans for Weight Loss and Comparison With Acceptable Macronutrient Distribution Ranges (AMDR) .......................................................... 27

Table 2: Summary of Low-Larbohydrate Diet Studies of Obese Humans ......................... 35

Table 3: Summary of Low-Carbohydrate Diet Studies in Rodent Models ....................... 53

Table 4: The Energy Proportion and Macronutrient Composition of Different Diets ..... 60

Table 5: Weights (Grams) of Fat Mass in Four Depots of Mice in SC-SC, HF-HF, HF-LC5, HF-LC10, HF-LC15, HF-SC Dietary Groups at the Conclusion of the Study .......... 82

Table 6: Organ Weights (Grams) of Mice in SC-SC, HF-HF, HF-LC5, HF-LC10, HF-LC15, HF-SC Dietary Groups at the Conclusion of the Study .............................................. 83

Table 7: Metabolic Parameters of Mice in SC-SC, HF-HF, HF-LC5, HF-LC10, HF-LC15, HF-SC Dietary Strategy Groups at the Conclusion of the Study ......................... 92

Table 8: Comparison of Present Study to a Similar Study ............................................. 112
LIST OF FIGURES

Figure 1: Timeline, and summary of the low-carbohydrate diets study………………………….62

Figure 2: Body weight (grams) of mice in SC-SC, HF-HF, HF-LC5, HF-LC10, HF-LC15, HF-SC dietary groups at the end of phase 1 of the study……………………………….68

Figure 3: Weight (grams) of mice in SC-SC, HF-HF, HF-LC5, HF-LC10, HF-LC15, HF-SC dietary groups at the conclusion of the study.……………………………………..69

Figure 4: Weight (grams) of mice in SC-SC, HF-HF, HF-LC5, HF-LC10, HF-LC15, HF-SC dietary groups during phase 2 of the study………………………………………..70

Figure 5: Body fat (grams) of mice in SC-SC, HF-HF, HF-LC5, HF-LC10, HF-LC15, HF-SC dietary groups during phase 2 of the study………………………………………73

Figure 6: Body lean mass (grams) of mice in SC-SC, HF-HF, HF-LC5, HF-LC10, HF-LC15, HF-SC dietary groups during phase 2 of the study……………………………………75

Figure 7: Body fluid (grams) measurements of mice in SC-SC, HF-HF, HF-LC5, HF-LC10, HF-LC15, HF-SC dietary strategy groups during phase 2 of the study………………77

Figure 8: Comparing fat, lean, and fluid accumulation to body weight accumulation in C-SC, HF-HF, HF-LC5, HF-LC10, HF-LC15, HF-SC dietary groups at the conclusion of the study.………………………………………………………………………………….79

Figure 9: Total energy consumption (kcal/animal) of mice in SC-SC, HF-HF, HF-LC5, HF-LC10, HF-LC15, HF-SC dietary groups in the last 4 weeks during phase 2 of the study……………………………………………………………………………………………………86
CHAPTER 1: INTRODUCTION

The incidence of obesity has risen substantially worldwide and reached epidemic proportions in most affluent societies. In the United States, 66% of adults are either overweight (BMI of 25-29.9 kg/m²) or obese (BMI of 30 kg/m² and above; C. L. Ogden, Yanovski, Carroll, & Flegal, 2007). Youth are not immune to this trend. Among children and teens, the prevalence of overweight increased to 16% with nearly 9 million American youth ages 6 to 19 being overweight (J. Ogden & Flanagan, 2008).

Obesity is associated with a number of health problems. The risk of heart attack, congestive heart failure, sudden cardiac death, and angina or chest pain is increased in persons who are overweight or obese. High blood pressure is more common in adults who are obese than in those who are at a healthy weight. Atherosclerosis, or narrowing of the arteries, which is an important precondition of many strokes, can be accelerated or worsened by obesity, especially morbid obesity. Moreover, one of the most common risk factors for insulin resistance is obesity, especially central abdominal obesity. Higher body weights are also associated with increases in all-cause mortality (Ho et al., 2008).

Overweight and obesity and their associated health problems have a significant economic impact on the U.S. health care system. According to a study of national costs attributed to both overweight (BMI 25–29.9) and obesity (BMI greater than 30), medical expenses accounted for 9.1 percent of total U.S. medical expenditures in 1998 and may have reached as high as $78.5 billion. Approximately half of these costs were paid by Medicaid and Medicare (Finkelstein, Fiebelkorn, & Wang, 2003).
Weight loss is an important concern for many individuals, including the obese. Research suggests that changes in diet and physical activity are important to long-term success of weight loss (National Institutes of Health, National Heart, Lung, and Blood Institutes, Obesity Education Initiative, 1998; Pronk & Wing, 1994). Both the US Department of Agriculture’s Dietary Guidelines and the National Heart, Lung, and Blood Institute’s Clinical Guidelines recommend decreasing calorie intake and increasing physical activity at least 30 minutes or more on all or most days of the week (Dietary Guidelines Advisory Committee, 1995; National Institutes of Health, National Heart, Lung, and Blood Institutes, Obesity Education Initiative, 1998). Although people spend an additional $33 billion in weight control services and products per year trying to lose body weight or prevent weight gain in the United States (Cleland, et al., 2001), fewer than 25% of obese people who attempt to lose weight actually reduce caloric intake and increase exercise as currently recommended (Serdula et al., 1999). For those that do attempt to lose weight, the preferred means of achieving the goal is typically by solely modifying diet (Bish et al., 2005).

A number of diet plans have been developed outside the medical and nutritional mainstreams that are marketed directly to the public as weight loss strategies. Low-carbohydrate, high-fat diets have been introduced as a revolutionary weight-loss program because many studies show the short-term benefits of this diet on weight loss and blood lipids (Brehm, Seeley, Daniels, & D’Alessio, 2003; Meckling, O’Sullivan, & Saari, 2004; Segal-Isaacson, Johnson, Tomuta, Cowell, & Stein, 2004). The Atkins diet is the most
well known of the low-carbohydrate, high-fat dietary regimens. The Atkins diet books have been popular for weight loss for over 40 years. However, several studies indicate that an Atkins style diet has no long-term effects on weight loss when subjects were under treatment over 12 months (Foster, et al., 2003), and subjects sometimes suffered serious side effects, such as halitosis, muscle cramps, and constipation (Yancy, Olsen, Guyton, Bakst, & Westman, 2004). More severe restriction of carbohydrates has been utilized, but not really evaluated for their impact on weight loss. Some more severe, ketogenic diets were originally used in medicine over 80 years as an anti-convulsant for controlling seizures (Wilder, 1921) and have also been shown to cause body weight loss (Kossoff, Rowley, Sinha, & Vining, 2008).

Regardless of the form of carbohydrate restriction, elevated blood ketone levels are thought to contribute to the weight loss. Ketone bodies in the form of acetoacetic acid, 3- hydroxybutyric acid, or acetone can be oxidized as a fuel to meet the body’s energy needs when cells have insufficient energy or carbohydrates. The low-carbohydrate diets that increase ketone levels have been implicated to promote weight loss in clinical trials (Brehm et al., 2003; Meckling, Gauthier, Grubb, & Sanford, 2002). Furthermore, the elevated ketone levels caused by a low-carbohydrate diet have been shown to improve lipid profiles, reduce blood glucose and serum insulin levels, which collectively could reduce the risk of cardiovascular diseases and diabetes (Meckling et al., 2004; Willi, Oexmann, Wright, Collop, & Key, 1998).
Conducting long-term studies establishing the efficacy and safety of these diet plans is somewhat problematic in human populations. That is, some of these diets likely have serious long-term side effects, compliance would be very challenging, and cost would be prohibitive. In addition, some measurements and testing would not be possible because of their invasiveness or risk. Therefore, establishing an animal model to study the benefits and risks of low-carbohydrate diets is essential. In mice, the standard Atkins type diet is not effective in promoting weight loss possibly due to a higher percentage of protein in this diet increases gluconeogenesis and inhibits the ketogenesis (Mobbs, et al., 2007). Likewise, according to a previous study in our lab, low-carbohydrate diets that are consisted with the nutrient composition of the human Atkins diet failed to promote weight loss in obese mice. Furthermore, this low-carbohydrate diet resulted in weight gain in lean mice (List, et al., 2005). However, some studies have found that lower carbohydrate and protein levels than typically used for low-carbohydrate diets in humans appear to be very effective in promoting weight loss in mice (Mobbs, et al., 2007). In fact, low-carbohydrate diets that promote ketogenesis in mice were shown to promote weight loss by increasing the expression of genes involved in lipid catabolism and decreasing the expression of genes involved in lipid synthesis (Kennedy, et al., 2007).

Despite several studies reporting weight loss in mice using highly restrictive low-carbohydrate diets, no study has been published that attempted to optimize the levels of macronutrients, as will be done in this study. Although low-carbohydrate diets with a very low percentage of protein were reported to be effective at promoting weight loss and
ketosis in mice (Kennedy, et al., 2007), it is important to determine how restrictive protein levels need to be in order to elicit ketogenesis and weight loss, as one would assume that severe protein restriction may also induce protein malnutrition.

Statement of the Problem

The incidence of obesity in the United States has escalated along with its physiological and psychological comorbidities. The need for effective weight loss methods has stimulated the promotion of numerous alternative diet plans. One approach to weight loss that has gained recognition in the face of modest supportive scientific evidence is the low-carbohydrate diet. Recent studies have shown that the more restrictive low-carbohydrate, low-protein diets can promote weight loss and ketogenesis (Kennedy, et al., 2007). Likewise, studies in humans also point to the need for ketogenesis for low-carbohydrate diets to be effective for weight loss (Brehm et al., 2003; Meckling et al., 2002). However, studies have not compared directly various low-carbohydrate, low-protein diets in mice as to their effectiveness for weight loss. It is also unclear how the low-carbohydrate diets would affect the biochemical parameters that are associated with obesity and the organs in the body. Obese animal models are excellent for studying these effects because of the possible health risks and logistical problems involved with administering various low-carbohydrate diets in clinical studies. Also, research with animal models allows for the collection of many different parameters that would be difficult if not impossible to obtain using humans. Compared to human studies,
animal models also offer the researcher the ability to control factors pertaining to their external and internal environments such as diet, temperature and humidity, light/dark cycles, time of procedures and genetic similarity. The C57BL/6J mouse strain has evolved as a useful model for obesity as these mice develop obesity when fed a high-fat diet, and remain lean on a low-fat diet. Furthermore, they display the same distribution and deposition of fat as seen in humans with high-fat feeding (Rebuffe-Scrive, Surwit, Feinglos, Kuhn, & Rodin, 1993; Surwit, Kuhn, Cochrane, McCubbin, & Feinglos, 1988). Thus, this mouse strain is valuable when studying parameters associated with obesity and diabetes and will be used to optimize the low-carbohydrate diet and evaluate the metabolic consequences of this diet.

**Research Questions**

1. Which low-carbohydrate diet causes the most significant change in body weight, fat mass, and lean mass in male, obese C57BL/6J mice?

2. Do any of the low-carbohydrate diets cause any difference in total energy consumption as compared to control mice fed high-fat or low-fat diets?

3. Do the low-carbohydrate diets cause any difference in the urinary ketone bodies, blood ketone bodies, blood glucose, insulin, free fatty acids, triglycerides, total cholesterol, HDL, LDL, IGF-1, and adiponectin among male, obese C57BL/6J mice as compared to mice fed control (standard chow and high fat) diets?
4. How do the various low-carbohydrate diets affect tissue/organ weights compared to control mice fed standard chow or high-fat diets?

**Purpose of the Study**

Recently, many studies have proved that low-carbohydrate, high-fat diets had significant effects on weight loss and improvements on several physiological parameters in human subjects. However, the effectiveness and safety of this diet still remain unclear and controversial. More restrictive low-carbohydrate, low-protein diets have been popular for clinical seizure control for many years. Therefore, the hypothesis for this study is low-carbohydrate, low-protein diets would cause significant weight loss in mice without impairing health of mice, and the most restrictive protein percentage would cause the most body weight loss.

The purpose of this study is to investigate the effectiveness of various low-carbohydrate diets, which differ in protein and fat composition, on weight loss, and to evaluate the safety and effectiveness of this dietary manipulation by measuring several physiological parameters. There are a few studies using mice that assess whole body composition changes with the low-carbohydrate diet treatment, and no study has attempted yet to optimize protein percentage in diets to compare promoting ketosis in mice. Based on a previous study in our lab, standard low-carbohydrate diets with high protein cannot promote weight loss in mice. In this study, we will use low-carbohydrate diets with differing protein percentages to treat obese mice, evaluate the effectiveness and
safety of these low-carbohydrate diets, and compare their effects on promoting body
weight loss in mice. In this study, not only will whole body composition be measured to
assess the obese status, but also physiological parameters, such as blood glucose, insulin,
lipid profile, IGF-1, and adiponectin levels, will be measured to assess the impact of the
dietary manipulation. Tissue collection at the conclusion of the study will also allow for
further investigations related to tissue function. The low-carbohydrate diet may serve as
an aid to weight loss. However, because of the high percentage of fat in this diet, it is still
unclear as to whether this type of treatment would be beneficial or harmful to manage
obesity. This research will provide valuable insight for possible outcomes of this type of
treatment on obese human subjects.

Limitations/Delimitations

1. This study uses mice and may not be fully generalized to the human population.
2. Mice in this study were bred to be genetically similar; however, some variations
   still exist.
3. Multiple animals were housed in one cage possibly leading to hierarchical
   changes that may affect measurements taken.
4. Stress levels of the animals during the study may fluctuate due to experimental
   procedures. This may cause variations in results unrelated to the treatment.
Definition of Terms

*Adiponectin*: A protein hormone produced and secreted mainly by adipocytes that regulates the metabolism of lipids and glucose. Adiponectin also influences the body’s response to insulin and has anti-inflammatory effects on the cells lining the walls of blood vessels. High blood levels of adiponectin are associated with a reduced risk of heart attack. Low levels of adiponectin are found in people who are obese (Guerre-Millo, 2008).

*Blood ketones*: Ketone bodies are present in the blood. Ketones are chemicals with a carbonyl unit that has 2 alkyl or aromatic substituents bonded to the carbon atom. They include acetoacetic acid, 3-hydroxybutyric acid, and acetone. Higher blood ketones levels are caused when the body metabolizes body fat or specific amino acids for energy purposes due to starvation or high-fat diets, instead of the usual glucose-from-carbohydrates.

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

*Insulin*: A hormone made by the pancreas that controls the level of the glucose in the blood. Insulin permits cell to use glucose for energy.

*Low carbohydrate diet*: A low-carbohydrate diet is a low-carbohydrate, high-fat diet that could promote ketosis in the body, which was originally used in medicine as an
anti-convulsant for controlling seizures. Recently, people begin to use it as a diet plan for weight loss. In the present study, low-carbohydrate diets consisted of low-carbohydrate, high fat, and restrictive protein.

*NADH*: A principal electron donor for the respiratory chain in mammalian cells. NADH acts as electron carriers which receive electrons from the oxidation of fuel molecules (glucose, ketone bodies) and transport them to the mitochondrial electron-transport chain (ETC) for ATP-synthesis.

*Obesity*: Obesity is a condition in which excess body fat has accumulated to such an extent that health may be negatively affected. It is commonly defined as Body Mass Index (BMI, weight divided by height squared) of 30 kg/m² or higher. Obesity is associated with various diseases, particularly cardiovascular diseases, diabetes mellitus type 2.
CHAPTER 2: REVIEW OF LITERATURE

The high rates of overweight and obese individuals present many social and economic problems. With the number of obese adults approaching epidemic proportions, research has focused on ways to treat and prevent obesity. Low-carbohydrate, high-fat diets have been used for body weight loss for many years. However, their impact on long-term weight loss, and the potentially harmful consequences that may accompany this type of diet remain controversial. This review will include an overview of obesity, dietary strategies to combat obesity, and the effect of low-carbohydrate, high-fat diets on reversing obesity in both humans and rodent models.

**Obesity**

Obesity is defined as having excess body fat. Usually, obesity or overweight status for an adult is assessed by measuring body mass index (BMI), which takes into account body weight and height. A BMI between 25 kg/m² and 29.9 kg/m² is considered overweight and greater than 30 kg/m² is considered obese (Centers for Disease Control and Prevention, 2006). The individual’s waist circumference and waist to hip ratio are also indicators for assessing body weight status. If a waist circumference is greater than 35 inches in women or 40 inches in men, or the waist to hip ratio is greater than 1, they would be at increased risk of health problems related to overweight or obesity (National Heart, Lung, and Blood Institute, 2000). In addition to these means of assessing weight and risk status, there are a number of methods to assess body composition that specifically address the proportion of lean versus fat tissue. While a better indicator of
health status, body composition tend to be more cumbersome and limited to research studies.

Many health problems have been identified as comorbidities with obesity. The incidence of type 2 diabetes, cardiovascular disease, hypertension, dyslipidemia, metabolic syndrome, gallbladder disease, osteoarthritis, sleep apnea and certain cancers have been shown to increase with advancing obesity (Wyatt, Winters, & Dubbert, 2006). Understandably, there is a significant association between extreme obesity and early death (Bender, Trautner, Spraul, & Berger, 1998).

Obesity is a leading public health concern in the United States today. Obesity among adults age 20-74 years has increased from 15% in 1976-1980 to 32.9% in 2003-2004 (Centers for Disease Control and Prevention, 2006). Equally, alarming are the growing rates of childhood obesity. In the United States, the CDC estimates that in the same 20 year time frame (1976-1980 through 2003-2004) the number of overweight children increased from 5.0% to 13.9% in ages 2-5, 6.5% to 18.8% in ages 6-11, and 5.0% to 17.4% in ages 12-19 (Centers for Disease Control and Prevention, 2006). Moreover, the WHO estimates that of people age 15 and older, approximately 1.6 billion are overweight and 400 million are obese globally (World Health Organization, 2006b). By the year 2015, the WHO estimates that 2.3 billion adults will be overweight and 700 million will be obese (World Health Organization, 2006b). These alarming statistics indicate a growing public health concern of epidemic proportions.
The high incidence of obesity has an economic impact. It is estimated that in the United States, the annual medical expenses for the treatment of overweight, obesity and associated diseases is between $51.5-$78.5 billion (Finkelstein et al., 2003). Due to the prevalence of obesity, its related complications and the economic strain it causes, there is an urgent need for research in obesity prevention and treatment.

**The Diet Plans for Obesity**

As the prevalence of obesity increases, the search for an effective weight loss strategy becomes more critical. Although long-term weight loss is difficult to achieve, research suggests that changes in both diet and physical activity are important to long-term success (King & Tribble, 1991; Pronk & Wing, 1994). In fact, both the United States Department of Agriculture’s Dietary Guidelines and the National Heart, Lung, and Blood Institute’s Clinical Guidelines recommend decreasing calorie intake and increasing physical activity for losing and maintaining weight loss (Dietary Guidelines Advisory Committee, 1995; National Institutes of Health, National Heart, Lung, and Blood Institutes, Obesity Education Initiative, 1998).

Statistics related to weight loss practices in the US suggest that the recommended changes in both calorie intake and physical activity are rarely followed. The reported prevalence of individuals in the US trying to lose weight was 33% for men and 46% for women in 2000 (Bish et al., 2005). Women, even those with lower BMI, are more likely to try to lose weight than men within every sociodemographic and weight category.
Interestingly, among the people who were trying to lose weight, only 19% of women and 22% of men reported using the combination of decreased calorie intake and increased physical activity. On the other hand, the majority of individuals (56% of women and 53% of men) reported modifying diet alone for weight loss (Bish et al., 2005). In addition to modifying diet alone, consumers place their hope on various products for weight loss. It is estimated that Americans spend $33 billion annually for weight loss products and services, and this spending is growing at a rate of 6 to 7 percent a year (Cleland, et al., 2001). Because of the considerable desire on the part of obese individuals to lose weight and because few of them like to increase their physical activity as well as decrease their dietary intake, a number of diet plans have been developed outside the medical and nutritional mainstreams that are marketed directly to the public as weight loss strategies.

*Low-Fat, High-Carbohydrate, Calorie-Restricted Diet Plan*

According to obesity management guidelines of the National Heart, Lung, and Blood Institute (National Institutes of Health, National Heart, Lung, and Blood Institutes, Obesity Education Initiative, 1998), and guidelines from American Heart Association (Krauss et al., 2000), the recommended diet for weight loss is a high-carbohydrate, low-fat, calorie restricted diet. With this dietary strategy, total calorie is restricted sufficiently to create a deficit of 500 calories per day, with 55-60% of total calories from carbohydrate, 30% or less of total calories derived from fat, 10% of calories from saturated fat, and 300 mg of cholesterol intake per day. Furthermore, low-fat grains, vegetables, fruits, and legumes intakes are encouraged and additional fats, sweets, and
high-fat snacks consumption are limited. Meanwhile, increasing exercise and behavior modification strategies are still considered indispensable for weight loss.

Besides the diets recommended by most health organizations, there are other diet plans that are popular for weight management. These diet plans vary according to diet composition, macronutrient makeup, and food choices. Several of these include the Zone diet (lower in carbohydrate; Sears & Lawren, 1995), the Ornish diet (very low in fat; Ornish, 2001), and the Atkins diet (very low in carbohydrate; Atkins, 2002). Books promoting these diet plans have been listed on the New York Times Bestseller during the past 5 years because of their tremendous popularity. Moreover, several trials that had compared these diet plans for weight loss were published (Dansinger, Gleason, Griffith, Selker, & Schaefer, 2005; Gardner, et al., 2007). A summary and comparison of these popular diet plans is presented on Table 1.
### Table 1

**Summary of Popular Diet Plans for Weight Loss and Comparison With Acceptable Macronutrient Distribution Ranges (AMDR)**

<table>
<thead>
<tr>
<th>Diet Plan</th>
<th>Macronutrient</th>
<th>Recommendation for Nutrients Intake</th>
<th>Amount of Energy Consumed</th>
<th>Anticipated Goal</th>
<th>Actual Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zone Diet (Sears &amp; Lawren, 1995)</td>
<td>40% carbohydrate, 30% protein, 30% fat.</td>
<td>The ratio of protein intake to carbohydrate intake must conform to 0.75. Limiting grain and starch intake.</td>
<td>Energy intake is restricted according to the BMI and body fat percentage in subjects.</td>
<td>Weight loss and benefitting overall health.</td>
<td>Weight loss sometimes due to energy intake restriction. No significant effectiveness on health (Cheuvront, 2003; Linn et al., 2000).</td>
</tr>
<tr>
<td>Ornish Diet (Ornish, 2001)</td>
<td>70% carbohydrates, 20% protein, 10% fat.</td>
<td>Increasing legumes and high-fiber carbohydrates intake. Limiting cholesterol and saturated fat intake. Limited Animal Products (only egg whites and nonfat dairy products acceptable).</td>
<td>Energy intake is not restricted.</td>
<td>Weight loss and preventing heart disease.</td>
<td>No more significant improvement in body weight loss and lipid profile than low-carbohydrate diet (Gardner et al., 2007).</td>
</tr>
<tr>
<td>Atkins Diet (Atkins, 2002)</td>
<td>5-8% carbohydrate, 27-35% protein, 60-65% fat.</td>
<td>Increasing fat intake and decreasing carbohydrate intake.</td>
<td>Energy intake is not restricted.</td>
<td>Weight loss and improvement in lipid profile.</td>
<td>Weight loss and lipid profile improvement in short term (Meckling, Gauthier, Grubb, &amp; Sanford, 2002; Meckling et al., 2004).</td>
</tr>
<tr>
<td>AMDR (Barr, 2006)</td>
<td>45-65% carbohydrate, 10-35% protein, 20-35% fat.</td>
<td>Saturated + trans fats ≤10%, n-6 PUFA 5-10%, n-3 PUFA 0.6-1.2%, Necessary vitamin and minerals intake</td>
<td>Related to age, height, weight, and intensity of physical activity.</td>
<td>Chronic disease prevention, such as obesity, diabetes, cardiovascular disease.</td>
<td>Can be used for prevention of chronic diseases and keep body in health.</td>
</tr>
</tbody>
</table>

AMDR: Acceptable Macronutrient Distribution Ranges
Zone Diet Plan

The Zone Diet is a diet defined as a 40% carbohydrate, 30% protein and 30% fat that specifically advocates sparing use of grains and starchy carbohydrates (Sears & Lawren, 1995). Dr. Sears believes that dietary intake make people obese not due to fat consumption alone, but eating too many fat-free carbohydrates or too many calories. Overeating of fat-free carbohydrates and calories will increase plasma insulin levels greatly, which is the key factor promoting physiological anabolic processes in the human body. What is the zone? The zone is the balance of hormonal responses that occurs every time you eat. The zone tackles what Dr. Sears believes is the major cause of the spiraling obesity plague: excess insulin levels. The zone plan is a dietary strategy to keep the hormone insulin in a zone that is not too high and not too low, and the zone is a metabolic state that the body works at a peak efficiency (Sears & Lawren, 1995).

There are several fundamental principles in the Zone Diet plan. First, it is very important for people who follow this diet plan to eat the correct combination of low-fat protein (such as lean chicken or pork), the right type of carbohydrate such as fruits and/or vegetables (starchy carbohydrates such as breads or potatoes are not as good), and a dash of “good” fat (such as olive oil). Secondly, people should not let more than 5 hours go by without a Zone meal, even if they are not hungry. This keeps their blood sugar levels stable. Moreover, drinking a minimum of 8 glasses of water every day is very important as well. According to Zone Diet doctrine, in every meal, the ratio of protein intake to carbohydrate intake (mainly derived from vegetable and fruits, not grains and starchy carbohydrates)
must conform to 0.75 in order to control insulin levels and realize the purported health benefits, while fat intake makes up the remaining 30% of total energy. The Zone Diet claims that the ratio 0.75 of protein to carbohydrate would reduce the insulin to glucagon ratio, which inhibits the dietary fat store and promotes excess body fat to be “burned” leading to weight loss (Sears & Lawren, 1995). Moreover, supplementing the diet with omega-3 fatty acids, such as using olive oil to round off your meal, is recommended in the Zone Diet in order to help the body produce “good” eicosanoids. While eicosanoids are extremely powerful biological agents, the Zone diet claims to promote the specific production of “good” eicosanoids leading to some of the diet’s purported health benefits (Sears & Lawren, 1995). In the Zone, no foods are completely off limits. That means people can eat anything, as long as it has the right balance of protein, carbohydrates, and fat. Wine is even allowable because of the substantial amount of antioxidants it contains.

However, little evidence shows the ratio 0.75 of protein to carbohydrate in the Zone Diet has a significant effect on promoting fat burning or weight loss when compared to a conventional diet with a ratio 0.24 of protein to carbohydrate (Linn, et al., 2000). With regard to body weight control, any weight loss experienced by adherence to the Zone Diet prescription is easily explained by the severe energy restriction of the diet rather than enhanced fat metabolism resulting from manipulations in the ratio of dietary protein and carbohydrate (Cheuvront, 2003). Moreover, the American Heart Association does not recommend the Zone Diet due to high protein, lack of essential nutrients, and little information on long-term effects (American Heart Association, 2007).
Ornish Diet Plan

The Ornish diet, which is 10% fat, 20% protein, and 70% carbohydrate, is developed with the goal of reversing and preventing heart disease. This diet was developed by Dr. Dean Ornish M.D., which emphasizes foods that are very low in fat yet filling, including high-fiber grains and legumes (beans and peas). Actually, the Ornish plan is vegetarian diet, which eliminates all kinds of meat. The source of carbohydrate consumption is multiple, such as vegetables, fruits, grains and beans. It allows non-fat dairy foods and processed or refined foods in moderation. Cholesterol and saturated fat are strictly limited, and all animal products (except egg whites and nonfat dairy products) are excluded. Energy is not restricted, but the participant is directed to confine dietary intake to the recommended foods (Ornish, 2001). Legumes and high fiber carbohydrates are the foundation of the plan. The following can be eaten at any time by people who are sticking to the Ornish diet: beans and legumes, fruits, grains, and vegetables. All kinds of meat, oils and oil-containing products, avocados, olives, dairy products, sugar and simple sugar derivatives should be avoided. A controversial part of the Ornish diet is its prohibition of nuts and fish.

Dr. Ornish presents two diets: the Reversal Diet and the Prevention Diet. The Reversal Diet is for people with known heart disease who want to reverse its effects and lower their heart attack risk. The Prevention Diet is recommended for people who do not have heart disease, but whose cholesterol levels are less than ideal. Both these diets are vegetarian diets. The diet supplies only 10% of calories from fat. It excludes cholesterol
and saturated fat, including all animal products (except egg whites and nonfat dairy products), nuts, seeds, avocados, chocolate, olives, and coconuts. Oils are eliminated except a small amount of canola oil for cooking, and oil that supplies omega-3 essential fatty acids. The Ornish diet also prohibits caffeine, but allows a moderate intake of alcohol, sugar and salt. There is no restriction on the calorie intake so long as the diet is confined within the recommended food.

The effectiveness of the Ornish diet remains controversial. The Ornish diet has been shown to result in higher body weight loss and to reduce the ratio of low density lipoprotein to high density lipoprotein in a 12-month trial (Dansinger et al., 2005). However, in the same trial dietary adherence was lower on the Ornish diet plan. In another 12-month trial, the body weight loss effect of the Ornish Diet was less than that of low-carbohydrate diet plan and the improvement in metabolic effects related to the risk of cardiovascular disease was no better than the low-carbohydrate diet plan (Gardner, et al., 2007). Thus, the utility of such an extreme diet may be difficult to follow and may not be necessary to elicit weight loss and the associated metabolic improvements.

**Low-Carbohydrate, High-Fat Diet Plan**

Low-carbohydrate diets have been popular since 1860s, when William Banting claimed he lost 21 kg without feeling hunger (Banting, 1869). The impact of this dietary manipulation is the central focus of this thesis and will be more fully discussed in the subsequent section.
As a well-known low-carbohydrate, high-fat diet, the "Atkins Nutritional Approach" has re-emerged as a revolutionary weight-loss program in the last several decades. The Atkins diet books have sold more than 45 million copies over 40 years, and this diet and accompanying Atkins food products have become increasingly popular as the obesity rate escalates. Atkins diet is a very low-carbohydrate, high-fat diet, which consists of 5-8% carbohydrate, 27-35% protein, and 60-65% fat (Atkins, 2002). The diet claims to be effective at producing weight loss despite *ad libitum* consumption of fatty meat, butter, and other high-fat dairy products, restricting only the intake of carbohydrates to under 30g a day (Atkins, 2002). Based on the difference in the ratio of fat, carbohydrate and protein in this diet, other forms of low-carbohydrate diets also exist, such as those that are even more restrictive in carbohydrate and protein that elicit production of higher levels of blood ketones.

**Clinical Trials Testing Low-Carbohydrate Diets**

Numerous studies in recent years have studied low-carbohydrate, high-fat diets, such as the Atkins diet. A summary of these studies is provided in table 2. Comparing the results of these studies is challenging. That is, different dietary regimes were followed in most. For example, the severity of the carbohydrate restriction has been as low as 20 grams (Sondike, Copperman, & Jacobson, 2003) or as high as 50-100g/day (Meckling et al., 2004). Second, the foods utilized to achieve low-carbohydrate consumption varied, altering levels of other nutrients. For example, 20-30g carbohydrate intake can be comprised of green vegetables and salad (Dashti, et al., 2003), while in other trials, the
carbohydrate can consist of low sugar fruits, berries, and vegetables (Meckling et al., 2002). Moreover, the protein and fat sources in these two trials were also not the same. One was fowl, eggs, shellfish, and the other was lean meats, cheese, sugar free or low sugar desserts. Thus, there are major differences in nutrient intake beyond the carbohydrate restriction. Third, the maintenance of the low-carbohydrate, high-fat diet interventions also differed. That is, subjects were often asked to maintain a low-carbohydrate level over the whole study or were sometimes instructed to modify the restriction over time. For example, in one study, participants were asked to eat less than 20g/day carbohydrate for 2 weeks and then increase to 40-60g/day for the rest period of the trial (Brehm et al., 2003). While in another trial, the participants were asked to reduce and maintain their carbohydrate intake from 100g/day to 50g/day for the entire study period (Meckling et al., 2004).

Besides dietary differences, there other important differences in these trials, making interpretation of results difficult. Different inclusion and exclusion characteristics of subjects were used in these studies. For example, in different trials, the ages and genders of subjects are not the same, and the degree of obesity is distinct. Furthermore, some trials have diabetic patients (Stern, et al., 2004), while other trials have hyperlipidemic patients (Yancy et al., 2004). The different time frames of the studies also make interpretation difficult. For example, some trials were relatively short term, like those only lasting 8 weeks (Meckling et al., 2002) while others were more long term continuing for years (Shai, et al., 2008). Finally, not all studies analyzed similar clinical
parameters that might be important. For example, ketone levels appear to be important predictors of diet adherence and effectiveness but many studies did not report or assess ketone levels.

Some studies have demonstrated impressive evidence as to the effectiveness of the low-carbohydrate diet. For example, 311 free living, overweight/obese nondiabetic, premenopausal women were assigned to Atkins, Zone, and Ornish diet for 12 months, providing the unique opportunity to directly compare these diet plans. Interestingly, the women in the Atkins diet group had the most significant body weight loss, and experienced more favorable overall metabolic effects at 12 months (Gardner, et al., 2007). Other studies had confirmed that Atkins diets are also effective for weight loss in different ages and for improving some of the comorbidities that accompany obesity. For example, in 2003, 30 youth from 12 to 18 years of age were divided into 2 groups. The experimental group was instructed to consume less than 20g of carbohydrate per day for 2 weeks, then less than 40g/day for 10 weeks. The control group was instructed to consume less than 30% of energy from fat for 12 weeks. After a period of 12 weeks of treatment, the experimental group lost more weight than the control group and had improvement in non-HDL cholesterol and triglyceride levels (Sondike et al., 2003). Overall, a significant effect of the low-carbohydrate, high-fat diet on body weight loss in humans can be found in many studies as summarized in table 2.
### Table 2

**Summary of Low-Carbohydrate Diet Studies of Obese Humans (Table 2 continued on page 36, 37, 38)**

<table>
<thead>
<tr>
<th>Reference</th>
<th>Subjects (age and health status)</th>
<th>Length of Study</th>
<th>Diets</th>
<th>Presence of measurable ketones</th>
<th>Body Weight</th>
<th>Fat Mass</th>
<th>Lean Mass</th>
<th>Fasting Insulin</th>
<th>Fasting Glucose</th>
<th>IGF-1</th>
<th>Leptin</th>
<th>Adiponectin</th>
<th>Total Cholesterol</th>
<th>Triglyceride</th>
<th>Free Fatty Acids</th>
<th>Low density lipoprotein</th>
<th>High density lipoprotein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Willi, Oexmann, &amp; Wright, 1998</td>
<td>6 people, 12-15 YO, Morbidly obese</td>
<td>8 weeks</td>
<td>High-protein, low-carbohydrate, low-fat ketogenic diet 645-725 calories (80-100g protein, 13oz lean meat, 25g carbohydrate, 3 cups of low-calorie vegetables)</td>
<td>Yes</td>
<td>↓</td>
<td>NR</td>
<td>NS</td>
<td>NR</td>
<td>NR</td>
<td>↑</td>
<td>Correlated with weight and fat content</td>
<td>NR</td>
<td>↓</td>
<td>NR</td>
<td>NR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meckling, Gauthier, &amp; Grubb, 2002</td>
<td>20 women, 20-52 YO, Obese</td>
<td>8 weeks</td>
<td>Hypocaloric, low-carbohydrate diet (lean meats, low-sugar fruits and berries, vegetables, cheese, sugar free or low sugar desserts)</td>
<td>Yes</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NS</td>
</tr>
<tr>
<td>Sondike, et al., 2003</td>
<td>16 people, Mean age 14.4 YO, Obese</td>
<td>12 weeks</td>
<td>Low carbohydrate, high fat diet, 20g/d carbohydrate for first 2 weeks, then increased to 40g/d.</td>
<td>Yes</td>
<td>↓</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Brehm, et al., 2003</td>
<td>22 women mean age 44.22 YO, obese</td>
<td>6 months</td>
<td>Low carbohydrate, high-fat diet, 20g/d carbohydrate for first 2 weeks, then increased to 40-60g/d</td>
<td>Yes</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>NR</td>
<td>↓ at 3 months</td>
<td>NR</td>
<td>↓ at 3 months</td>
<td>NR</td>
<td>↓ at 3 months</td>
<td>at 6 months</td>
<td></td>
</tr>
<tr>
<td>Reference</td>
<td>Subject (age and health status)</td>
<td>Length of Study</td>
<td>Diets</td>
<td>Presence of measurable ketones</td>
<td>Body Weight</td>
<td>Fat Mass</td>
<td>Lean Mass</td>
<td>Fasting Insulin</td>
<td>Fasting Glucose</td>
<td>IGF-1</td>
<td>Leptin</td>
<td>Adiponectin</td>
<td>Total Cholesterol</td>
<td>Triglyceride</td>
<td>Free Fatty Acids</td>
<td>Low density lipoprotein</td>
<td>High density lipoprotein</td>
</tr>
<tr>
<td>----------------------------</td>
<td>---------------------------------</td>
<td>----------------</td>
<td>----------------------------------------------------------------------</td>
<td>--------------------------------</td>
<td>------------</td>
<td>----------</td>
<td>-----------</td>
<td>-----------------</td>
<td>-----------------</td>
<td>-------</td>
<td>--------</td>
<td>-------------</td>
<td>-------------------</td>
<td>---------------</td>
<td>----------------</td>
<td>------------------------</td>
<td>------------------------</td>
</tr>
<tr>
<td>Foster, et al., 2003</td>
<td>33 people mean age 44 YO, obese</td>
<td>1 year</td>
<td>Low carbohydrate, high fat, high protein, 20g/d carbohydrate for the first 2 weeks.</td>
<td>Present at first 3 months</td>
<td>↓ at 6 months, NS at 12 months</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Dashti, et al., 2003</td>
<td>102 people, obese, mean age 40.8 YO</td>
<td>12 weeks</td>
<td>Low carbohydrate, high fat, 20-30g carbohydrate intake in green vegetables and salad, 80-100 g of proteins, in meat, fish, fowl, eggs shellfish.</td>
<td>NR</td>
<td>↓</td>
<td>NR</td>
<td>NR</td>
<td>↓</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>↓</td>
<td>↓</td>
<td>NR</td>
<td>↓</td>
<td>↑</td>
<td></td>
</tr>
<tr>
<td>Samaha, et al., 2003</td>
<td>64 people, obese, 26 with diabetes, Age: 53±9 YO.</td>
<td>6 months</td>
<td>Low carbohydrate, high-fat diet, 30g or less carbohydrate diet</td>
<td>NR</td>
<td>↓</td>
<td>NR</td>
<td>NR</td>
<td>↓</td>
<td>in diabetic subjects</td>
<td>NR</td>
<td>↓</td>
<td>NR</td>
<td>NS</td>
<td>↓</td>
<td>NR</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Stern, et al., 2004</td>
<td>44 people Mean 55 Obese, 83% have diabetes or the metabolic syndrome</td>
<td>1 year</td>
<td>Low carbohydrate, high-fat diet, 30g/d carbohydrate intake.</td>
<td>NR</td>
<td>↓ at 6 months significantly</td>
<td>NR</td>
<td>NR</td>
<td>↓</td>
<td>↓ in diabetes subjects</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NS</td>
<td>↓</td>
<td>NR</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Yancy, et al., 2004</td>
<td>34 subjects, mean age 44.1 overweight or hyperlipidemic</td>
<td>24 weeks</td>
<td>Low carbohydrate high-fat diet, 20g/d or less carbohydrate intake (meat, fish, fowl, eggs, shellfish, cheese, 2 cups of salad vegetables)</td>
<td>Yes</td>
<td>↓</td>
<td>↓</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>↓</td>
<td>NR</td>
<td>NS</td>
</tr>
<tr>
<td>Reference</td>
<td>Subjects (age and health status)</td>
<td>Length of Study</td>
<td>Diets</td>
<td>Presence of measurable ketones</td>
<td>Body Weight</td>
<td>Fat Mass</td>
<td>Lean Mass</td>
<td>Fasting Insulin</td>
<td>Fasting Glucose</td>
<td>IGF-1</td>
<td>Leptin</td>
<td>Adiponectin</td>
<td>Total Cholesterol</td>
<td>Triglyceride</td>
<td>Free Fatty Acids</td>
<td>Low density lipoprotein</td>
<td>High density lipoprotein</td>
</tr>
<tr>
<td>--------------------</td>
<td>----------------------------------</td>
<td>-----------------</td>
<td>----------------------------------------------------------------------</td>
<td>-------------------------------</td>
<td>-------------</td>
<td>----------</td>
<td>-----------</td>
<td>-----------------</td>
<td>-----------------</td>
<td>-------</td>
<td>--------</td>
<td>-------------</td>
<td>---------------------</td>
<td>--------------</td>
<td>----------------</td>
<td>------------------------</td>
<td>------------------------</td>
</tr>
<tr>
<td>Meckling et al., 2004</td>
<td>15 subjects, mean age 41.2, obese</td>
<td>10 weeks</td>
<td>Low carbohydrate, high-fat diet, restricting carbohydrate from 100g/d to 50g/d at 5 days and kept on it.</td>
<td>Present at 2 and 4 weeks</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>NS</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>↑</td>
</tr>
<tr>
<td>Brehm, et al., 2005</td>
<td>20 women, mean age 44.8, obese</td>
<td>4 months</td>
<td>Low carbohydrate, high-fat diet, 20g carbohydrate/day for 2 weeks, then increased to 40-60g carbohydrate/day.</td>
<td>NR</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>NS</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>↑</td>
</tr>
<tr>
<td>Nickols-Richardson., 2005</td>
<td>13 women, mean age 38.8, overweight premenopausal</td>
<td>6 weeks</td>
<td>Low carbohydrate, high-fat diet, less than 20g/d carbohydrate intake, increased to 40 g/d at 6 weeks.</td>
<td>Yes</td>
<td>↓</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Seshadri, et al., 2005</td>
<td>40 people, obese, 17 with diabetes</td>
<td>6 months</td>
<td>Low carbohydrate, high-fat diet, 30g or less carbohydrate diet</td>
<td>NR</td>
<td>↓</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>↓</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Dashti, et al., 2006</td>
<td>66 obese subjects, 35 with high cholesterol level, and 31 not with high cholesterol</td>
<td>1 year</td>
<td>Low carbohydrate, high-fat diet, 20 g/d carbohydrate intake in the form of green vegetables and salad, then increased to 40g/d gradually</td>
<td>NR</td>
<td>↓</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>↓</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>↑</td>
</tr>
<tr>
<td>Reference</td>
<td>Subjects (age and health status)</td>
<td>Length of Study</td>
<td>Diets</td>
<td>Presence of measurable ketones</td>
<td>Body Weight</td>
<td>Fat Mass</td>
<td>Lean Mass</td>
<td>Fasting Insulin</td>
<td>Fasting Glucose</td>
<td>IGF-1</td>
<td>Leptin</td>
<td>Adiponectin</td>
<td>Total Cholesterol</td>
<td>Triglyceride</td>
<td>Free Fatty Acids</td>
<td>Low density lipoprotein</td>
<td>High density lipoprotein</td>
</tr>
<tr>
<td>-----------------------------------------------</td>
<td>----------------------------------</td>
<td>-----------------</td>
<td>----------------------------------------------------------------------</td>
<td>--------------------------------</td>
<td>-------------</td>
<td>----------</td>
<td>-----------</td>
<td>----------------</td>
<td>----------------</td>
<td>--------</td>
<td>--------</td>
<td>------------</td>
<td>-------------------</td>
<td>---------------</td>
<td>-----------------</td>
<td>--------------------------</td>
<td>--------------------------</td>
</tr>
<tr>
<td>Westman, et al., 2006</td>
<td>59 subjects, mean age 44, overweight</td>
<td>6 months</td>
<td>Low-carbohydrate high-fat diet, less than 28g carbohydrate/day initially</td>
<td>NR</td>
<td>↓</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NS</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Cardillo, Sesardi, &amp; Iqbal, 2006</td>
<td>27 people, obese, 9 with diabetes</td>
<td>36 months</td>
<td>Low carbohydrate, high-fat diet, 30g/d or less carbohydrate</td>
<td>NR</td>
<td>↓ at 6 months, NS at 36 months</td>
<td>NR</td>
<td>NR</td>
<td>↑</td>
<td>NS</td>
<td>NR</td>
<td>NR</td>
<td>NS</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>MR</td>
<td>NR</td>
</tr>
<tr>
<td>Dastani, et al., 2007</td>
<td>64 obese subjects with high blood glucose or without high blood glucose.</td>
<td>1 year</td>
<td>Ketogenic diet, 20g/d carbohydrate, 80-100g/d of protein</td>
<td>NR</td>
<td>↓</td>
<td>NR</td>
<td>NR</td>
<td>↓</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NS</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Keogh, Brinkworth, &amp; Clifton, 2007</td>
<td>13 obese subjects (high dropout rate)</td>
<td>52 weeks</td>
<td>33% carbohydrate, 27%-fat(7%-SF,6%-PUFA,13%-MUFA),40%-protein, calorie restricted (&lt;30%)</td>
<td>NR</td>
<td>↓</td>
<td>NR</td>
<td>↓</td>
<td>NS</td>
<td>NR</td>
<td>↑</td>
<td>↓</td>
<td>NS</td>
<td>NR</td>
<td>↓</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Dyson, Beatty, &amp; Matthews, 2007</td>
<td>32 diabetic subjects and 13 non-diabetes</td>
<td>3 months</td>
<td>Low carbohydrate diet, less than 40g/day carbohydrate intake</td>
<td>Yes</td>
<td>Decreased more significantly in diabetic subjects</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Shai, et al., 2008</td>
<td>109 moderately obese subjects</td>
<td>2 years</td>
<td>Low carbohydrate diet. Less than 20g/d carbohydrate intake first 2 months</td>
<td>Yes</td>
<td>Decreased in subjects with diabetes</td>
<td>NR</td>
<td>NR</td>
<td>↓</td>
<td>NR</td>
<td>NR</td>
<td>↓</td>
<td>NR</td>
<td>↓</td>
<td>NR</td>
<td>NR</td>
<td>NS</td>
<td>↑</td>
</tr>
</tbody>
</table>

*Note. Only statistically significant changes are reported. NS=No significant difference from baseline or control; NR= Not reported.*
Several Clinical Trials Show No Long-Term Effectiveness for Weight Loss

A significant unresolved issue with low-carbohydrate, high-fat diets is whether the diet is effective at maintaining weight loss and the safety of the diet if sustained for a long time. For cost and compliance reasons, many studies mentioned thus far have been relatively short-term diet intervention studies. However, longer studies have been conducted. In 2003, two randomized trials reported the longer term (12 months) effect of diets. In the first study (Stern, et al., 2004), severely obese individuals were randomized to either an ad-libitum low-carbohydrate diet or an energy restricted low-fat diet for 12 months. Those on the low-carbohydrate diet had lost more body weight after 6 months, but at 12 months the difference was no longer significant. The second study over 36 months randomized 132 obese adults to either a low-carbohydrate diet (carbohydrate intake less than 30g per day) or caloric restricted low-fat diet (reduced by 500 calories per day and less than 30% of calories from fat; Cardillo, Seshadri, & Iqbal, 2006). Although there was a greater weight reduction at 6 months in subjects who followed a low-carbohydrate diet as compared to a caloric restricted low-fat diet, the difference in weight loss was not significant at 36 months.

Although these two studies provide evidence that a low-carbohydrate diet does produce increased weight loss over a 3 to 6 month period and might be superior to the recommended calorie-reduced low-fat diet in short term, the longer studies have shed doubt on the effectiveness of these diets. While these two longer studies are important, more evidence is needed to determine whether low-carbohydrate diets are superior to the
energy-restricted low-fat diets over extended periods of time. It is also important to note that these two longer studies either did not assess ketone status or did not measure ketones throughout the study, which may be a key factor to the diet’s effectiveness.

**Low-Carbohydrate Diets That Have Assessed Ketone Bodies Production**

Ketosis is a state characterized by elevated levels of ketone bodies in the blood, occurring when the liver converts fat into fatty acids and ketone bodies (which can be used by brain and muscle for energy as an alternative to glucose). Ketosis usually happens when the body is in starvation (Cahill, 2006), diabetes (Balasubramanyam, Nalini, Hampe, & Maldonado, 2008), or treated with low-carbohydrate diets (Adam-Perrot, Clifton, & Brouns, 2006). Ketones include acetoacetic acid, 3-hydroxybutyric acid, and acetone. Blood ketones are produced in times of glucose deprivation when the body metabolizes body fat for energy purposes. The restriction in carbohydrate forces the body to rely on ketones as an alternative fuel source for glucose. As some amino acids can be used in the production of glucose in the body, diets that are more restrictive in both protein and carbohydrate are more likely to promote ketone production. Thus, a low-carbohydrate diet is a form of a ketogenic diet. As suggested by others (Mobbs, et al., 2007), elevated ketone levels appear to be indispensible for subjects to lose body weight.

While many studies have evaluated weight loss with low-carbohydrate feeding, fewer studies have addressed the relative ketosis produced by these diets. Discussed below are four studies that have assessed the effectiveness of low-carbohydrate diets on weight loss and also evaluated ketone status. The first study included 53 obese females
fed a low-carbohydrate diet or a calorie restricted low-fat diet for 6 months (Brehm et al., 2003). The low-carbohydrate diet group were instructed to follow an *ad libitum* diet with a maximum intake of 20g carbohydrate for 2 weeks and then permitted to increase their intake of carbohydrate to 40-60g/d after 2 weeks. The other group of dieters was instructed to consume a calorie-restricted, moderately low-fat diet with a recommended macronutrient distribution of 55% carbohydrate, 15% protein, and 30% fat. From the beginning to the end of the study, the low-carbohydrate group had higher levels of blood beta-hydroxybutyate levels (a blood ketone) with peak beta-hydroxybutyrate levels at 3 months. Thus, they were able to promote ketone production and sustain these higher ketone levels over time, which likely contributed to the favorable outcome on weight.

In the second trial (Yancy et al., 2004), 120 overweight, hyperlipidemic participants were randomly assigned to either a low-carbohydrate diet group or a low-fat, low-cholesterol, reduced-calorie diet group for 6 months. The intervention for both groups included group meetings, diet instruction, and an exercise recommendation. Body composition, lipid profile, and ketone levels were assessed. At the end of this study, weight loss was greater in the low-carbohydrate diet group than in the low-fat diet group. Subjects in both groups lost substantially more fat mass. Furthermore, the subjects in the low-carbohydrate group made great improvements in their lipid profile, such as triglyceride and HDL, than those subjects in the low-fat group. The ketone levels were measured throughout the study to monitor for adherence to the low-carbohydrate diet.
The results showed a higher level of ketones was present throughout the trial in the low-carbohydrate diet and the ketone levels reached the maximum after 2 weeks on the diet.

The next two trials discussed were longer trials and thus provide valuable information about the long-term efficacy of this diet relative to ketone levels. The third trial had the longer experimental treatment period with 1 year dietary intervention (Foster, et al., 2003). As described before, at the first 6 months, the low-carbohydrate diet produced a greater weight loss than did the conventional diet and also elevated significant ketone levels until 12 weeks. After 12 weeks, the difference in urinary ketone levels between two groups was no longer significant. Furthermore, after 12 months of dietary intervention, the difference in body weight loss between two groups was also not significant. In the fourth study to date assessing low-carbohydrate diets and ketone status, a 2-year trial was recently reported with 109 moderately obese subjects (Shai, et al., 2008). The subjects were treated with 20g of carbohydrate per day for the initial 2 months and a gradual increase to a maximum of 120g per day. In this low-carbohydrate, high-fat diet group, the proportion of participants with detectable urinary ketones at 24 months was higher and the weight loss of the subjects was significant throughout the trial. Collectively, these four studies in which ketone status was assessed indicate strongly that higher ketone status is critical in promoting the initial weight loss and higher levels must be sustained in order to see continued benefit in body weight.
How Do Low-Carbohydrate Diets Produce Body Weight Loss?

How a low-carbohydrate diet elicits weight loss remains unclear. However, it is suggested that the low-carbohydrate diet works by restricting total calorie intake and promoting ketone production. Restriction of total calories could be a direct effect of the macronutrient composition of the diet or secondary to the lack of food choices and the monotony of the diet. Regardless, the low-carbohydrate diet along with the calorie restriction should produce a metabolic altered state that promotes glycogen depletion and ketosis. The loss of glycogen and its associated bound water could account for some of the reported weight loss although most trials report that the weight loss is mainly attributable to fat loss and not lean or fluid loss, which is also supported by improvement in metabolic factors (Meckling et al., 2002; Meckling et al., 2004; Willi et al., 1998).

So how might ketone levels induce weight loss? Some have suggested that the ketone levels could alter hypothalamic glucose-sensing neurons to alter post-prandial thermogenesis and increase resting energy expenditure (Almind & Kahn, 2004; Westerterp, 2004). In support of this view, chronic infusion of ketones into the brain of rats reduces their body weight (Davis, Wirtshafter, Asin, & Brief, 1981). Ketone status has also been shown to alter the expression of many key genes in fat metabolism (Kennedy, et al., 2007). That is, microarray analysis of liver tissue shows a unique pattern of gene expression in low-carbohydrate, ketogenic mice, with increased expression of genes pivotal in fatty acid oxidation pathways, such as adenosine monophosphate (AMP) kinase activity, and reduction in lipid synthesis pathways, such as acetyl-CoA
carboxylase (ACC) activity, which plays a pivotal role in lipogenesis. Thus, the ketogenic state does result in significant metabolic changes in at least rodent models that likely contribute to the observed changes in clinical trials. Ultimately, the mechanism for how low-carbohydrate diets and ketones alter weight still needs to be resolved.

The Low-Carbohydrate Diet on Body Weight Loss and Metabolic Parameters

Numerous studies show that low-carbohydrate diets have significant effects on reducing body weight, improving the lipid profile, decreasing blood glucose and serum insulin levels and ameliorating IGF-1 levels accompanied with remarkably elevated ketone body levels. In addition, there are a few trials that show low-carbohydrate diets had significant effects on reducing body weight and improving metabolic syndrome. A summary of the relevant findings for each of these clinical features is provided below.

**Body weight, body mass index (BMI), and body composition.** Low-carbohydrate diets, have a significant effect on decreasing body weight, BMI, and improving body composition with both short-term and long-term trials.

Several 8- to 12-week trials provide strong evidence for low-carbohydrate diets promoting weight loss. For example, 6 adolescents aged 12 to 15 years old, weighing an average of 147.8 kg and having an average body mass index of 50.9 kg/m², consumed the low-carbohydrate diet for 8 weeks (Willi et al., 1998). Subjects lost 15.4 ± 1.4 kg during the diet intervention. Body mass index decreased 5.6 ± 0.6 kg/m². Body composition analysis indicated that weight was lost equally from all areas of the body and was predominantly fat. Lean body mass was not significantly affected. In another study, 20
overweight women were fed hypocaloric, low-carbohydrate diet for 8 weeks (Meckling et al., 2002). The average weight loss was 5.0 kg, with a net decrease in body mass index of 1.82 kg/m² and 3.4% body fat loss. In another 10-week trial (Meckling et al., 2004), which compared a low-fat and low-carbohydrate diet on weight loss, the low-carbohydrate group lost a similar level of body weight as the low-fat group did. However, the low-fat group better preserved lean body mass when compared with the low-carbohydrate group. In a 12-week study (Dyson, Beatty, & Matthews, 2007), type 2 diabetic subjects and nondiabetic subjects were randomly allocated to either a low-carbohydrate diet or a healthy eating conventional diet with the greater weight loss (6.9 vs. 2.1 kg) seen in the low-carbohydrate group.

Longer trials, those lasting around 6 months, show equally impressive improvements in weight and body composition. For example, obese female subjects fed a very low-carbohydrate diet group showed greater weight loss and body fat loss than a calorie-restricted low-fat diet group (Brehm et al., 2003). Further, overweight, hyperlipidemic subjects lost more weight on a low-carbohydrate diet group than the subjects who ate a low-fat reduced-calorie diet although both groups lost substantial amounts of fat mass (Yancy et al., 2004). A 1-year trial, participants with high cholesterol levels also had a significant weight loss after they had been treated a low-carbohydrate diet with only 20 g carbohydrate per day intake for 12 months (Dashti, et al., 2006). In a 2-year trial, moderately obese subjects were randomized into three diets: low-fat, restricted-calorie; Mediterranean, restricted-calorie; or low-carbohydrate, non-restricted-
calorie ketogenic diet (Shai, et al., 2008). The low-carbohydrate group consumed the smallest amount of carbohydrates, but a similar total energy intake over 2 years among 3 dietary regimes. The results showed that the Mediterranean diet and the low-carbohydrate groups had more body weight loss than the low-fat group.

Overall, the trials discussed above were from short term (8-12 weeks) to long term (2 years). In these trials, low-carbohydrate diets have significant effects on body weight loss and BMI decline. Some trials also showed that the body weight loss induced by the low-carbohydrate diet was mainly attributable to the fat mass loss, not lean mass.

*The low-carbohydrate diet on lipid profile.* Obesity can cause dyslipidemia. Therefore, changes in lipid profile are major factors to consider when evaluating the effectiveness of the low-carbohydrate diet. Surprisingly, multiple studies have suggested that these high-fat diets are surprisingly effective at improving blood lipid levels despite their high-fat content. For example, 6 morbidly obese adolescents, aged 12 to 15 years, reduced their serum cholesterol from 162 ± 12 to 121 ± 8 mg/dL in the initial 4 weeks after low-carbohydrate diet intervention (Willi et al., 1998). In another study (Meckling et al., 2002), the total cholesterol of 20 women decreased 1.2 mM after they had been consuming a low-carbohydrate diet for 8 weeks. Moreover, total triglyceride decreased 0.6 mM and the ratio of triglyceride/HDL also significantly decreased. Longer trials also show improvements in blood lipids. In a 6-month trial (Yancy et al., 2004), subjects fed the low-carbohydrate diet had greater decreases (-0.84 mM/L) in serum triglyceride and increased HDL by 0.14 mM/L. In another 6-month trial (Westman, Yancy, Olsen, Dudley,
& Guyton, 2006), the low-carbohydrate diet intervention resulted in a shift from small, dense LDL to large, buoyant LDL in 59 subjects.

Longer studies have also shown improvements in lipid levels. A 1-year trial reported a significant increase in HDL levels and significant decrease in total cholesterol, triglyceride, and LDL (Dashti, et al., 2006). In the 2-year trial discussed before (Shai, et al., 2008), the greatest increase of HDL levels and decrease of triglyceride levels occurred in the low-carbohydrate diet group. Moreover, the low-carbohydrate diet group also had the most significant improvement, with a relative decrease of 20%, in the ratio of total cholesterol to HDL cholesterol. In short, both the short-term and long-term trials show that low-carbohydrate diets provide significant improvements in lipid profile. However, it should be noted that at least one study in children contradicts these benefits. In this study, children with difficult to treat seizures were fed low-carbohydrate diets for 6 months and had significantly increased mean plasma levels of total cholesterol, LDL, VLDL, and non-HDL cholesterol, while mean HDL decreased significantly (Kwiterovich, Vining, Pyzik, Skolasky, & Freeman, 2003). While this study may be specific to children, it does demonstrate that some groups or some extremes in the low-carbohydrate diet may elevate blood lipids.

The low-carbohydrate diet on blood glucose level and serum insulin levels. As obesity is typically accompanied by insulin resistance, evaluating glucose and insulin levels with these diets is important. Again, data are overwhelmingly positive. For example, circulating insulin concentrations of 15 subjects who consumed low-
carbohydrate diet for 10 weeks were reduced from $23.7 \pm 2.7 \mu\text{IU/ml}$ to $16.9 \pm 1.9 \mu\text{IU/ml}$ (Meckling et al., 2004). In another 6-month study talked before (Brehm et al., 2003), the blood fasting glucose levels decreased significantly from $99.1 \text{ mg/dL}$ to $90.1 \text{ mg/dL}$, while the serum insulin levels decreased from $16.9 \mu\text{IU/ml}$ to $14.4 \mu\text{IU/ml}$ significantly. Interestingly, the blood glucose levels were decreased more significantly in diabetic patients after the dietary intervention with low-carbohydrate (Samaha, et al., 2003; Seshadri et al., 2005; Stern, et al., 2004).

Some trials are less promising with respect to low-carbohydrate diets on improving glucose and insulin levels. For example, an 8-week trial in overweight women (Meckling et al., 2002) and a 36-month trial, failed to show improvement (Cardillo et al., 2006). Thus, like lipid profiles, there is some evidence that not all benefit from specific low-carbohydrate regimes.

The low-carbohydrate diet on IGF-1. Insulin-like growth factor I (IGF-I) is an important growth and differentiation factor that also has potent metabolic, insulin-like actions. IGF-I is considered to be important for skeletal growth and is also a marker of the patient’s nutritional status, being decreased during starvation (Wang, Zhou, Cheng, Kopchick, & Bondy, 2004).

Although not well studied, low-carbohydrate diets increase IGF-1 from $317 \pm 13 \text{ ng/ml}$ to $415 \pm 54 \text{ ng/ml}$ after 8 weeks dietary intervention with 6 morbidly obese adolescents (Willi et al., 1998). In another trial, there was no significant changes in IGF-1
levels in overweight women who consumed a low-carbohydrate diet for 8 weeks (Meckling et al., 2002).

*The low-carbohydrate diet on Adipokine levels.* Adipokines are secreted from adipocytes and they have a wide variety of endocrine, paracrine, and autocrine effects, including regulation of satiety, energy metabolism, immune function, and angiogenesis (Tilg & Moschen, 2006). Leptin and adiponectin are the first discovered and best characterized adipokines to date. Leptin is generally considered to be a proinflammatory adipokine, whereas adiponectin functions as a potent anti-inflammatory molecule. Increasing obesity leads directly to elevation of circulation concentrations of leptin but a decrease in serum adiponectin levels (Arita, et al., 1999; Santosa, Demonty, Lichtenstein, Cianflone, & Jones, 2007).

Few studies have assessed adiponectin status with low-carbohydrate diets. In 2007, it was reported that 37 obese subjects were randomized to a low-carbohydrate diet and high-carbohydrate diet both low in saturated fat for 52 weeks. The adiponectin was increased at the end of the study and change in adiponectin was correlated with change in HDL (Keogh, Brinkworth, & Clifton, 2007). However, in another 3-year trial, a low-carbohydrate diet had no significant effect on adiponectin compared to a low-fat diet at 36 months (Cardillo et al., 2006). The same outcome for a 6-month trial, either in diabetic patients or non-diabetes subjects, the circulating adiponectin levels had no significant changes after low-carbohydrate dietary intervention.
In a previous discussed 6-month trial with obese subjects (Brehm et al., 2003), the serum leptin levels was decreased as the subjects lost their body weight at first 3 months. In the other two 6-month trials with diabetic patients and not diabetic subjects discussed already (Samaha, et al., 2003; Seshadri et al., 2005), the serum leptin levels were decreased significantly, specifically in diabetic patients. Even in the 2-year trial, the serum leptin levels also were declined (Shai, et al., 2008).

**Rodent Models in Obesity and Diet Research**

Animal models are valuable tools to address disease states or to study factors relevant to human health. In many cases, animal models allow for more invasive procedures, such as organ analysis, that would be considered unethical to inflict on a human. The merits of animal research are widely accepted by scientists and largely appreciated by the general public. Not surprising, animal models can also be useful in dietary intervention studies.

*C57BL/6J Mice – A Demonstrated Animal Model for Research Related to Diet-Induced Obesity*

Rat and mouse models are commonly used to study disease states or dietary interventions. An important mouse strain for studying both diet-induced obesity and diet strategies for reversing obesity is the C57BL/6J mouse. For obesity, this model has been shown to progress through the same stages of obesity and type 2 diabetes as seen in humans. Previously, it has been reported that on a diet consisting of 20.5% protein,
35.8% fat and 36.8% carbohydrate, C57BL/6J mice become obese and gain weight faster than a similar strain, C57BL/AJ (Surwit et al., 1988). When glucose and insulin were measured in these animals, the obese C57BL/6J mice were found to be significantly more hyperglycemic and hyperinsulinemic than lean C57BL/6J, as well as lean and obese C57BL/A/J mice (Surwit et al., 1988). Thus, these mice progress through a metabolic state similar to humans when provided *ad libitum* access to high-fat or low-fat diets (Collins, Martin, Surwit, & Robidoux, 2004). In other words, when C57BL/6J mice are raised on a low-fat diet, these mice are lean and euglycemic with normal insulin levels and blood pressure, while these mice would develop obesity, hyperinsulinemia, hyperglycemia and hypertension when fed a high-fat diet. Other strains of mice, such as the A/J mouse or the C57BL/KsJ, are relatively resistant to the effects of a high-fat diet (Rebuffe-Scrive et al., 1993). In addition, the diet-induced diabetes and obesity in the C57BL/6J mice is characterized by selective deposition of fat in the mesentery (visceral adiposity), an observation consistent with the finding that abdominal obesity is an independent risk factor for diabetes in humans (Rebuffe-Scrive et al., 1993). Moreover, the high-fat diet induced obesity, hyperglycemia, and hyperglycemia are independent of increased caloric intake, which means the caloric restricted high-fat diet might attenuate but cannot prevent the rise of body weight, especially in fat tissue, and blood glucose levels in C57BL/6J mice (Petro et al., 2004).
Rodent Models and Low-Carbohydrate Diet Studies

Rodent models have been used to study the effectiveness and safety of low-carbohydrate diets. Table 3 summarizes the published studies in this area.
### Table 3

**Summary of Low-Carbohydrate Diet Studies in Rodent Models**

<table>
<thead>
<tr>
<th>Reference</th>
<th>Animals</th>
<th>Length of Study</th>
<th>Diets</th>
<th>Presence of measurable ketones</th>
<th>Body Weight</th>
<th>Fat Mass</th>
<th>Lean Mass</th>
<th>Fasting Insulin</th>
<th>Fasting Glucose</th>
<th>IGF-1</th>
<th>Leptin</th>
<th>Adiponectin</th>
<th>Total Cholesterol</th>
<th>Triglyceride</th>
<th>Free Fatty Acids</th>
<th>Low density lipoprotein</th>
<th>High density lipoprotein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cheng, et al., 2003</td>
<td>Sprague Dawley rats</td>
<td>7 days</td>
<td>Ketogenic diet, calorie restricted, composed of fat (78%), protein (10%), carbohydrate (2%)</td>
<td>Yes</td>
<td>NS</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Thio, et al., 2006</td>
<td>C57BL/6J mice</td>
<td>50 days</td>
<td>Ketogenic diet 92% calories from fat, 3% from carbohydrate, 5% from protein.</td>
<td>Yes</td>
<td>Slow weight gain</td>
<td>NR</td>
<td>NR</td>
<td>↓</td>
<td>↓</td>
<td>NR</td>
<td>↑</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Williams et al., 2007</td>
<td>C57BL/6 mice</td>
<td>14 weeks</td>
<td>High-fat diet (20% protein, 20% carbohydrate, 60% fat), 14 weeks, low-carbohydrate diet (35% protein, 5% carbohydrate, 60% fat) 7 weeks after 7 weeks of high-fat diet.</td>
<td>NS</td>
<td>↑</td>
<td>NS, compared with high-fat group</td>
<td>NS, compared with high-fat group</td>
<td>↓, comparred with high-fat group</td>
<td>↓, compared with high-fat group</td>
<td>NS, compared with high-fat group</td>
<td>NS, compared with high-fat group</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kennedy, et al., 2007</td>
<td>C57BL/6 mice</td>
<td>9 weeks</td>
<td>Ketogenic diet, 95% fat, 5% protein, 0% carbohydrate</td>
<td>Yes</td>
<td>↓</td>
<td>Similar as conventional diet group</td>
<td>Similar as conventional diet group</td>
<td>↓</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NS</td>
<td>NS</td>
<td>NR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Koide, et al., 2007</td>
<td>Otsuka Long Evans Tokushima Fatty rats</td>
<td>8 weeks</td>
<td>Calorie-restricted low-carbohydrate diet, total calories intake restricted to 70% of the average intake from each diet.</td>
<td>NR</td>
<td>↓</td>
<td>NR</td>
<td>NR</td>
<td>↓</td>
<td>NS</td>
<td>NR</td>
<td>↑</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
</tbody>
</table>

**Note.** Only statistically significant changes are reported. NS=No significant difference from baseline or control; NR= Not reported.
Impact of Low-Carbohydrate Diets on Mice

There are several studies using the well characterized C57BL/6 mice, which are prone to diet induced obesity. In one study (Kennedy, et al., 2007), 4 groups of mice were fed either a conventional low-carbohydrate diet (16.7% fat, 26.8% protein, and 56.4% carbohydrate in calories) as a control group, a conventional low-carbohydrate but with 66% caloric restriction, a high-fat diet (45% fat, 24% protein, and 35% carbohydrate in calories), or a high-fat, ketogenic diet (95% fat, 0% carbohydrate, and 5% protein in calories) for 9 weeks (Kennedy, et al., 2007). Except for the caloric restriction group, all groups of mice were allowed access to food ad libitum. Over the course of the 9 weeks, there was no significant difference in energy consumption between the conventional diet, high-fat diet and high-fat, ketogenic diet groups. Not surprising, the high-fat, ketogenic diet had the highest ketone levels. The conventional diet control group had a little weight gain and the high-fat group had the highest weight gain. The caloric restriction group, as expected, had a significant weight loss throughout the whole study. Interestingly, the high-fat, ketogenic diet group had decreased serum leptin levels and similar significant weight loss as the caloric restriction group did, although the high-fat, ketogenic diet group consumed a similar level of energy as the high-fat group. Moreover, there was no significant difference in the body composition between the high-fat, ketogenic diet group and caloric restriction group. In this study, the researchers also studied the impact of high-fat, ketogenic diet on obese mice caused by 12 weeks of high-fat diet pretreatment. After 5 weeks of high-fat, ketogenic diet treatment of these obese mice, the results
showed that the high-fat, ketogenic diet significantly reversed the obesity in these mice. This same study also compared gene expression by microarray analysis in livers of C57Bl/6J mice fed the high-fat diet, conventional diet, and calorie restricted high-fat diet in order to find out the difference in metabolic gene expression levels. The microarray studies indicated that the expression of genes in fatty acid synthesis pathways, such as fatty acid synthase and stearoyl-CoA desaturase-1, decreased, and in contrast, the genes in fatty acid oxidation pathways, such as hydroxybutyrate dehydrogenases increased. Moreover, the activity of AMP activated protein kinase (AMPK), which leads to decreased fatty acid synthesis and increased fatty acid oxidation, was increased, while the activity of acetyl-CoA carboxylase (ACC), which increases fatty acid synthesis and decreases the fatty acid oxidation, was decreased. Thus, key genes in liver fat metabolism are altered in a favorable manner, presumably, when mice were fed a high-fat, ketogenic diet.

In another study, C57BLKS/J mice were fed a low-carbohydrate diet (Thio, Erbayat-Altay, Rensing, & Yamada, 2006). Animals on the low-carbohydrate diet received 92% of their calories from fat, 3% from carbohydrate, and 5% from protein. In the control group, the mice received 12% calories from fat, 65% from carbohydrate, and 24% from protein. After a period of 50 days of dietary treatment, mice on the low-carbohydrate diet had a slower weight gain than the control group. Interestingly, the serum leptin levels of the low-carbohydrate diet group were higher than control groups. These increased leptin levels seen in this study with C57BLKS/J mice was similar to the
results reported for calorie restriction with low-carbohydrate diets in Otsuka Long Evans Tokushima fatty rats (Koide, Oyama, Miyashita, & Shirai, 2007). However, in another study, the serum leptin levels in C57BL/6 mice, as expected, were decreased after they were fed high-fat, ketogenic diet for a period of time (Kennedy, et al., 2007). In all three mouse studies, fasting serum insulin levels and blood glucose levels were all decreased or tend to be decreased with low-carbohydrate feedings (Kennedy, et al., 2007; Koide et al., 2007; Thio et al., 2006). As for lipids, total cholesterol levels were significantly decreased in Otsuka Long Evans Tokushima fatty rats (Koide et al., 2007), not significant in C57BL/6 mice (Kennedy, et al., 2007), while the triglyceride levels were all decreased in C57BL/6 mice and Otsuka Long Evans Tokushima fatty rats (Kennedy, et al., 2007; Koide et al., 2007). Higher levels of ketone bodies were found in C57BLKS/J and C57BL/6 mice and significant body weight loss and slower body weight gain were also found in these studies (Kennedy, et al., 2007; Thio et al., 2006). For body composition, the C57BL/6 mice fed low-carbohydrate diets have a similar fat mass and lean mass as the conventional diet group (Kennedy, et al., 2007).

However, there are two published studies with negative outcomes with low-carbohydrate diets. One study was using sprague dawley rats (Cheng et al., 2003). In this study, and the ketone levels were elevated but the body weight did not change too much. The shorter dietary interference (7 days) might account for the inability to detect body weight diminution. In another study using C57BL/6 mice, body weights of the mice were increased with low-carbohydrate feeding while the elevated ketone levels were not
significant. Actually, according to a previous study of low-carbohydrate diet on mice in our lab, a traditional low-carbohydrate diet (low carbohydrate, high fat, high protein) cannot promote weight loss efficiently in obese mice with a 14-week dietary treatment. On the contrary this low-carbohydrate diet increased body weight in lean mice significantly (List, et al., 2005). This suggested the high percentage protein in diets would contribute to gluconeogenesis during low-carbohydrate diet treatments. Therefore, the low-carbohydrate diets that will be used in our study are very low in protein content, which might be effective on promoting body loss in mice.

**Conclusion**

Obesity and related comorbidities have become a major health concern in the United States and worldwide. The search for a viable treatment has led many to consider using low-carbohydrate diets. This is an attractive choice due to its low cost and convenience. Overview of recent research shows that the low-carbohydrate diet has significant effects on body weight loss in human subjects and animals. Moreover, low-carbohydrate diets can improve metabolic parameters, such as dyslipidemia and insulin resistance. However, not all studies show benefits and some report adverse effects.

C57Bl/6J mice are an ideal animal model that can be used to better evaluate the effects of low-carbohydrate diets on body weight loss and metabolic parameters. Also, using mice allows for the ability to assess changes in organ function, biochemistry and physiology, which would be difficult or impossible in human studies. Thus far,
researchers have not compared different low-carbohydrate diets on their ability to
promote a healthy and sustainable weight loss in mice. This comparison is the purpose of
this study.
CHAPTER 3: MATERIALS AND METHODS

Previous studies have shown significant effects of the low-carbohydrate diet on body weight loss in obese mice (Kennedy, et al., 2007). In this thesis, various compositions of low-carbohydrate diets were used to evaluate their effectiveness on reducing body weight in mice. Physiological parameters such as blood glucose, insulin, blood lipid profile, blood ketone levels, IGF-1, as well as organ weights were measured to help determine the physiological impact and possible harmful effects of this type of dietary treatment.

Animals

Mice in the C57BL/6J background were used for this study. Previous studies have characterized this model as useful for the study of diet-induced obesity due to their human-like progression from obesity to type 2 diabetes when fed a high-fat diet (R. S. Surwit et al., 1988). 50 male C57BL/6J mice were obtained from The Jackson Laboratory (Bar Harbor, ME) at 6 weeks age. Once at Ohio University, mice were maintained in a temperature and humidity-controlled room and exposed to a 14/10 hour light/dark cycle. Mice were housed 2 per cage and allowed ad libitum access to food and water. All studies were approved by the Ohio University Institutional Animal Care and Use Committee and fully complied with federal state and local policies.
Diets

Five separate diets were used in this study, a standard chow (SC) (LabDiet® product, Richmond, IN), a high-fat (HF) diet without restrictive carbohydrate or protein (Diet 12492, Research Diets, New Brunswick, NJ), and three low-carbohydrate diets. The low-carbohydrate diets specially formulated by Research Diets: LC5 (D07100603), LC10 (D07100604), LC15 (D07100605). Details of these diets are provided in Table 4.

Table 4

The Energy Proportion and Macronutrient Composition of Different Diets

<table>
<thead>
<tr>
<th>Diets</th>
<th>Protein Energy (%)</th>
<th>Carbohydrates Energy (%)</th>
<th>Protein Weight (%)</th>
<th>Carbohydrates Weight (%)</th>
<th>Protein Fat Energy (%)</th>
<th>Carbohydrates Fat Weight (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard chow (SC)</td>
<td>26.0</td>
<td>60.0</td>
<td>23.5</td>
<td>56.0</td>
<td>14.0</td>
<td>6.5</td>
</tr>
<tr>
<td>High-fat diet (HF)</td>
<td>20.0</td>
<td>20.0</td>
<td>26.0</td>
<td>26.0</td>
<td>60.0</td>
<td>36.0</td>
</tr>
<tr>
<td>LC5</td>
<td>5.0</td>
<td>2.0</td>
<td>9.0</td>
<td>4.0</td>
<td>93.0</td>
<td>71.0</td>
</tr>
<tr>
<td>LC10</td>
<td>10.0</td>
<td>2.0</td>
<td>16.0</td>
<td>3.0</td>
<td>88.0</td>
<td>64.0</td>
</tr>
<tr>
<td>LC15</td>
<td>15.0</td>
<td>2.0</td>
<td>24.0</td>
<td>3.0</td>
<td>83.0</td>
<td>58.0</td>
</tr>
</tbody>
</table>

Protocols

There were 2 phases in this study. An overview of the 2 phases and the measurements taken are provided in schematic form in Figure 1. In phase 1, 40 mice at 6 weeks age were placed on HF diet, and 10 mice also at 6 weeks age were placed on SC. Phase 1 lasted for a period of 4 months. After 4 months, mice on the HF diet had gained
excess body weight compared with SC (see results section). In phase 2, the mice on the SC were maintained on the SC (SC-SC), while the HF diet fed mice were divided into 5 groups of 8 and switched to either one of three low-carbohydrate diets (LC5, LC10 or LC15), to the SC diet, or maintained on the HF diet. Therefore, there were 6 groups in this study, SC-SC, HF-HF, HF-LC5, HF-LC10, HF-LC15, and HF-SC. Phase 2 of the study continued for 6 weeks, whereupon all mice were sacrificed, and tissues were taken for analysis.

**Weight and Body Composition Measurements**

Body weight was measured weekly throughout both phases of the study using a standard scale to the tenth of a gram. Body composition was assessed using the Bruker Minispec (The Woodlands, TX) prior to and during phase 2 of the study. This machine uses NMR technology to assess the total fat mass, lean mass, and fluid mass in grams of live, awake animals (Barac-Nieto & Gupta, 1996). The measurements were performed at the same time each week in duplicate. A summary of all body composition and weight measurements are represented in Figure 1.
Figure 1. Timeline and summary of the low-carbohydrate diet study. Five types of diet were used in the present study: standard chow (SC), high-fat (HF), low-carbohydrate diet with 5% protein (LC5), low-carbohydrate diet with 10% protein (LC10), low-carbohydrate diet with 15% protein (LC15). Phase 1 was the obesity inducing period. Forty mice were placed on a HF diet and 10 mice, as control, were placed on a SC diet for 4 months. Phase 2 was the experimental dietary period of the study. HF diets were switched to the various experimental diets (LC5, LC10, LC15) or a SC diet and, as a control, one group of mice were maintained on the high-fat diet. There were six groups in Phase 2: SC-SC, HF-HF, HF-LC5, HF-LC10, HF-LC15 and HF-SC. Weights, body composition were measured weekly. Blood glucose levels were measured biweekly. Energy consumption was measured in the last 4 weeks. Urinary ketone levels were measured at the fifth week. At the conclusion of this study, blood ketone levels, insulin, IGF-1, total cholesterol, triglycerides, free fatty acids, HDL and LDL, and adiponectin were assessed. Tissues weights were measured at the end of the study after dissection.
**Blood Glucose Measurements and Collection of Plasma**

Blood glucose measurements and plasma collection began in phase 2 of diet treatment and continued every other week to the end of the study (see Figure 1). Prior to glucose measurements and collection, the mice were fasted for 12 hours overnight. The measurements and collections were completed between 9:00 a.m. and 12:00 p.m. on the next day. An infrared heating lamp was used to enhance blood collection. To measure the blood glucose and collect blood, the tip of the tail was removed and blood glucose measurements were taken using a LifeScan OneTouch glucometer (Milpitas, CA) with the first drop of blood. Immediately after the blood glucose measurements, blood was collected using Chase Natelson heparinized capillary tubes (VWR International, Bridgeport NJ) and stored on ice. Whole blood was then spun at 4°C for 10 minutes at 7,000 x g. Plasma was collected and stored at -80°C until further analysis.

To obtain more accurate blood glucose measurements, glucose measurements were repeated in triplicate at 6th week. The tip of the tail was cut and the first three drops of blood were used for plasma glucose measurements taking triplicate measurements. The three values were averaged for the final blood glucose concentration.

**Energy Intake**

Total food intake/cage was assessed every 3 or 4 days in the last 4 weeks of phase 2 in the study by subtracting the total grams of food remaining in the cage from the total grams of food added to the cage. These measurements are reported as the average number
of kilocalories consumed by each animal in each experimental group. Food was measured on a standard scale.

**Urinary Ketone Levels**

Mouse urine was collected by gently massaging the bladder area of a male mouse over a clean petri dish at the 15th week of phase 2 in this study. Care was taken to avoid fecal contamination of the urine. The reagent end of the Bayer Reagent Strips (Bayer Corporation, Elkhart, IN) were immersed in fresh urine and removed immediately. Exactly 15 seconds after removing from specimen, the urinary ketone levels were obtained by comparing reagent side of test area with corresponding color chart provided with the strips.

**Tissue Collection**

All mice were sacrificed by cervical dislocation after 6 weeks of dietary treatment in phase 2. Inguinal subcutaneous adipose tissue, epididymal adipose tissue, retroperitoneal adipose tissue, mesenteric adipose tissue, kidney, heart, and liver were collected immediately after the mice were killed. All tissues collected were weighed using a standard laboratory scale and flash frozen in cryogenic vials using liquid nitrogen. They were then stored at -80°C until further use.
Serum Measurements

Total plasma cholesterol, HDL, LDL and triglycerides, free fatty acids were assayed using enzymatic kits (Wako Pure Chemicals Ltd., Osaka 541, Japan), following the manufacturer’s instructions. Enzyme-linked immunosorbent assay kits were used to measure plasma insulin (ALPCO, Salem, NH), and free IGF-1 (Diagnostic Systems Laboratories, Webster, TX), adiponectin (ALPCO, Salem, NH) levels. Blood total ketone bodies levels were determined using enzymatic kits (Wako Pure Chemicals Ltd., Osaka 541, Japan).

Statistical Analysis

Statistics were performed on all body composition measurements, glucose measurements, insulin, energy intake, total cholesterol, free fatty acids, triglyceride, blood ketone, urinary ketone concentrations, and tissue weights using SPSS version 14.0 software (Chicago, IL). Groups were reported as SC-SC (SC diet in phase 1 and phase 2), HF-HF (HF diet in phase 1 and phase 2), HF-LC5 (HF diet in phase 1 and LC5 diet in phase 2), HF-LC10 (HF diet in phase 1 and LC10 diet in phase 2). HF-LC15 (HF diet in phase 1 and LC15 diet in phase 2) and HF-SC (HF diet in phase 1 and LF diet in phase 2). All variables were analyzed using one way analysis of variance (ANOVA) and group means were reported as means ± SEM. A value of $p<0.05$ were regarded as statistically significant.
CHAPTER 4: RESULTS

Weight Gain in Phase 1

In phase 1 of the study, body weight gain varied significantly ($F(5,44)=13.02$, $p < .001$). The body weight of C57BL/6J mice placed on the HF diet increased quickly and significantly as compared to controls fed SC. Weight gain data of all 6 groups at the end of phase 1 of the study are shown in Figure 2. At the end of the phase 1, all HF groups had higher body weights than the SC-SC group ($p < .001$), and there was no significant difference in body weight among the HF groups (HF-HF vs. HF-LC5, $p = .997$. HF-HF vs. HF-LC10, $p = 1.000$. HF-HF vs. HF-LC15, $p = 1.000$. HF-HF vs. HF-SC, $p = 1.000$). At the end of phase 1 of the study, the body weight were 30.13 ± 0.75g in SC-SC group, 42.43 ± 2.48g in HF-HF group, 43.50 ± 1.42g in HF-LC5 group, 43.04 ± 2.03g in HF-LC10 group, 42.66 ± 0.82g in HF-LC15 group, and 42.27 ± 1.28g in HF-SC group.

Weight and Weight Accumulation

Body weight change over the course of phase 2 of the study was different between treatment groups ($F(5,44)=16.20$, $p < .001$). Body weight data at the conclusion of the study are shown in Figure 3. HF-HF group had the highest body weight (HF-HF vs. SC-SC, $p < .001$. HF-HF vs. HF-LC5, $p < .001$. HF-HF vs. HF-LC10, $p < .001$. HF-HF vs. HF-LC15, $p = .001$. HF-HF vs. HF-SC, $p < .001$) and SC-SC, HF-LC5, and HF-SC groups had lower body weight among all six groups ($p < .05$). There was no significant difference in body weight among SC-SC, HF-LC10, HF-LC15, and HF-SC groups ($p >$
The body weight of each group at the conclusion of the study was 29.90 ± 0.87g in SC-SC, 43.9 ± 2.19g in HF-HF, 29.07 ± 0.61g in HF-LC5, 35.06 ± 1.81g in HF-LC10, 35.19 ± 1.22g in HF-LC15, 32.58 ± 0.58g in HF-SC.

Weight accumulation over the course of phase 2 of the study was different among treatment groups ($F(5,44)=63.14, p < .001$). Weight accumulation data from weekly measurements in phase 2 of the study are shown in Figure 4. All low-carbohydrate diet treatment groups and the HF-SC group started to lose weight by the end of the first week of phase 2 of the study. By week 2, HF-LC5, HF-LC10, HF-LC15, and HF-SC groups continued to lose body weight progressively while there was a slight increase in body weight for SC-SC and HF-HF groups. The HF-LC5 group started to lose more body weight than HF-LC10 and HF-LC15 groups, and had similar weight loss as compared to HF-SC group. After 3 weeks of dietary treatment, the SC-SC and HF-HF
Figure 2. Body weight (grams) of mice in SC-SC, HF-HF, HF-LC5, HF-LC10, HF-LC15, HF-SC dietary groups at the end of phase 1 of the study. Data are expressed as mean ± SEM. Values with a common letter do not differ, p > .05.
Figure 3. Weight (grams) of mice in SC-SC, HF-HF, HF-LC5, HF-LC10, HF-LC15, HF-SC dietary groups at the conclusion of the study. Data are expressed as mean ± SEM. Values with a common letter for a particular time point do not differ, p > .05.
Figure 4. Weight Accumulation (grams) of mice in SC-SC, HF-HF, HF-LC5, HF-LC10, HF-LC15, HF-SC dietary groups during phase 2 of the study. Data are expressed as mean ± SEM. Values with a common letter for a particular time point do not differ, p > .05.
groups still had a slight increase in their body weight. HF-LC5 group had the most significant weight loss than HF-LC10, HF-LC15 and HF-SC groups (p < .05). HF-SC lost more body weight than HF-LC15 (p < .05), and there was no significant difference in body weight loss between the HF-SC and HF-LC10 groups (p > .05). From week 4 to week 6, all 6 dietary treatment groups had the same trend in body weight change as in week 4. SC-SC and HF-HF groups had little change their body weights. HF-LC5 lost more body weight than HF-LC10, HF-LC15 and HF-SC groups (HF-LC5 vs. HF-LC10, p < .001. HF-LC5 vs. HF-LC15, p < .001. HF-LC5 vs. HF-SC, p = .001), and there was no significant difference in body weight loss among HF-LC10, HF-LC15 and HF-SC groups (p > .05). The total weight accumulation after 6 weeks dietary treatment was -0.23 ± 0.21g in SC-SC group, 1.47 ± 0.43g in HF-HF group, -14.43 ± 1.05g in HF-LC5 group, -7.98 ± 1.06g in HF-LC10 group, -7.47 ± 0.72g in HF-LC15 group, and -9.69 ± 0.87g in HF-SC group.

**Total Fat Mass Accumulation**

The accumulation of fat mass over the course of phase 2 of the study was altered with different dietary treatments ($F(5,44)=59.63$, p < .001). Fat accumulation data from all time points in phase 2 are shown in Figure 5. SC-SC group had very little fat mass change for all of phase 2. HF-HF group had an increase in their body fat mass in the first 3 weeks and, eventually, HF-HF group had 1.54 ± 0.31g fat mass increase totally during phase 2 of the study.
For the HF-LC5, HF-LC10, HF-LC15, and HF-SC groups, they began to lose their fat mass after 1 week of dietary treatment. At the end of week 1, HF-SC group lost more fat mass than HF-LC5, HF-LC10, and HF-LC15 (p < .05). There was no significant difference in fat mass loss among HF-LC5, HF-LC10, HF-LC15 groups (p > .05). After 2 weeks treatment, more fat mass was lost in each group. HF-LC5 group began to catch up with HF-SC group in fat mass loss and there was no significant difference in fat mass loss between HF-LC5 and HF-SC at this time point (p > .05). In subsequent weeks, additional fat mass was lost progressively by these groups but the trend was similar to week 2 for each group. That is, HF-LC5 and HF-SC both lost more fat mass than HF-LC10 and HF-LC15 groups (HF-LC5 vs. HF-LC10, p = .001, HF-LC5 vs. HF-LC15, p < .001. HF-SC vs. HF-LC10, p = .002. HF-SC vs. HF-LC15, p < .001). There was no significant difference in fat mass loss between HF-LC5 and HF-SC groups (p > .05), and there was no significant difference between HF-LC10 and HF-LC15 groups (p > .05). HF-LC5 and HF-SC groups lost the most fat mass at -10.99 ± 0.89g and -10.72 ± 0.97g fat mass, respectively, followed by -6.66 ± 0.91g of fat mass lost in the HF-LC10 group and 5.80 ± 0.62g of fat mass lost in the HF-LC15 group.
Figure 5. Body fat (grams) of mice in SC-SC, HF-HF, HF-LC5, HF-LC10, HF-LC15, HF-SC dietary groups during phase 2 of the study. Data are expressed as mean ± SEM. Values with a common letter for a particular time point do not differ, p > .05.
**Total Lean Mass Accumulation**

The lean mass accumulation over the course of phase 2 of the study was altered with dietary treatment ($F(5,44)=20.62$, $p < .001$). Lean mass accumulation data from phase 2 of the study is shown for all time points in Figure 6. From week 1 to week 6, the lean mass change of SC-SC and HF-HF groups fluctuated in a very small range. After the first 2 weeks dietary treatments, the lean mass accumulation in HF-SC group was negative. However, from week 3 to week 6, the lean mass accumulation of HF-SC group was continuously positive. Low-carbohydrate diet treatment groups were continuously negative throughout phase 2 of the study. The lean mass loss of low-carbohydrate diet treatment groups peaked at week 2, and did not change much for the remaining time of phase 2 of the study. After 6 weeks of dietary treatment, the lean mass accumulation was positive in only the HF-SC and HF-HF groups, and there was no significant difference in lean mass accumulation between them ($p > .05$). Among the HF-LC5, HF-LC10 and HF-LC15 groups, there was a negative lean mass accumulation. The HF-LC5 group lost more lean mass than HF-LC10 and HF-LC15 groups (HF-LC5 vs. HF-LC10, $p = .002$. HF-LC5 vs. HF-LC15, $p = .006$), and there was no significant difference in lean mass lost between HF-LC10 and HF-LC15 groups ($p > .05$). At the conclusion of the study, the accumulation of lean mass was $-0.36 \pm 0.11$g in SC-SC, $0.31 \pm 0.19$g in HF-HF, $-2.63 \pm 0.38$g in HF-LC5, $-0.85 \pm 0.33$g in HF-LC10, $-1.04 \pm 0.37$g in HF-LC15, and $1.38 \pm 0.34$g in HF-SC.
Figure 6. Body lean mass (grams) of mice in SC-SC, HF-HF, HF-LC5, HF-LC10, HF-LC15, HF-SC dietary groups during phase 2 of the study. Data are expressed as mean ± SEM. Values with a common letter for a particular time point do not differ, p > .05.
Fluid Mass Accumulation

Fluid mass accumulation over the course of phase 2 of the study varied with different dietary treatments \((F(5,44)=19.78, p < .001)\). The fluid mass accumulation data from phase 2 of the study is shown for all time points in Figure 7. For the whole phase 2 of the study, the fluid mass accumulation of SC-SC and HF-HF was never negative and they fluctuated in a very small range. The low-carbohydrate diet treatment groups and HF-SC group were continuously negative from week 1 to week 6. However, fluid mass loss of low-carbohydrate diet groups and HF-SC group peaked at week 2 and did not change much for the remaining time of phase 2. After 6 weeks dietary treatment, the fluid mass accumulation was about 0g in SC-SC group and positive in HF-HF group. Among the HF-LC5, HF-LC10, HF-LC15 and HF-SC groups with negative fluid mass accumulation, HF-LC5 lost more fluid mass than the HF-LC10 and HF-LC15 groups \((HF-LC5 \text{ vs. } HF-LC10, p = .001, HF-LC5 \text{ vs. } HF-LC15, p = .01)\) and had no significant difference in fluid mass loss as compared to HF-SC group. At the conclusion of the study, fluid mass loss was \(0 \pm 0.03g\) in SC-SC, \(0.18 \pm 0.06g\) in HF-HF group, \(-0.76 \pm 0.10g\) in HF-LC5, \(-0.29 \pm 0.09g\) in HF-LC10, \(-0.37 \pm 0.08g\) in HF-LC15, and \(-0.50 \pm 0.08g\) HF-SC group.
Figure 7. Body fluid (grams) measurements of mice in SC-SC, HF-HF, HF-LC5, HF-LC10, HF-LC15, HF-SC dietary strategy groups during phase 2 of the study. Data are expressed as mean ± SEM. Values with a common letter for a particular time point do not differ, p > .05.
Fat, Lean, and Fluid Accumulation in Body Weight Accumulation

At the conclusion of the study, fat, lean, and fluid accumulation was compared to body weight accumulation (see Figure 8). There was no weight loss in SC-SC and HF-HF groups. The HF-LC5, HF-LC10, HF-LC15, and HF-SC groups had weight loss. In HF-LC5 group, fat mass, lean mass, and fluid mass accumulation were 76%, 18%, and 5% of body weight accumulation, respectively. In HF-LC10 group, fat mass, lean mass, and fluid mass accumulation were 83%, 10%, and 4% of body weight accumulation, respectively. In HF-LC15 group, fat mass, lean mass, and fluid mass accumulation were 76%, 14%, and 5% of body weight accumulation, respectively. In HF-SC group, fat mass and fluid mass accumulation were 111%, and 5% of body weight accumulation, respectively. Lean mass accumulation was positive and increased with SC diet in this group during phase 2 of the study. Fat mass loss in HF-LC5, HF-LC10, HF-LC15 and HF-SC groups was major in body weight loss.
Figure 8. Comparing fat, lean, and fluid accumulation to body weight accumulation in SC-SC, HF-HF, HF-LC5, HF-LC10, HF-LC15, HF-SC dietary groups at the conclusion of the study. Data are expressed as mean ± SEM.
Weights of Four Fat Depots

Fat mass in subcutaneous, epididymal, retroperitoneal and mesenteric depots varied with different dietary treatments at the conclusion of the study. Weights of four fat depots are shown in Table 5. The subcutaneous depot varied in different groups ($F(5,44)=14.07, p < .001$). HF-HF had higher fat mass than all other five groups (HF-HF vs. SC-SC, $p < .001$. HF-HF vs. HF-LC5, $p < .001$. HF-HF vs. HF-LC10, $p = .001$. HF-HF vs. HF-LC15, $p = .009$. HF-HF vs. HF-SC, $p < .001$). HF-LC15 had higher fat mass than SC-SC and HF-SC groups (HF-LC15 vs. SC-SC, $p = .029$. HF-LC15 vs. HF-SC, $p = .046$), and had no significant difference in fat mass as compared to HF-LC5 and HF-LC10 groups ($p > .05$). There was no significant difference in weights of subcutaneous fat mass among SC-SC, HF-LC5, HF-LC10 and HF-SC groups ($p > .05$). Using the HF-HF group as the standard control group, the percent change in weight of the subcutaneous fat mass of the HF-LC5, HF-LC10, HF-LC15 and HF-SC groups was -80.65%, -53.00%, -45.21% and -82.82%, respectively. The epididymal fat pads varied with different dietary treatments ($F(5,44)=13.54, p < .001$). HF-HF and HF-LC15 groups had higher fat mass than SC-SC, HF-LC5 and HF-SC groups (HF-HF vs. SC-SC, $p = .001$. HF-HF vs. HF-LC5, $p < .001$. HF-HF vs. HF-SC, $p < .001$. HF-LC15 vs. SC-SC, $p = .015$. HF-LC15 vs. HF-LC5, $p < .001$. HF-LC15 vs. HF-SC, $p < .001$) and had no significant difference as compared to HF-LC10 ($p < .05$). There was no significant difference in weights of epididymal fat mass among SC-SC, HF-LC5 and HF-SC groups ($p > .05$). Using the HF-HF group as the standard control group, the percent change in weight of the epididymal...
fat mass of the HF-LC5, HF-LC10, HF-LC15 and HF-SC groups was -65.41%, -30.25%, -11.08% and -68.85%, respectively. The retroperitoneal fat pads varied with different dietary treatments too ($F(5,44)=18.48$, $p < .001$). HF-HF group had more fat mass than the other five groups (HF-HF vs. SC-SC, $p < .001$. HF-HF vs. HF-LC5, $p < .001$. HF-HF vs. HF-LC10, $p < .001$. HF-HF vs. HF-LC15, $p = .009$. HF-HF vs. HF-SC, $p < .001$). Both HF-LC10 and HF-LC15 had more fat mass than HF-LC5 and HF-SC groups (HF-LC10 vs. HF-LC5, $p = .019$. HF-LC10 vs. HF-SC, $p = .018$. HF-LC15 vs. HF-LC5, $p = .001$. HF-LC15 vs. HF-SC, $p = .001$). There was no significant difference in weights of retroperitoneal fat pad mass among SC-SC, HF-LC5 and HF-SC groups ($p > .05$). Using the HF-HF group as the standard control group, the percent change in weight of the retroperitoneal fat mass of the HF-LC5, HF-LC10, HF-LC15 and HF-SC groups was -78.69%, -45.87%, -35.32% and -79.08%, respectively. The mesenteric fat pads varied with different dietary treatments ($F(5,44)=20.54$, $p < .001$). Among the 6 groups, the HF-HF group had more fat mass than the other five groups (HF-HF vs. SC-SC, $p < .001$. HF-HF vs. HF-LC5, $p < .001$. HF-HF vs. HF-LC10, $p < .001$. HF-HF vs. HF-LC15, $p < .001$. HF-HF vs. HF-SC, $p < .001$) and there was no significant difference in weights of mesenteric fat mass among SC-SC, HF-LC5, HF-LC10, HF-LC15 and HF-SC groups ($p > .05$). Using the HF-HF group as the standard control group, the percent change in weight of the mesenteric fat mass of the HF-LC5, HF-LC10, HF-LC15 and HF-SC groups were -81.56%, -63.70%, -60.84% and -84.68%, respectively.
Table 5

Weights (Grams) of Fat Mass in Four Depots of Mice in SC-SC, HF-HF, HF-LC5, HF-LC10, HF-LC15, HF-SC Dietary Groups at the Conclusion of the Study

<table>
<thead>
<tr>
<th>Depots</th>
<th>SC-SC</th>
<th>HF-HF</th>
<th>HF-LC5</th>
<th>HF-LC10</th>
<th>HF-LC15</th>
<th>HF-SC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subcutaneous Fat</td>
<td>0.49±0.07a</td>
<td>2.09±0.46b</td>
<td>0.56±0.15ac</td>
<td>1.37±0.26ac</td>
<td>1.59±0.31c</td>
<td>0.50±0.09a</td>
</tr>
<tr>
<td>Epididymal Fat</td>
<td>0.08±0.10ac</td>
<td>1.54±0.11b</td>
<td>0.53±0.08a</td>
<td>1.08±0.18b</td>
<td>1.37±0.13b</td>
<td>0.48±0.07b</td>
</tr>
<tr>
<td>Retroperitoneal Fat</td>
<td>0.24±0.03a</td>
<td>0.52±0.04c</td>
<td>0.11±0.022a</td>
<td>0.28±0.06a</td>
<td>0.34±0.04c</td>
<td>0.11±0.02a</td>
</tr>
<tr>
<td>Mesenteric Fat</td>
<td>0.37±0.04a</td>
<td>1.54±0.22b</td>
<td>0.28±0.03a</td>
<td>0.56±0.11a</td>
<td>0.60±0.08a</td>
<td>0.24±0.03a</td>
</tr>
</tbody>
</table>

Note. Shown is mean ± SEM. Values with a common letter for a particular fat depot do not differ, p > .05.

Organ Weights

Organ weights did not vary very much between the different dietary treatments. Organ weights of mice in different groups are shown in Table 6. For heart ($F(5,44)=3.31$, $p = .013$), HF-HF group had higher weights among 6 groups (HF-HF vs. SC-SC, $p = .02$). HF-HF vs. HF-LC5, $p = .025$. HF-HF vs. HF-LC10, $p = .616$. HF-HF vs. HF-LC15, $p = .076$. HF-HF vs. HF-SC, $p = .654$), and there is no significant difference in heart weights among the other 5 groups ($p > .05$). For liver ($F(5,44)=9.17$, $p < .001$), the HF-HF group had higher weights of liver tissue than HF-LC5, HF-LC10, HF-LC15, and HF-SC groups (HF-HF vs. HF-LC5, $p < .001$. HF-HF vs. HF-LC10, $p < .001$. HF-HF vs. HF-LC15, $p < .001$. HF-HF vs. HF-SC, $p = .004$). There was no significant difference in liver weights
among HF-LC5, HF-LC10, HF-LC15, and HF-SC groups (p > .05). For kidney (F(5,44)=4.47, p = .002), HF-SC groups had higher weights than the SC-SC and HF-LC5 groups (HF-SC vs. SC-SC, p = .007. HF-SC vs. HF-LC5, p = .001), with no significant difference in kidney as compared to HF-HF, HF-LC10, HF-LC15 groups (p > .05).

Table 6

Organ Weights (Gram) of Mice in SC-SC, HF-HF, HF-LC5, HF-LC10, HF-LC15, HF-SC Dietary Groups at the Conclusion of the Study

<table>
<thead>
<tr>
<th>Organs</th>
<th>SC-SC</th>
<th>HF-HF</th>
<th>HF-LC5</th>
<th>HF-LC10</th>
<th>HF-LC15</th>
<th>HF-SC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>0.14±0.00a</td>
<td>0.16±0.00b</td>
<td>0.14±0.00a</td>
<td>0.15±0.00ab</td>
<td>0.14±0.01ab</td>
<td>0.15±0.01ab</td>
</tr>
<tr>
<td>Liver</td>
<td>1.89±0.12abc</td>
<td>2.34±0.22a</td>
<td>1.39±0.05b</td>
<td>1.54±0.09bc</td>
<td>1.41±0.05b</td>
<td>1.68±0.06bc</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.32±0.01a</td>
<td>0.34±0.01ab</td>
<td>0.31±0.01a</td>
<td>0.34±0.01ab</td>
<td>0.34±0.01ab</td>
<td>0.37±0.01b</td>
</tr>
</tbody>
</table>

Note. Data are expressed as mean ± SEM. Values with a common letter for a particular organ does not differ, p > .05.

Energy Intake

Energy intake varied in different dietary treatments during phase 2 of the study (F(5,44)=17.11, p < .001). Total energy consumption of each group in the last 4 weeks is
showed in Figure 9. HF-SC groups had more energy intake than the SC-SC, HF-HF, HF-LC5, HF-LC10 and HF-LC15 groups (HF-SC vs. SC-SC, p < .001. HF-SC vs. HF-HF, p = .001. HF-SC vs. HF-LC5, p < .001. HF-SC vs. HF-LC10, p < .001. HF-SC vs. HF-LC15, p < .001). HF-LC15 had less energy intake than the SC-SC, HF-HF, HF-LC5, HF-SC groups (HF-LC15 vs. SC-SC, p = .025. HF-LC15 vs. HF-HF, p = .001. HF-LC15 vs. HF-LC5, p = .006. HF-LC15 vs. HF-SC, p < .001) and had no difference in energy intake as compared to HF-LC10 (p > .05). There was no significant difference in total energy intake between the SC-SC, HF-HF, HF-LC5 and HF-LC10 groups (p > .05). In the last 4 weeks of the study, the total energy consumption of each mouse was 298.23 ± 2.84kcal in SC-SC, 311.05 ± 6.36kcal in HF-HF, 300.41 ± 12.28kcal in HF-LC5, 286.80 ± 8.36kcal in HF-LC10, 240.35 ± 6.43kcal in HF-LC15, and 382.76 ± 21.91kcal in HF-SC.

**Blood Glucose**

The blood glucose levels of mice over the course of phase 2 of the study varied with different dietary treatment ($F(5,44)=24.99$, $p < .001$). Blood glucose levels at the conclusion of phase 2 of the study are shown in Table 7. After 6 weeks of dietary treatment, HF-HF group had higher blood glucose levels as compared to SC-SC, HF-LC5, HF-LC10, HF-LC15 (HF-HF vs. SC-SC, $p = .015$. HF-HF vs. HF-LC5, $p < .001$. HF-HF vs. HF-LC10, $p < .001$. HF-HF vs. HF-LC15, $p < .001$), and had no significant difference in blood glucose levels as compared to HF-SC ($p > .05$). HF-LC5 had the
lowest blood glucose levels among the 6 groups (HF-LC5 vs. SC-SC, p < .001. HF-LC5 vs. HF-HF, p < .001. HF-LC5 vs. HF-LC10, p = .039. HF-LC5 vs. HF-LC15, p < .001. HF-LC5 vs. HF-SC, p < .001). The blood glucose levels of HF-LC10 were lower than that of SC-SC, HF-SC groups (HF-LC10 vs. SC-SC, p = .007. HF-LC10 vs. HF-SC, p < .001), and did not differ from that of HF-LC15 (p > .05). There was no significant difference in blood glucose levels between the SC-SC and HF-SC groups (p > .05), and there was no significant difference between the SC-SC and HF-LC15 groups (p > .05).
Figure 9. Total energy consumption (kcals/animal) of mice in SC-SC, HF-HF, HF-LC5, HF-LC10, HF-LC15, HF-SC dietary groups in the last 4 weeks during phase 2 of the study. Data are expressed as mean ± SEM. Values with a common letter for a particular time point do not differ, p > .05.
**Plasma Insulin Levels**

Plasma insulin levels varied with different dietary treatments at the end of the study ($F(5,44)=9.97, p < .001$). Plasma insulin levels of each group at the conclusion of the study are shown in Table 7. HF-HF group had higher plasma insulin levels than SC-SC, HF-LC5, HF-LC10, and HF-SC groups (HF-HF vs. SC-SC, $p = .001$. HF-HF vs. HF-LC5, $p < .001$. HF-HF vs. HF-LC10, $p < .001$. HF-HF vs. HF-SC, $p = .001$), and had no significant difference in plasma insulin levels as compared to HF-LC15 ($p > .05$). Moreover, there was no significant difference in plasma insulin levels among SC-SC, HF-LC5, HF-LC10, and HF-SC groups ($p > .05$).

**Urinary Ketone Levels and Blood Ketone Levels**

Urine ketone levels varied with different dietary treatments during phase 2 of the study ($F(5,44)=50.92, p < .001$). Urine ketone levels of each group are shown in Table 7. HF-LC5 had higher urine levels than HF-LC10 ($p = .027$), and HF-LC10 had higher urine levels than SC-SC, HF-HF, HF-LC15, and HF-SC groups (HF-LC10 vs. SC-SC, $p < .001$. HF-LC10 vs. HF-HF, $p < .001$. HF-LC10 vs. HF-LC15, $p = .027$. HF-LC10 vs. HF-SC, $p < .001$). There was no significant difference in urine ketone levels among the SC-SC, HF-HF, HF-LC15, and HF-SC groups ($p > .05$). Blood ketone levels were also measured. Most likely due to 12 hours fasting before blood collection, the blood ketone levels were not consistent with urine ketone levels. HF-SC group had the highest blood ketone levels than SC-SC, HF-HF, and HF-LC5 group (HF-SC vs. SC-SC, $p = .006$. HF-
SC vs. HF-HF, p = .004. HF-SC vs. HF-LC5, p = .015). The highest levels of blood ketone were 4.17 ± 0.41 mg/dL in the HF-SC group and the lowest levels were 2.61 ± 0.20 mg/dL in the HF-HF group. The blood ketone levels were 2.75 ± 0.18 mg/dL in SC-SC group, 2.81 ± 0.27 mg/dL in HF-LC5 group, 3.63 ± 0.21 mg/dL in HF-LC10 group, and 3.82 ± 0.22 mg/dL in HF-LC15 group.

**Plasma IGF-1 Levels**

Plasma IGF-1 levels of each group varied with different dietary treatments ($F(5,44)=12.10$, $p < .001$). Plasma IGF-1 levels are showed in Table 7. HF-LC5 group had the lowest levels of IGF-1 among the six groups (HF-LC5 vs. SC-SC, $p < .001$. HF-LC5 vs. HF-HF, $p < .001$. HF-LC5 vs. HF-LC10, $p < .001$. HF-LC5 vs. HF-LC15, $p < .001$. HF-LC5 vs. HF-SC, $p < .001$). There was no significant difference among the remaining groups ($p > .05$).

**Plasma Adiponectin Levels**

Plasma adiponectin levels varied with different dietary treatments ($F(5,44)=14.54$, $p < .001$). Plasma adiponectin levels are shown in Table 7. HF-HF group had higher plasma adiponectin levels than SC-SC, HF-LC5, HF-LC10, HF-LC15, and HF-SC groups (HF-HF vs. SC-SC, $p < .001$. HF-HF vs. HF-LC5, $p < .001$. HF-HF vs. HF-LC10, $p < .001$. HF-HF vs. HF-LC15, $p < .001$. HF-HF vs. HF-SC, $p < .001$). There was no significant difference in plasma adiponectin levels among SC-SC, HF-LC5, HF-LC10,
HF-LC15, and HF-SC groups (p > .05). Adiponectin secretion is mainly associated with adipose tissue (Guerre-Millo, 2008). Circulating adiponectin levels can be changed not only by the production of adipocytes, but also by the number of adipocytes (Guerre-Millo, 2008). Therefore, the plasma adiponectin levels were also corrected by body adipose tissue to show true production in mice after weight change. The results showed normalized plasma adiponectin levels varied with different dietary treatment as well (F(5,44)=5.64, p < .001). Normalized plasma adiponectin levels are also shown in Table 7. HF-LC5 group had higher normalized plasma adiponectin levels than SC-SC, HF-HF, HF-LC10, HF-LC15 groups (HF-LC5 vs. SC-SC, p = .027. HF-LC5 vs. HF-HF, p = .024. HF-LC5 vs. HF-LC10, p = .027. HF-LC5 vs. HF-LC15, p = .001), and did not significantly differ as compared to HF-SC group (p > .05). There was no significant difference in normalized plasma adiponectin levels among SC-SC, HF-HF, HF-LC10, and HF-LC15 groups (p > .05).

**Plasma HDL, LDL, Total Cholesterol, Triglyceride, and Free Fatty Acids Levels**

Plasma lipid profile varied with different dietary treatments at the end of the study. Plasma lipid profile of each group is showed in Table 7. Plasma total cholesterol levels varied with different dietary treatments (F(5,44)=18.56, p < .001). HF-HF group had the highest levels of total cholesterol among all six groups (HF-HF vs. SC-SC, p < .001. HF-HF vs. HF-LC5, p < .001. HF-HF vs. HF-LC10, p < .001. HF-HF vs. HF-LC15, p = .002. HF-HF vs. HF-SC, p < .001). HF-LC5 had lower levels of total cholesterol than
HF-LC15 and HF-HF groups (HF-LC5 vs. HF-LC15, p = .018. HF-LC5 vs. HF-HF, p < .001). There was no significant difference in plasma total cholesterol levels among SC-SC, HF-LC5, HF-LC10 and HF-SC groups (p > .05). Plasma HDL levels varied with each group ($F(5,44)=21.64, p < .001$). The levels of HDL were higher in HF-HF group than that of SC-SC, HF-LC5 and HF-SC (HF-HF vs. SC-SC, p < .001. HF-HF vs. HF-LC5, p < .001. HF-HF vs. HF-SC, p < .001), and there was no significant difference in plasma HDL levels among HF-HF, HF-LC10, and HF-LC15 groups (p > .05). HF-LC5 had lower plasma HDL levels than HF-LC10, HF-LC15 and HF-HF (HF-LC5 vs. HF-LC10, p = .003. HF-LC5 vs. HF-LC15, p = .004. HF-LC5 vs. HF-HF, p < .001), and there was no significant difference in HDL levels between HF-LC5 and SC-SC groups (p > .05) and between the HF-LC5 and HF-SC groups (p > .05). Plasma LDL levels varied with different dietary treatments ($F(5,44)=14.69, p < .001$). HF-HF group had the highest levels of LDL among the six groups (HF-HF vs. SC-SC, p < .001. HF-HF vs. HF-LC5, p < .001. HF-HF vs. HF-LC10, p < .001. HF-HF vs. HF-LC15, p < .001. HF-HF vs. HF-SC, p < .001), and there was no significant difference in LDL levels among SC-SC, HF-LC5, HF-LC10, HF-LC15, and HF-SC groups (p > .05). Triglyceride levels varied with different dietary treatments ($F(5,44)=8.36, p < .001$). HF-LC5 had lower triglyceride levels than SC-SC and HF-SC groups (HF-LC5 vs. SC-SC, p < .001. HF-LC5 vs. HF-SC, p = .003). There was no significant difference among SC-SC, HF-HF, HF-LC15 and HF-SC groups (p > .05). Free fatty acids levels varied with different dietary treatments ($F(5,44)=18.13, p < .001$), SC-SC group had higher free fatty acids levels than HF-HF,
HF-LC5, HF-LC10, HF-LC15 and HF-SC groups (SC-SC vs. HF-HF, p < .001. SC-SC vs. HF-LC5, p < .001. SC-SC vs. HF-LC10, p < .001. SC-SC vs. HF-LC15, p < .001. SC-SC vs. HF-SC, p < .001). There was no significant difference among HF-HF, HF-LC5, HF-LC10, HF-LC15 and HF-SC groups (p > .05).
Table 7

Metabolic Parameters of Mice in SC-SC, HF-HF, HF-LC5, HF-LC10, HF-LC15, HF-SC Dietary Strategy Groups at the Conclusion of the Study

<table>
<thead>
<tr>
<th>Metabolic Parameters</th>
<th>SC-SC</th>
<th>HF-HF</th>
<th>HF-LC5</th>
<th>HF-LC10</th>
<th>HF-LC15</th>
<th>HF-SC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Glucose (mg/dL)</td>
<td>183.4±7.56&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>228.25±7.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>92.38±9.19&lt;sup&gt;c&lt;/sup&gt;</td>
<td>134.75±11.77&lt;sup&gt;d&lt;/sup&gt;</td>
<td>158.38±8.52&lt;sup&gt;de&lt;/sup&gt;</td>
<td>199.38±12.51&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Plasma Insulin (ng/ml)</td>
<td>0.90±0.12&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>1.92±0.25&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.33±0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.76±0.15&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>1.2±0.24&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>0.84±0.09&lt;sup&gt;ad&lt;/sup&gt;</td>
</tr>
<tr>
<td>Urinary Ketone (mg/dL)</td>
<td>0.10±0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.50±0.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.88±0.35&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.63±0.33&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.00±0.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.13±0.29&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Blood Ketone (mg/dL)</td>
<td>2.75±0.18&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>2.61±0.20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.81±0.27&lt;sup&gt;bd&lt;/sup&gt;</td>
<td>3.63±0.21&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>3.82±0.22&lt;sup&gt;acd&lt;/sup&gt;</td>
<td>4.17±0.41&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>IGF-1 (ng/ml)</td>
<td>230.36±13.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>287.68±17.98&lt;sup&gt;a&lt;/sup&gt;</td>
<td>101.87±10.84&lt;sup&gt;b&lt;/sup&gt;</td>
<td>228.93±25.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>250.94±21.96&lt;sup&gt;a&lt;/sup&gt;</td>
<td>276.4±21.39&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Adiponectin Levels</td>
<td>33.71±2.97&lt;sup&gt;a&lt;/sup&gt;</td>
<td>112.87±21.14&lt;sup&gt;b&lt;/sup&gt;</td>
<td>33.21±2.89&lt;sup&gt;a&lt;/sup&gt;</td>
<td>29.76±1.66&lt;sup&gt;a&lt;/sup&gt;</td>
<td>33.96±1.64&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.56±1.75&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Normalized Adiponectin by Body Fat (ug/ml/g)</td>
<td>7.49±0.575&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>7.00±0.75&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>14.86±2.69&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.09±2.42&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>4.29±0.71&lt;sup&gt;c&lt;/sup&gt;</td>
<td>12.83±1.88&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>41.6±3.86&lt;sup&gt;a&lt;/sup&gt;</td>
<td>92.2±2.64&lt;sup&gt;b&lt;/sup&gt;</td>
<td>53.48±3.34&lt;sup&gt;ac&lt;/sup&gt;</td>
<td>76.45±4.73&lt;sup&gt;bd&lt;/sup&gt;</td>
<td>76.22±6.09&lt;sup&gt;be&lt;/sup&gt;</td>
<td>62.94±1.91&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
<tr>
<td>LDL(mg/dL)</td>
<td>19.39±0.72&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36.68±4.66&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18.36±1.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.32±1.45&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.21±1.90&lt;sup&gt;e&lt;/sup&gt;</td>
<td>12.14±0.85&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total Cholesterol (mg/dL)</td>
<td>77.15±1.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>187.52±17.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>83.24±6.99&lt;sup&gt;a&lt;/sup&gt;</td>
<td>116.12±10.37&lt;sup&gt;d&lt;/sup&gt;</td>
<td>130.1±11.98&lt;sup&gt;de&lt;/sup&gt;</td>
<td>90.02±2.54&lt;sup&gt;ac&lt;/sup&gt;</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>77.49±5.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>61.87±3.56&lt;sup&gt;b&lt;/sup&gt;</td>
<td>33.77±5.17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>46.32±4.56&lt;sup&gt;bd&lt;/sup&gt;</td>
<td>58.06±5.75&lt;sup&gt;be&lt;/sup&gt;</td>
<td>64.63±7.19&lt;sup&gt;ac&lt;/sup&gt;</td>
</tr>
<tr>
<td>Free Fatty Acids (mEq/L)</td>
<td>0.78±0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.44±0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.29±0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.23±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.24±0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.36±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Note.* Data are expressed as mean ± SEM. Values with a common letter for a particular metabolic parameter do not differ, p > 0.05.
CHAPTER 5: DISCUSSION

Obesity has become a serious chronic disease in both developing and developed countries (Grundy & Barnett, 1990; Simopoulos & Van Itallie, 1984). The increasing incidence of obesity and related complications has prompted intensive research into prevention and treatment of this condition, now considered to be a major epidemic. Recent studies from various laboratories and clinical centers have shown that low-carbohydrate diets are quite effective in reducing body weight and risk factors associated with various chronic diseases (Dashti, et al., 2003; Stern, et al., 2004). However, there are other studies that report no significant effects of low-carbohydrate diets either on reducing body weight or improving metabolic parameters (Hamdy, Turner, Pyzik, & Kossoff, 2007; Segal-Isaacson et al., 2004). Therefore, the efficacy and safety of low-carbohydrate diets remain controversial and unknown.

Previous studies in our laboratory showed that low-carbohydrate diets (5.1% carbohydrate or 0% carbohydrate) with high fat and high protein failed to promote weight loss in mice (List, et al., 2005). Importantly, these particular low-carbohydrate diets also elevated blood glucose, insulin levels, and impaired glucose tolerance in mice. The low-carbohydrate diets that were used in this study were low in carbohydrate and high in fat as before, but more restrictive in protein. These diets were chosen to examine the effects of severe carbohydrate restriction on body weight change while at the same time limiting gluconeogenesis from dietary protein. Additionally, it is reported that the protein requirement for rats is at least 5% g/kg of diets for body mass maintenance and 15% g/kg
to promote grow (National Research Council, 1995). Previous studies had used low-carbohydrate diets with 5% protein or 8% protein in calories to promote weight loss in mice (Kennedy, et al., 2007; Van der Auwera, Wera, Van Leuven, & Henderson, 2005). Therefore, the diets in our study can likely meet the protein need of adult mice.

In the present study, mice were placed on a total of six dietary strategies (SC-SC, HF-HF, HF-LC5, HF-LC10, HF-LC15, HF-SC). Three of these groups were controls and included mice that remain on either a HF diet (HF-HF) or lower fat diet (SC-SC) and those that went from the high-fat control diet switched to the lower fat diet (HF-SC). These mice were used for comparison purposes for the three low-carbohydrate diets. As for general findings, body composition analyses revealed that the mice in HF-LC5 and HF-SC had the greatest weight loss and fat mass loss. Importantly, the HF-SC group not only had significant weight loss but also had the greatest lean mass increase. For all groups that lost weight, weight loss was primarily due to fat mass loss and not fluid and lean loss. Daily food intake measurement showed limiting calorie intake was not the main means by which these mice lost weight. The LC5 and LC10 diets were the most effective for inducing ketosis, and blood glucose levels were lowered in all low-carbohydrate diet groups. Plasma insulin levels in the three low-carbohydrate diet groups were also similar to that seen in SC-SC and HF-SC groups at the conclusion of the study. Moreover, LC diets had significant improvements in total cholesterol, LDL, triglyceride and HDL levels, and did not change liver, kidney and heart weights as compared to SC-SC groups. IGF-1 levels were decreased in LC5 groups. Thus, all the LC diets in this study were
effective at reducing weight in obese mice and improving several metabolic parameters after 6 weeks of treatment.

Although this study, and many of clinical and laboratory studies have shown low-carbohydrate diets had significant effects on weight loss, the mechanisms related to how these diets promote weight loss are still controversial. Proposed mechanisms for weight loss include altered appetite regulation (Volek & Westman, 2002), limited energy consumption and reduced -availability of foods (Brehm et al., 2003), loss of total body water at least initially (Bray, 2003), or elevated blood ketone levels caused by low-carbohydrate diets (Mobbs, et al., 2007). However, there is limited evidence for these mechanisms and not every published study agrees with these mechanisms.

Few animal studies have been conducted assessing the effectiveness of low-carbohydrate diets on obesity, increasing concern over the safety of their use in humans. Therefore, it is imperative to establish an animal system, like a mouse model, to evaluate their effectiveness and safety in long term, as well as explore mechanisms of how low-carbohydrate diets cause weight loss. Diet is an important variable when conducting studies on obesity. In most human studies involving dietary treatments, the patient’s adherence to a specific diet is difficult to control (Brehm et al., 2003). Animal studies offer the advantage of being able to totally control macronutrient composition of diets and to more fully assess impact of such diets on organs and specific metabolic parameters.
Low-Carbohydrate Diets on Weight Loss

In terms of weight loss, different dietary strategies caused distinct changes in body weight in this study. For the three low-carbohydrate diets, they all reduced body weight and fat mass in C57L/6J obese mice. LC5 diet had the most effect on reducing body weight, even better than standard chow (Relatively low-fat diet), which was the second most effective on weight loss. These positive results on body weight loss of low-protein, low-carbohydrate diets in mice have been reported previously in studies in which 5% protein, low-carbohydrate diet caused significant weight loss in 2 months treatment (Kennedy, et al., 2007), and 4.2% protein, low-carbohydrate diet led to weight loss in 16 days treatment (Caton et al., 2008). However, to the best of our knowledge, no studies have compared the effects of low-carbohydrate diets on weight loss when the protein percentage has been manipulated. In our study, as the protein in the diets decreased, the loss of body weight increased in these three low-carbohydrate diet groups. Among these three low-carbohydrate diet treatments, LC5 had the most body weight loss and an even greater loss than HF-SC dietary strategy (-14.4±1.0g vs. -9.7±0.9g). Importantly, there was some loss in lean mass loss in low-carbohydrate groups, which also contributed to the significant body weight loss in these groups. The mice in these three LC groups started to lose their body weight at the first week of phase 2 of the study, and the body weight loss effect was consistent throughout phase 2 of the dietary treatments and may have continued if the dietary treatments continued for longer than 6 weeks.
Low-Carbohydrate Diets Changed Body Composition

The diets resulted in major changes in body composition. During the phase 2 of the study, fat mass and lean mass were reduced in these three low-carbohydrate diets. While HF-SC group had decreased fat mass and increased lean mass. Fat mass and lean mass loss increased as the amount of the protein decreased in three low-carbohydrate diets, which also indicated that reducing protein in these diets directly contributed to the fat and lean mass loss. However, fat mass loss was significantly more than lean mass loss during the whole stage of phase 2 of the study. Loss of fat mass began by the first week of phase 2 and continued throughout phase 2 of the study, which also indicated that the trend of fat mass loss may have continued if the dietary treatments continued for longer than 6 weeks. However, for all these three LC groups, the lean mass loss started at week 1, and reached its peak at week 2, and fluctuated in a very small range during the rest of phase 2 of the study. This suggested that the loss of lean mass would not have continued and began to stabilize. Furthermore, in the same group of three LC diet groups, the loss in lean mass was always less than the loss in fat mass at any time point of phase 2 of the study. At the end of the study, the fat mass loss vs. lean mass loss was about 10.00g vs. 2.63g in LC5, 6.66g vs. 0.85g in LC10, and 5.80g vs. 1.04g in LC15. Therefore, the weight loss was mainly due to the fat mass loss. HF-SC was the only group that had weight loss and fat mass loss while gaining lean mass. As this would be considered favorable, this suggests that the switch from a HF to SC diet may prove to be the most prudent means for weight loss in this animal model.
Low-Carbohydrate Diets and Organ Weights

Significant differences in weights of adipose depots were found in this study after the different diet treatments. HF-LC5 and HF-SC groups had the greatest decrease in the adipose depots measured, with varying decreases seen in the remaining groups. The subcutaneous and mesenteric depots appeared to be the most responsive to diet treatments with a decrease of 80.65% and 81.56%, respectively in HF-LC5 versus HF-HF. This result agreed with a previous study that subcutaneous depot fat mass were more responsive to low-carbohydrate diet treatments than epididymal depot fat mass in rats (Caton et al., 2008). However, the remaining adipose depots were also highly affected by the diet treatments in the present study, with all depots in both HF-LC5 and HF-SC groups decreasing significantly in size. This suggests that all depots respond to the dietary treatment.

Other studies have shown that high-fat diet treatment caused larger heart and liver in mice, and high-fat diet also led to larger heart and liver in HF-HF group of our study due to lipid overload of internal organs (Ehrich, Kenney, Vaughn, Pletscher, & Cheverud, 2003; Mathieu et al., 2008). In our study, there was no significant difference in weights of heart among SC-SC, HF-SC, HF-LC5, HF-LC10, and HF-LC15 groups, and there was no significant difference in weights of liver among HF-SC, HF-LC5, HF-LC10, and HF-15 groups. Thus, all low-carbohydrate diets did not influence the heart, liver and kidney weights in our study.
**Water Loss and Limited Energy Consumption Did not Account for Weight Loss**

Weight loss with low-carbohydrate diets in some studies is attributed to water loss in subjects (Bray, 2003) or limited food intake (Brehm et al., 2003). However, studies with pair feeding between low-carbohydrate diet groups and standard chow groups in mice, showed low-carbohydrate diets caused more body weight loss (Caton et al., 2008; Kennedy, et al., 2007). In this thesis, low-carbohydrate diets did cause a slight loss of fluid that stabilized by 2 weeks of treatment. At the conclusion of the study, LC5 diet caused fluid loss at 0.76g, which is about 5% of the total weight lost. In other groups, fluid loss was 0.29g (3.6% of total body weight loss) in HF-LC10, 0.37g (5.0% of total body weight loss) in HF-LC15. Therefore, fluid loss did not account for the significant weight loss in low-carbohydrate diet groups. It should be noted that the HF-SC groups also had loss of fluid that is comparable to that experienced by the LC groups (0.5g or 5% of the total body weight loss), which suggests that weight loss by any means may result in water loss as well.

According to energy consumption measurements in the last 4 weeks of the phase 2 of the study, HF-HF group had similar energy consumption as compared to SC-SC group even though they had the most weight gain. This may not be surprising considering this strain of mice has been shown in previous studies to promote obesity with high-fat diet feeding and to be protected from obesity with isocalorical levels of low-fat diets (Surwit, et al., 1995; Woods, Seeley, Rushing, D'Alessio, & Tso, 2003). Although HF-
LC5 and HF-LC10 groups had more significant weight loss, these two groups had similar amount of energy intake as compared to SC-SC and HF-HF groups. This suggested limited energy intake was not the reason that these two groups lost their body weight. This outcome was in agreement with some human studies that demonstrated potential benefits of low-carbohydrate diets were over and above caloric restriction (Manninen, 2004; Seshadri & Iqbal, 2006). There are several studies that have reported similar energy intake between low-carbohydrate diets and normal diets in human or standard chow in mice and these studies have shown that, low-carbohydrate diets can cause more significant weight loss (Kennedy, et al., 2007; Keogh et al., 2007). Remarkably and consistent with previous studies (Surwit, et al., 1995), HF-SC group consumed the most energy and lost significant body weight while the HF-HF group did not consume any greater energy and had significant weight gain during phase 2 of the study. These all suggested that not only the total energy consumption, but also the proportion of specific macronutrients has a significant effect on body weight (Rebuffe-Scrive et al., 1993).

**Higher Ketone Bodies Levels in Low-Carbohydrate Diet Groups**

Elevated ketone levels have been implicated in causing weight loss on LC diets. Low ketone levels can alter hypothalamic glucose-sensing neurons to promote post-prandial thermogenesis and increase resting energy expenditure (Almind & Kahn, 2004; Westerterp, 2004). However, a previous study also found that elevated ketone levels did not relate to promoting weight loss (Coleman & Nickols-Richardson, 2005). In our study,
urine ketone levels were elevated significantly in LC5 and LC10 diets and there was no significant difference in levels between the groups of SC-SC and HF-LC15. In a previous study in our laboratory, obese mice were fed low-carbohydrate diets with higher percentage of protein. This diet regimen did not promote loss of body weight or elevated ketones (List, et al., 2005). Other laboratories have reported similar findings. That is, research showed that low-carbohydrate diets (carbohydrate 20%, protein 20%, fat 60%) did not promote ketosis in mice presumably because plasma glucose can be synthesized through gluconeogenesis (Mobbs, et al., 2007; Williams, Perkins, Smith, Hursting, & Lane, 2007). Some of low-carbohydrate diets in this study were able to promote ketosis, at least as indicated by urinary ketone levels. The highest ketone levels in mice were found in the HF-LC5 group, which had the greatest loss in body weight and the least percentage of protein in the diet. The second highest ketone levels were found for HF-LC10 and they had the second lowest percentage of protein in the diet and the second greatest loss in body weight loss. This implies that ketone levels increase with decreasing percentage of protein in the diets and that higher ketones, in some manner, contribute to the weight loss in mice. Although we did not measure resting energy expenditure or lipid metabolic parameters in these mice, ketone status has also been shown to increased expression of genes pivotal in fatty acid oxidation pathways and reduction in lipid synthesis pathways, which has been suggested increase energy expenditure and lead to weight loss in mice (Kennedy, et al., 2007). Blood ketone bodies levels were measured as well in our study. Due to the overnight fasting before blood collection in mice, blood
ketone bodies levels were not consistent with the urinary ketone bodies levels. The highest blood ketone bodies levels were in HF-SC group.

Weight Loss Caused by Low-Carbohydrate Diets Led to Improved Glucose Homeostasis

The relationship of obesity to insulin resistance is a long-recognized phenomenon with fundamentally important scientific and clinical implications. In most cases, insulin resistance is associated with obesity, and weight loss can increase insulin sensitivity (Kahn & Flier, 2000). Insulin resistance is characterized by high levels of insulin and glucose in the blood. In this study, the HF-HF group had significant higher blood glucose and plasma insulin with higher body weights similar to what has been reported previously in C57BL/6J mice (Surwit, et al., 1995). After phase 2 of the study, there was a significant decrease in plasma insulin levels in the SC-SC, HF-LC5, HF-LC10 and HF-SC groups as compared to the HF-HF group. The drop in insulin levels seemed to correlate with the loss in body weight as the groups with the greatest weight loss had the greatest decrease in insulin levels. This suggests that weight loss improved insulin resistance in obese mice and weight loss was more important than dietary composition.

At the conclusion of this study, HF-HF had the highest blood glucose levels, as well as body weight, than other groups. The LC5 had the lowest blood glucose levels and LC10 had the second lowest blood glucose among the six groups and both of these two groups had lower blood glucose levels even those mice that had remained on the standard
chow diet for life (SC-SC group). These results are not surprising as weight loss can contribute to better blood glucose control by improving insulin resistance (Westman et al., 2006). In addition to weight loss, the low-carbohydrate diets in this study likely improved blood glucose control due to their reduced-glycemic index. A reduced-glycemic index diet without weight loss can also lead to improvement in blood glucose control (Westman et al., 2006). In contrast, the SC diet in this study has a higher glycemic index diet and might explain why the HF-SC had higher blood glucose levels than low-carbohydrate diets group. A previous study also showed low-carbohydrate diets (5% protein, 3% carbohydrate and 92% fat) lowered blood glucose levels, as well as insulin levels, in Sprague-Dawley rats (Thio et al., 2006). Likewise, human studies also show beneficial effects on glucose homeostasis with diabetic subjects (Dashti, et al., 2007; Dyson et al., 2007).

**IGF-1 Levels was Only Decreased in LC5 Groups**

Nutritional status is one of the main regulators of circulating insulin-like growth factor 1 (IGF-I) levels, and serum IGF-I concentrations are markedly lowered by energy or protein deprivation ((Maxwell, Butterwick, Batt, & Camacho-Hubner, 1999; Thissen, Ketelslegers, & Underwood, 1994)). The decline of IGF-I levels results from a postreceptor defect in the GH action at the hepatic level, by which IGF-I gene expression is diminished (Thissen et al., 1994). In addition, protein restriction also leads to changes in the levels of circulating IGF-1 binding proteins, which contribute to decreased serum
IGF-1 (Ketelslegers, Maiter, Maes, Underwood, & Thissen, 1995). One previous study also showed that low-carbohydrate diets reduced IGF-1 mRNA levels in Juvenile rats (Cheng et al., 2003). In this study, HF-LC5 had the lowest IGF-1 levels and there was no significant difference in IGF-1 levels among all other treatment groups. Due to the very low percentage of protein in the low-carbohydrate diets, a negative nitrogen balance or protein malnutrition may be a possibility in all the low-carbohydrate groups, which could contribute to the alteration of IGF-1 levels (Fraser, et al., 2000). However, only the HF-LC5 group had significantly lowered IGF-1 levels. IGF-1 is also an important growth and differentiation factor that had potent metabolic, insulin-like actions. Studies have shown that increased insulin levels are associated with increased IGF-1 gene transcription (Kaytor, Zhu, Pao, & Phillips, 2001). However, the HF-LC5 group in the present study had the lowest plasma insulin levels among all six treatment groups. Interestingly, HF-LC10 and HF-LC15 groups had similar IGF-1 levels as compared to SC-SC, HF-HF, and HF-SC groups. This suggested that IGF-1 production is not very sensitive to the change of protein percentage in diets or that only severe restriction of protein (LC5) results in protein malnutrition.

**Adiponectin Production Changed With LC5 and SC Diets**

Several studies have examined the effect of weight loss on adiponectin with conflicting results (Abbasi et al., 2004; Dvorakova-Lorenzova, et al., 2006; Kopp et al., 2005). Plasma levels of adiponectin have been reported to be significantly reduced in
mice and humans with established obesity/diabetes (Arita, et al., 1999; Yamauchi, et al., 2001). In our study, absolute circulating plasma adiponectin levels were higher in HF-HF groups, and there was no significant difference in circulating adiponectin levels between all the five treatment groups. The outcomes that high-fat diets treatment led to higher adiponectin levels in C57BL/6J mice agreed with a previous study using the same strain mice (Bullen, Bluher, Kelesidis, & Mantzoros, 2007). In this previous study, circulating adiponectin levels were corrected to total body fat mass in order to show adiponectin production in total fat mass. Although serum adiponectin levels were elevated in HF-HF groups during phase 2 of the study, once adiponectin levels were corrected for total body fat, HF-LC5 and HF-SC group had significant higher adiponectin production per gram of adipose tissue in our study. This suggested that change of adiponectin secretion levels from total body adipose tissue accounted for the alteration of circulating adiponectin levels, and this also indicated that the highest weight loss in HF-LC5 and HF-SC groups was associated with increased adiponectin secretion levels in adipose tissue. Moreover, increased adiponectin production can relieve insulin resistance in obesity (Yamauchi, et al., 2001), increases energy expenditure and fatty acid oxidation in liver and skeletal muscle by activating AMP-activated protein kinase (Wu et al., 2003; Yamauchi, et al., 2002). This suggested that weight loss, decreased blood glucose and plasma insulin levels were associated with increased adiponectin production in HF-LC5 and HF-SC groups.
Lipid Profile Improved With Low-Carbohydrate Diets in Mice

In this study, low-carbohydrate diets resulted in an overall improved lipid profile. Specifically, low-carbohydrate diets led to greater reduction in plasma LDL, total cholesterol, and triglyceride as compared to HF-HF group, and an increase in HDL as compared to SC-SC group. Several studies have shown LDL levels to be highly predictive of progression of coronary artery disease, and a small LDL level >30mg/dL in humans is associated with 9-fold increased risk of CAD progression (Lamarche, et al., 1997; Rosenson, Otvos, & Freedman, 2002). In our study, the HF-HF group had significant higher LDL levels as compared to the other five treatment groups, and there was no significant difference between these five treatment groups. This suggested that, like standard chow diets, low-carbohydrate diets or the weight loss from these diets have the ability to reduce LDL levels. Further, this effect is independent of protein percentage of protein in low-carbohydrate diets. Actually, there are several studies that show low-carbohydrate diets with higher percentage of protein had significant effect on reducing LDL levels in human (Dashti, et al., 2003; Dashti, et al., 2006; Dashti, et al., 2006; Keogh et al., 2007).

Triglyceride is increasingly though to be important in the pathogenesis of atherosclerosis, and treatments that lower triglyceride levels has been shown to reduce major coronary events (Asztalos et al., 2008; Carlson & Rosenhamer, 1988). High triglyceride levels promote the formation of small LDL and increase circulating LDL levels (Eisenberg, Gavish, Oschry, Fainaru, & Deckelbaum, 1984). Based on our study,
the LC5 diet significantly reduced triglyceride levels. However, the level of protein did seem to impact the ability of the diet to reduce triglyceride levels because triglyceride levels increased with increasing protein content with no difference noted for the HF treatment group and LC15 treatment group. Studies using isotopically labeled very low-density lipoprotein (VLDL) -triacylglycerol tracers in humans suggest that a low-carbohydrate diet would increase hepatic fatty acid oxidation, leading to decreased hepatic fatty acids availability for triglyceride synthesis and hence decreased hepatic VLDL-triglyceride formation and secretion (Rashid, Uffelman, & Lewis, 2002).

Although difficult to explain, it should be noted that the SC treatment group had a similar triglyceride levels as HF diet treatment group. This outcome agreed with a previous study in which C57BL/6 mice fed standard chow had a similar triglyceride levels as mice in a high-fat diet group after 9 weeks treatment (Kennedy, et al., 2007).

High total cholesterol levels and low HDL levels are other strong indicators of those individuals that are prone to coronary heart disease. In our study, HF diet caused significantly higher total cholesterol level as compared to other five treatment groups. In LC diet treatment groups, the total cholesterol levels were reduced progressively and LC5 diet had the lowest total cholesterol levels. There was no significant difference in total cholesterol levels between the groups of SC-SC, HF-LC5, HF-LC10, and HF-SC. This suggested that low-carbohydrate diets, especially LC5, have the same effects on reducing total cholesterol levels as standard chow. In a previous study, high levels of total cholesterol caused by high-fat diet were also decreased by low-carbohydrate diets in mice.
during 5 weeks treatment (Kennedy, et al., 2007). Moreover, calorie-restricted low-carbohydrate diets were more effective on reducing total cholesterol levels than calorie-restricted standard chow in rats even though a similar calorie was consumed in these two diets (Koide et al., 2007). In terms of HDL, several published randomized trials have shown a more favorable response of HDL levels on a low-carbohydrate diet when compared with low-fat diet after 6 months (Foster, et al., 2003) and after 1 year (Foster, et al., 2003; Stern, et al., 2004). In our study, HDL levels were elevated significantly in HF-HF, HF-LC10, HF-LC15 and HF-SC groups while HDL levels were not improved in HF-LC5 group, when they were compared to SC-SC group. The mechanism by which serum HDL levels increase on a low-carbohydrate diet is unknown. However, the increase in HDL on a low-carbohydrate diet could partly result from the increased intake of saturated and unsaturated fats, both of which are known to increase HDL, as well as elevated HDL levels in HF diet in our study (Kris-Etherton & Yu, 1997). In our study, SC-SC group had the highest plasma free fatty acids levels among the six dietary treatment groups and there was no significant difference in plasma free fatty acids levels between the other five treatment groups. Previous study also showed that low-fat diets caused higher plasma free fatty acids levels than high-fat diets did, but with the levels of free fatty acids in adipose tissue and liver (Belury, Moya-Camarena, Liu, & Vanden Heuvel, 1997). The reason for this is unknown but higher levels of linoleic acid in high-fat diets than that of standard chows may contribute to decreased plasma lipids because of increase of linoleic acid- dependent hepatic fatty acid oxidation (Belury et al., 1997;
Faulconnier, Arnal, Patureau Mirand, Chardigny, & Chilliard, 2004). In the HF-SC group, as well as low-carbohydrate diets, significant weight loss might account for the decreased free fatty acids levels in these groups.

**Comparing to Other Published Studies in Mice**

There are several published studies using low-carbohydrate, restricted protein diets in rodent animals (Caton et al., 2008; Kennedy, et al., 2007; Thio et al., 2006; Van der Auwera et al., 2005). All these studies showed that low-carbohydrate, restricted protein diets caused significant weight loss or slowed weight gain in rodent animals with different dietary strategies. All these diets had also been shown to improve physiological parameters in rodent animals, such as lipid profile and blood glucose, etc. Yet, these studies did not agree the possible mechanism of low-carbohydrate diets on body weight loss. Increased leptin levels, elevated ketone body levels, and raised fatty acid oxidation discussed in these studies were all potential factors that accounted for weight loss in rodent animals. Due to different animals and dietary strategies used in these studies, it is hard to put them together and analyze data as a whole. Based on experiments designed and animals used in these studies, Kennedy’s study had lots of factors in common with our study. In order to estimate changes of some variables that we did not measure in the present study, and design a more reasonable experiment for our future continued studies with the same mice model, this study and our study are compared in Table 8. There were two protocols in this study (Kennedy, et al., 2007). The second protocol is compared to
our study. For the second protocol, 24 8-week old C57BL/6 male mice were split into three groups. Two groups fed high-fat diet and one fed standard chow for 12 weeks, and then one of two high-fat groups was switched to a ketogenic diet. These three groups were maintained on these diets for an additional 5 weeks. This experiment design is very similar to our study, which had 16 weeks diets induced obesity (phase 1) and 6 weeks dietary treatments (phase 2). Moreover, in this study, they used the same strain mice (C57BL/6J) with similar ages (6 weeks old in Kennedy’s study, 8 weeks old in our study), and almost the same standard chow and low-carbohydrate diets as we did. A direct comparison of this study with ours is shown in Table 8.

By comparison of our studies with this study, there are very similar outcomes in energy consumption, body weight, body composition change, lipid profiles, glucose homeostasis and insulin levels with low-carbohydrate diet treatments in mice. From Kennedy’s study, AMPK activity was increased and Acetyl-CoA Carboxylase activity was decreased in low-carbohydrate diets group. Energy expenditure and blood ketone bodies levels were increased with the low-carbohydrate diet. Hepatic gene analysis showed that gene related to fatty acid oxidation was increased and related to fatty acid synthesis was decreased in the low-carbohydrate diet group. From our study, the results showed that low-carbohydrate diets had no significant influence on organ weights. However, the effects of low-carbohydrate diets on body weight loss were decreased with the protein percentage increased in low-carbohydrate diets. The LC5 diet had the best effects on body weight loss in C57BL/6J mice, as well as on improving several
physiological parameters. Therefore, the LC5 diet can be used for future study in C57BL/6J mice to explore the exact mechanism of low-carbohydrate diets on body weight loss in mice and evaluate the safety of low-carbohydrate diets.
Table 8

**Comparison of Present Study to a Similar Study**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Kennedy’s study</th>
<th>This study</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diets</strong></td>
<td>SC(16.7% fat, 26.8% protein, 56.4% carbohydrate)</td>
<td>SC(14% fat, 26% protein, 60% carbohydrate)</td>
</tr>
<tr>
<td></td>
<td>HF(45% fat, 24% protein, 35% carbohydrate)</td>
<td>HF(60% fat, 20% protein, 20% carbohydrate)</td>
</tr>
<tr>
<td></td>
<td>LC(95% fat, 0% carbohydrate, 5% protein)</td>
<td>LC5(93% fat, 2% carbohydrate, 5% protein)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LC10(88% fat, 2% carbohydrate, 10% protein)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LC15(83% fat, 2% carbohydrate, 15% protein)</td>
</tr>
<tr>
<td><strong>Animals</strong></td>
<td>C57BL/6J mice, 6 weeks old</td>
<td>C57BL/6J mice, 8 weeks old.</td>
</tr>
<tr>
<td><strong>Dietary strategy</strong></td>
<td>Diet induce obesity-12 weeks (Phase 1)</td>
<td>Diet induce obesity-16 weeks (Phase 1)</td>
</tr>
<tr>
<td></td>
<td>Diet treatments-5 weeks (Phase 2)</td>
<td>Diet treatments-6 weeks (Phase 2)</td>
</tr>
<tr>
<td><strong>Energy consumption</strong></td>
<td>Energy consumption similar in each group.</td>
<td>SC-SC, HF-HF, HF-LC5, HF-LC10 had similar energy consumption.</td>
</tr>
<tr>
<td><strong>Body weight</strong></td>
<td>LC reduced body weight significantly</td>
<td>LC diets and HF-SC had significant weight loss.</td>
</tr>
<tr>
<td><strong>Fat mass</strong></td>
<td>Decreased in LC.</td>
<td>LC diets and HF-SC had significant fat loss.</td>
</tr>
<tr>
<td><strong>Lean mass</strong></td>
<td>No difference lean mass change between LC and SC.</td>
<td>Lean mass loss in LC diets, and increased in HF-SC.</td>
</tr>
<tr>
<td><strong>Energy expenditure</strong></td>
<td>Higher in LC.</td>
<td>Not measured</td>
</tr>
<tr>
<td><strong>Locomotor activity</strong></td>
<td>No difference among each group.</td>
<td>Not measured</td>
</tr>
<tr>
<td><strong>Insulin levels</strong></td>
<td>Decreased in LC.</td>
<td>Increased in HF, decreased in LC5, LC10 and HF-SC.</td>
</tr>
<tr>
<td><strong>Leptin levels</strong></td>
<td>Lower in LC than HF.</td>
<td>Not measured</td>
</tr>
<tr>
<td><strong>Ketone bodies</strong></td>
<td>Blood ketone increased in LC.</td>
<td>Urinary ketone increased in LC diets.</td>
</tr>
<tr>
<td><strong>Blood glucose</strong></td>
<td>Not measured</td>
<td>Decreased in LC diets and increased in HF.</td>
</tr>
<tr>
<td><strong>Glucagon</strong></td>
<td>Lower in LC than HF.</td>
<td>Not measured</td>
</tr>
<tr>
<td><strong>TSH</strong></td>
<td>Higher in LC than HF.</td>
<td>Not measured</td>
</tr>
<tr>
<td><strong>IGF-1</strong></td>
<td>Not measured</td>
<td>Lower in LC5.</td>
</tr>
<tr>
<td><strong>Adiponectin</strong></td>
<td>Not measured</td>
<td>Circulating adiponectin higher in HF.</td>
</tr>
<tr>
<td><strong>Free fatty acids</strong></td>
<td>Lower in LC than HF.</td>
<td>LC diets lower than control.</td>
</tr>
<tr>
<td><strong>Triglycerides</strong></td>
<td>Lower in LC than HF.</td>
<td>Lowed in LC5 group.</td>
</tr>
<tr>
<td><strong>Total cholesterol</strong></td>
<td>Lower in LC than HF.</td>
<td>Lower in LC5, LC10 than HF.</td>
</tr>
<tr>
<td><strong>HDL</strong></td>
<td>Not measured</td>
<td>LC10 and LC15 higher than SC-SC.</td>
</tr>
<tr>
<td><strong>LDL</strong></td>
<td>Not measured</td>
<td>Similar in LC diets and SC-SC.</td>
</tr>
<tr>
<td><strong>Organ weights</strong></td>
<td>Not measured</td>
<td>No difference in heart, liver, kidney among LC diets and SC-SC.</td>
</tr>
<tr>
<td><strong>Hepatic gene analysis</strong></td>
<td>Gene related to fatty acid oxidation increased in LC.</td>
<td>Not measured</td>
</tr>
<tr>
<td><strong>AMPK</strong></td>
<td>Increased in LC.</td>
<td>Not measured</td>
</tr>
<tr>
<td><strong>Acetyl-CoA carboxylase</strong></td>
<td>Decreased in LC.</td>
<td>Not measured</td>
</tr>
</tbody>
</table>
Future Studies

In our study, we were able to both confirm and extend existing knowledge regarding the effects of low-carbohydrate diets on body weight in mice (Kennedy, et al., 2007; Van der Auwera et al., 2005). Additional studies are still needed to resolve several issues raised in the present study. One of the most important unresolved issues relates to the mechanisms that caused the weight loss. In this study, limiting energy consumption and fluid loss do not appear to be the major mechanisms by which weight loss occurred. One possibility is that absorption of nutrients from these diets may vary, which could change body weight by altering true energy availability. Elevated ketone account for weight loss in some published articles (Mobbs, et al., 2007). In fact, a previous study showed infusion of ketones into the brain of rats reduced their body weight (Davis et al., 1981). This body weight loss was thought to be caused by elevated ketone levels altering hypothalamic glucose-sensing neurons to promote post-prandial thermogenesis and increase resting energy expenditure (Almind & Kahn, 2004; Westerterp, 2004). Therefore, detailed analysis of energy expenditure in these mice should be measured. The effectiveness and safety of long-term use of these low-carbohydrate diets remains questionable. Interestingly, previous research found, after treatment with low-carbohydrate diets, rats gained more weight back when put back onto to standard chow than mice maintained on standard chow diets (Caton et al., 2008). Body weight regain also happened in human subjects after low-carbohydrate diet treatments are discontinued (Sacks, et al., 2009). So what would happen if the low-carbohydrate groups in this study
were placed back on a SC diet? Would they increase weight and restore lean mass? In reality, few people would like to have low-carbohydrate diets continually after body weight loss. However, over time such body weight loss/gain cycles with low-carbohydrate diets might eventually lead to greater weight gain. Therefore, body weight change in mice after low-carbohydrate diet treatments still need to be evaluated when they are transferred to habitual diets for a period of time. Furthermore, in order to evaluate the safety of low-carbohydrate diets, the organ function of mice should be assessed, such as ALT and AST levels of liver, urea, creatinine levels and glomerular filtration rate of the kidney. Because long-term compliance with LC diets in human studies is low (Cardillo et al., 2006), the mice model established in the present study could be used for evaluating the effectiveness and safety of low term treatment on low-carbohydrates diets.

**Conclusions**

1. Obese mice lost a significant amount of weight when they were switched to LC5, LC10, LC15 and SC diets. The LC5 diet resulted in the greatest weight loss (33% of starting weight) after 6 weeks.

2. For groups that lost weight, weight loss was primarily due to fat mass loss and not fluid loss. However, loss of lean mass contributed to some of the weight lost for the HF-LC5, HF-LC10, and HF-LC15 groups. Only for the HF-SC group was weight loss accompanied by a significant increase in lean mass.
3. The LC5 and LC10 diets were the most effective for inducing ketosis as judged by urinary ketone levels, suggesting that high ketones are associated with weight loss. However, weight loss did not require high urinary ketones as LC15 diets and HF-SC had similar ketone levels as compared to SC-SC group.

4. During phase 2 of the study, the HF-SC group has the greatest energy intake and the HF-LC15 has the least energy intake. There is no significant difference in the energy intake between the HF-LC5, HF-LC10, SC-SC, and HF-HF groups. Thus, there was no relationship between weight loss and energy intake, indicating that limiting kcalorie intake was not the main means by which these mice lost weight.

5. LC5 and LC10 diets, as well as SC diet, had significant effects on improving insulin resistance in mice as they reducing body weight in mice. Moreover, possibly due to improved insulin sensitivity and extreme restriction in carbohydrate and protein intake, low-carbohydrate diets reduced blood glucose levels effectively even more than SC diet did in this study.

6. Lipid levels were improved in all groups except the mice that remained on the HF diet in phase 2 of the study. Improvements in LDL, triglycerides, and total cholesterol levels were seen with the most dramatic improvements seen for the more protein restrictive diets.

7. Low-carbohydrate diets did not influence heart and kidney weights. LC5 and LC15 led to decreased liver weights when compared to SC-SC group.
8. Adiponectin levels were the highest in HF-HF group. While corrected to body fat mass, adiponectin was highly produced in LC5 and HF-SC diet groups. This suggested weight loss caused by low-carbohydrate diets is associated with high production of adiponectin in LC5 diet group.

9. IGF-1 levels was the lowest in LC5 diet group, which implied that extreme low-protein percentage in diets affect the IGF-1 levels, which is associated with malnutrition to some degree in LC5 diet group. However, LC10 and LC15 had similar IGF-1 levels as compared to SC groups.

10. All the LC diets in this study were effective at reducing weight and improving several metabolic parameters although the LC5 diet was most effective at inducing ketosis. However, loss of lean mass with all LC diets suggests that the switch from a HF to SC diet may prove to be the most prudent means for weight loss in these animals.

We demonstrated that short-term exposure to low-carbohydrate diets with restrictive protein percentage results in body weight loss, as well as improvements in several physiological parameters, in mice even though similar energy was consumed in these mice, suggesting that merely altering the macronutrient composition of the diet is sufficient to achieve changes in body weight. We also found out that effects of low-carbohydrate diets on body weight loss were decreased when protein percentage was increased in these diets. The low-carbohydrate diet with 5% protein had the most significant effects on promoting weight loss in mice and improving several metabolic
parameters except IGF-1 in our study. For future studies, the LC5 diet is the best choice to be used for promoting weight loss, evaluating safety, and exploring mechanism of low-carbohydrate diets on weight loss in mice.
References


 Retrieved September, 10, 2008, from

http://www.cdc.gov/nccdphp/dnpa/obesity/index.htm


diet and physical activity induced weight reduction is associated with changes in plasma lipids, but not interleukin-6 or adiponectin. *Metabolism: Clinical and Experimental*, 55(3), 359-365.


and treatment of overweight and obesity in adults. *Obesity Research, 6*(Suppl 2), 51S-209S.


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


severely obese adults: One-year follow-up of a randomized trial. *Annals of Internal Medicine, 140*(10), 778-785.


