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# The Association Between Triglyceride and Leptin Levels in Obese Subjects Following a Low-Carbohydrate or Low-Fat Diet

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**The Association Between Triglyceride and Leptin Levels in Obese Subjects  
Following a Low-Carbohydrate or Low-Fat Diet**

Sarah Kim Herbst

University Scholar Project

University of Connecticut

2006

**The Association Between Triglyceride and Leptin Levels in Obese Subjects  
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Obesity has been cited as the second leading preventable cause of death in the United States. Studies have determined that at risk overweight and obese individuals have high circulating levels of leptin indicative of leptin resistance as well as elevated levels of plasma triglycerides. Postulates have been formed that link elevated levels of triglycerides with the inhibition of leptin across the blood-brain barrier. If a method of lowering triglycerides is achieved, leptin should be able to cross the blood-brain barrier and reach the hypothalamus, thus regulating body weight through a decrease in appetite and increase in energy expenditure. The primary aim of this study was to compare the effects of both carbohydrate and fat restricted diets on plasma triglycerides and leptin concentrations in overweight and obese subjects with hypertriglyceridemia. Forty subjects were randomly assigned to either the low carbohydrate or low fat diet group for a 12 wk period. Plasma triglyceride and leptin concentrations in the blood were analyzed before and after the 12 wk period to assess diet-induced changes. Both groups had a significant reduction in body weight, though the effect was much greater in the carbohydrate restricted diet group. Fasting and postprandial plasma triglycerides also

decreased to a greater extent in the low carbohydrate diet group. Similarly, leptin levels decreased to a greater extent in the carbohydrate restricted diet group. The changes in leptin levels were directly related to the changes in both fasting and postprandial triglyceride levels. The results from this study provide preliminary evidence of diet-induced triglyceride reductions as an underlying mechanism in lowering plasma leptin and improving leptin sensitivity. Further, they provide evidence that an increase in triglyceride levels is at least partially responsible for leptin resistance and reduced leptin sensitivity in obese hypertriglyceridemic individuals. This novel discovery is important as it raises the possibility that other methods of lowering triglycerides may improve the efficiency of leptin transport and ultimately improve fat metabolism in overweight and obese individuals.

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# *Chapter 1*

## **Introduction**

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Obesity has been cited as the second leading preventable cause of death in the United States <sup>1</sup> and is the most common metabolic condition among men, women, and children in North America <sup>2</sup>. The recent discovery of an anti-obesity hormone, leptin, in mice twelve years ago has intensified the research surrounding the etiology of obesity <sup>3</sup>. Produced predominantly by adipocytes in white adipose tissue, leptin is considered to play an important role in appetite control, fat metabolism, and regulation of body weight. The dual effects of leptin result in a decrease in appetite and increase in energy expenditure, ultimately leading to an increase in fat metabolism <sup>4</sup>. Leptin levels correlate with body mass index and percentage body fat and are elevated with increasing adiposity in both men and women. The relationship between serum leptin levels and several measures of adiposity demonstrates that leptin levels are elevated in obese patients <sup>5</sup>.

Obesity has also been linked to leptin resistance that arises in part from impaired leptin transport across the blood-brain barrier and defects in leptin receptor signaling, although the mediator of this resistance is unknown. It appears that leptin is unable to enter the cerebrospinal fluid to reach sites in the hypothalamus that regulate appetite <sup>5</sup>. Because serum triglycerides are elevated in obese patients, postulates have been formed that link triglycerides with inhibited leptin transport across the blood-brain barrier.

Results from previous experiments performed on mice have shown that serum triglycerides play a key role in the transport of leptin and that reduction of triglyceride levels by diet or pharmacological treatment could enhance leptin transport across the blood-brain barrier <sup>6</sup>.

Recent studies have shown that low carbohydrate diets are extremely effective at improving weight loss, specifically through the reduction of plasma triglycerides <sup>1</sup>.

Although the concept has not yet been tested in humans, it is believed that lower levels of triglycerides may improve the transport of leptin across the blood-brain barrier as well as leptin sensitivity, explaining, in part, why carbohydrate restricted diets have been successful in promoting satiety and augmenting fat loss <sup>4</sup>. In the following study, the preliminary support for the theory behind triglyceride-induced leptin resistance at the blood-brain barrier was investigated.

# *Chapter 2*

## **Review of Literature**

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### **Obesity Epidemic**

The prevalence of obesity is increasing worldwide and has evolved into a global health problem over the past few decades<sup>3</sup>. The etiology of obesity is multifactorial and incorporates genetic susceptibility along with social and psychological factors, but its main causes are an excess of energy intake and inadequate energy expenditure. Obesity, particularly visceral fat accumulation, is associated with dyslipidemia, impaired glucose tolerance, insulin resistance, and is a risk factor for the development of metabolic syndrome, type II diabetes mellitus, cancer, and cardiovascular disease<sup>2</sup>. The need to establish effective weight loss methods has led to a renewal of interest in alternative diets and modification of macronutrient content<sup>7</sup>.

Past dietary guidelines have advocated a decrease in fat consumption and increase in carbohydrate intake. A decrease in fat, as opposed to carbohydrate or protein, has been targeted because of its ability to concentrate energy and its preferential storage in adipose tissue. A diet high in fat also tends to lead to passive over-consumption because of the body's inability to make auto-regulatory adjustments in the utilization of fat after a high-fat meal<sup>8</sup>. However, the number of obese individuals in the United States, although



reportedly adhering to fat restricted diets, has risen steadily since the mid-1970s and our country is currently in the midst of a national epidemic of obesity, suggesting it is improbable the cause of obesity is related to the consumption of dietary fat. Additionally, an increase in carbohydrate intake, especially of a high-glycemic nature, raises insulin levels and is associated with weight gain. In accordance with obesity, at such physiologic concentrations insulin has an anti-lipolytic effect <sup>1</sup>.

## **Leptin**

Along with elevated levels of insulin, obesity is also associated with an increase in plasma leptin, a hormone that targets the central nervous system and is involved in the control of appetite, fat metabolism, and regulation of body weight <sup>4,5</sup>. A member of the cytokine family, leptin is a single chain 167 amino acid anti-obesity hormone secreted predominantly from white adipose tissue in an ultradian and circadian fashion. Due to the female distribution of body fat, women exhibit markedly higher levels of leptin than men for any given degree of fat mass <sup>9</sup>. Leptin circulates in the plasma in both the free form and bound to leptin-binding proteins and is regulated by numerous compounds including glucocorticoids, catecholamines, sex hormones, thyroid hormones, melatonin, and insulin. Although the highest expression of leptin receptors is in the brain, receptors are also expressed in peripheral tissues such as the kidney, liver, pancreas, lungs, adrenals, ovaries, and skeletal muscle <sup>3</sup>. Recent research has suggested leptin is involved in such processes as the immune response, haematopoiesis, angiogenesis, growth and development, intestinal transport, and reproduction, though the hormone is most widely known for its role in maintaining energy balance <sup>10</sup>.

## **Function of Leptin**

Leptin is crucial in the control of body metabolism and functions primarily by crossing the blood-brain barrier and binding with leptin receptors in the hypothalamus, the area of the brain involved with the control of hunger. The binding of leptin subsequently stimulates the pituitary gland to relay a signal to the thyroid and alter metabolism by inhibiting the activity of neurons containing the appetite stimulator neuropeptide Y, decreasing food intake, and increasing energy expenditure <sup>9</sup>.

Leptin injected centrally into the brain or bloodstream of obese rodents results in a reduction of food intake and body weight, with increasing concentrations correlating with greater metabolic effects. In isolated adipocytes, leptin-stimulated lipolysis is accompanied by the up-regulation of mRNA associated with fatty acid metabolism <sup>10</sup>. Leptin also inhibits the synthesis of free fatty acids, providing further protection against the build up of fat <sup>4</sup>.

## **Leptin and Obesity**

The gene expression of leptin appears to be related to adiposity, with leptin mRNA and circulating leptin levels increasing with body mass index and percentage body fat, even in obese individuals <sup>5</sup>. Abnormal serum leptin binding and abnormal leptin catabolism do not appear to be underlying mechanisms in the development of human obesity, given the half-life and biological activity of circulating leptin is relatively the same in both non-obese and obese individuals <sup>3</sup>.

Clinical trials world-wide found exogenous leptin was able to reduce body weight in normal sized mammalian rats, dogs, pigs, monkeys, and fish <sup>3</sup>. One such trial studied the effects of acute and chronic leptin administration in teleost goldfish. Results determined that the success of acute leptin administration over the course of two days was dependent on the amount dispensed, while chronic ten day treatment significantly suppressed spontaneous feeding, reduced body weight gain, and modified systems involving neuropeptide Y. Chronic leptin administration also increased lipid mobilization and carbohydrate storage as hepatic and muscle glycogen <sup>11</sup>.

Recently released data from a second study showed that daily subcutaneous administrations of leptin over the course of nine months to a leptin-deficient patient decreased body weight by almost fifteen kilograms and greatly improved the patient's metabolic profile. Leptin-induced weight loss was also found to be completely specific for the loss of adipose tissue, whereas diet-induced weight loss resulted in the loss of both adipose tissue and lean body mass. However, human studies have shown the administration of leptin is efficacious in only those individuals with leptin deficiency. Because obesity is associated with high levels of leptin, the usefulness of exogenous leptin on fat metabolism in obese individuals appears ineffective <sup>3</sup>.

### **Leptin Resistance**

In preliminary studies, rodents with diet-induced obesity were hyperleptinemic and showed signs of resistance when administered exogenous leptin <sup>9</sup>. Similarly, obesity in humans is believed to be associated with a resistance to, rather than a deficiency of, leptin that arises from impaired leptin transport across the blood-brain barrier, defects in

leptin receptor signaling, and blockades in downstream neural circuitries, although the mediator of this resistance is unknown. It appears that because of the relationship between cerebrospinal fluid and serum levels of leptin in obese subjects, defective blood-brain transport accounts for more of the leptin resistance than defects in receptors<sup>4</sup>. Leptin resistance is most prominently evidenced by the fact that obese individuals with elevated levels of leptin are unable to alter their fat metabolism or decrease body weight<sup>9</sup>.

Whether leptin resistance may be bypassed to achieve weight loss through the use of high doses of exogenous leptin is yet to be determined. It is possible that the approximate five to ten percent of obese individuals with relatively low levels of leptin may benefit from and be fully sensitive to such treatments<sup>3</sup>. However, given the majority of the obese population already has high levels of leptin that fail to produce weight loss, it is unlikely such treatment will prove successful in these leptin resistant individuals.

### **Leptin and Triglycerides**

Impaired leptin transport is believed to account for at least a portion of leptin resistance and may explain why there is an accumulation of leptin in obese individuals. Leptin is transported across the blood-brain barrier by a saturable transporter, though the process of regulation behind this is unknown. Given that other blood-brain barrier transporters are regulated noncompetitively, there may be a circulating factor interfering with and subsequently preventing the transport of leptin to the hypothalamus. Because

serum triglycerides are elevated in obese patients, postulates have been formed that link triglycerides with inhibited leptin transport <sup>4</sup>.

Previous experiments done in vivo with mice have shown that milk, for which fats are 98% triglycerides, immediately inhibited leptin transport across the blood-brain barrier, a phenomenon not seen when fat-free milk was administered, eliminating the possibility of milk proteins as the source of resistance. When intralipid, a soybean oil-based source of triglycerides containing the essential free fatty acids linolenic and linoleic acid, was administered, there was also no effect on leptin transport, suggesting plant triglycerides and essential free fatty acids are also non-limiting. Three of four commercially available triglycerides, specifically triolein, DPOG, and DSOG, when injected intravenously at a dose equivalent to the level of triglycerides in milk, further inhibited uptake of radioactive I-leptin <sup>4</sup>.

When the mice were subjected to conditions of elevated triglycerides through starvation or diet-induced obesity, there was also decreased transport of leptin across the blood-brain barrier as well as a reduction in brain uptake. Conversely, mice that had undergone short-term fasting had decreased blood triglycerides and increased leptin transport. Although increased triglyceride levels appear to inhibit leptin transport, it should be noted that their free fatty acid constituents did not have the same effect <sup>4</sup>.

However, leptin resistance can be reversed. Drugs, such as gemfibrozil that are clinically used to treat hypertriglyceridemia, specifically reduce triglyceride levels and have been able to improve leptin transport across the blood-brain barrier. Experiments with mice that reduced their body weight and triglycerides by pharmacological

intervention were also able to reverse leptin impairment and increase fat metabolism, no longer resisting the effects of the leptin hormone <sup>4</sup>.

### **Response of Leptin to Weight Loss**

Results from a recent body weight reduction study performed at the University of Milan in Italy support the theory behind triglyceride-induced leptin resistance. Ten obese subjects, after completing a three-week body weight reduction program consisting of an energy-restricted diet and exercise training, significantly reduced their body weight and body mass index. Upon analyzing their blood samples, leptin levels were found to have decreased dramatically from the start of the study for both fasting and postprandial conditions <sup>12</sup>. A second study found that fasting over several days induced a ten percent decrease in body weight and a fifty-three percent reduction in serum leptin <sup>9</sup>. An energy expenditure and body composition study performed on rats in the fed and fasted conditions showed that starvation accompanied by weight loss allowed for greater mobilization of fat and decreased leptin levels <sup>13</sup>.

Similar results were obtained from another weight loss study with seventeen obese women adhering to an energy-restricted 800 kcal/d diet for seven weeks. Upon completion of the diet, it was found that weight, triglycerides, and plasma leptin had all decreased significantly, suggesting lower levels of triglycerides correlate with lower levels of leptin and leptin resistance is caused by the accumulation of fat. Before and after the weight reduction diet, each woman was randomly given either a high-fat (eighty percent fat/twenty percent carbohydrate) or high-carbohydrate (eighty percent carbohydrate/twenty percent fat) test meal to determine the effect of satiety factors on

weight loss. Subsequent postprandial satiety and hunger ratings were recorded and it was determined that satiety ratings were significantly higher after weight loss for both the carbohydrate and fat test meals. With weight loss correlating to lower levels of triglycerides, leptin would be expected to cross the blood-brain barrier and bind with receptors in the hypothalamus to inhibit appetite stimulating factors and decrease food intake <sup>14</sup>.

### **Response of Leptin to Diet Composition**

Lower levels of leptin are also associated with low carbohydrate diets, as reported from studies performed on overweight women in the United Kingdom where subjects decreased leptin after consuming thirty-five percent of their energy from carbohydrates, fifty percent from fat, and fifteen percent from protein <sup>15</sup>. Even in non-obese subjects, leptin has been shown to increase with the consumption of foods high in dietary fat. Normal sized mice that consumed a high fat diet for eight weeks significantly increased their body fat, percent body fat, and serum leptin <sup>16</sup>.

Another study attempting to determine the influence of diet on plasma leptin focused on eight healthy, postmenopausal women in their sixties with an above average body mass index on a low-fat, high-carbohydrate diet with less than thirty percent of their total energy intake from fat. Results found that both fasting and postprandial states of plasma leptin concentrations were significantly higher after consuming the high carbohydrate diet after only three days. A strong positive, linear relationship between increasing levels of both postprandial insulin and leptin concentrations was also found, indicating a greater accumulation of fat corresponds to a high carbohydrate diet <sup>15</sup>.

## **Low Carbohydrate Diets**

Conversely, low carbohydrate diets have been shown to decrease the release of insulin, lessening its anti lipolytic effect and enhancing the suppression of appetite. Not surprisingly, carbohydrate restricted diets have led to long-term weight loss as well as weight maintenance. Individuals adhering to such diets reportedly decrease their caloric intake, though this is only partially due to a limited availability of carbohydrate restricted food choices. Fat and protein both have a higher satiety value than carbohydrates, and elevated levels of  $\beta$ -hydroxybutyrate, one of the primary ketones in blood, also act as a satiety factor <sup>1</sup>.

While there has been speculation that a carbohydrate restricted diet high in fat, especially saturated fat, may lead to insulin resistance, data from recent studies have shown carbohydrate restricted diets do not have an adverse effect on either glucose metabolism or insulin resistance. In fact, low carbohydrate diets alter the effects of insulin on oxidative and nonoxidative glucose disposal, favoring the storage of glucose as glycogen in muscle. Carbohydrate restricted diets also appear to prevent insulin-stimulated inhibition of lipid oxidation allowing triglyceride levels to fall and the subsequent reduction of body weight <sup>1</sup>.

## **Effects on Weight Loss**

With its altered metabolism, low carbohydrate diets have proven to be more effective for weight loss than low fat diets. Results from clinical trials and studies on low carbohydrate diets suggest there is a threshold level of dietary carbohydrate intake upon which ketogenesis and lipolysis are initiated if consumption falls short. Accompanying



lipolysis is the reduction of body weight and body mass index <sup>6</sup>. One university study reported that obese women randomly selected to follow a low carbohydrate diet lost more than twice the amount of weight as those following a low fat diet over a six month period though there were no differences in energy intake. Women following the carbohydrate restricted diet also lost approximately double the amount of body fat than those adhering to the fat restricted diet <sup>7</sup>.

A study using dual-energy x-ray absorptiometry to examine the change of body composition in subjects who had switched from their typical diet of forty-eight percent carbohydrate and thirty-two percent fat to eight percent carbohydrate and sixty-one percent fat for six weeks showed a significant decrease in fat mass and increase in lean body mass despite any change in level of physical activity. Although water may account for some of the initial and rapid weight loss on a carbohydrate restricted diet, it appears that fat loss is accelerated and lean tissue is maintained over extended periods of time <sup>1</sup>. Other diet and weight loss studies performed in Sweden showed similar results. Individuals following carbohydrate restricted diets demonstrated a much more rapid weight loss, especially after the three and six month periods, than those following fat restricted diets. Although both diets led to a decrease in risk factors for coronary heart disease, the low carbohydrate diet was associated with a greater decrease in serum triglycerides for both fasting and postprandial conditions <sup>17</sup>.

### **Effects on Triglycerides**

The most consistent and predictable lipemic response to a carbohydrate restricted diet has been a decrease in plasma triglycerides, with the most dramatic reductions seen

in individuals with moderate to severe hypertriglyceridemia. Fat restricted diets have been able to reduce plasma triglyceride levels to a small extent during active weight loss, but increases are seen when not associated with significant weight loss or combined with physical activity <sup>1</sup>. Results from a study involving sixty-three healthy obese women assigned to either an ad libitum low carbohydrate or calorie-restricted low fat diet for twelve months showed there was a significant seventeen percent reduction in triglyceride levels in those individuals adhering to the low carbohydrate diet group. However, women following the low fat diet for the one year duration on average slightly increased triglyceride levels by approximately one percent <sup>6</sup>.

The triglyceride lowering effect of a carbohydrate restricted diet may be due to a decreased synthesis/secretion of VLDL in the liver and/or an enhanced clearance mediated by lipoprotein lipase. Reducing carbohydrate intake also causes a decrease in the insulin/glucagon ratio thus inducing glucagon-mediated lipolysis and stimulating the breakdown of triglycerides to its glycerol and free fatty acid components <sup>6</sup>. Ultimately, low carbohydrate diets are able to reduce plasma triglycerides more effectively than low fat diets, which may prove significant in its relation to other anti-obesity factors.

# Research Aims & Hypotheses

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## Specific Aims

The primary purpose of the study was to compare the effects of both carbohydrate and fat restricted diets on plasma TAG and leptin concentrations in overweight and obese subjects with hypertriglyceridemia. Because previous studies have already indicated increased levels of TAG inhibit leptin from crossing the blood-brain barrier, a decrease in TAG should allow leptin to cross the blood-brain barrier and act on the hypothalamus to decrease appetite and increase fat metabolism, both of which would result in a decreased volume of adipocytes and thus a fall in leptin levels. Therefore, because it has been shown that a carbohydrate restricted diet results in lower TAG levels, subjects adhering to such a diet would be expected to have a resultant greater decrease in leptin levels in comparison to subjects following a fat restricted diet. Further, there may be an association between diet induced reductions in plasma TAG and decreases in circulating levels of leptin; such an association would provide indirect preliminary evidence for improved leptin sensitivity with reductions in plasma TAG in humans.

## Research Hypotheses

- 1.) A low carbohydrate diet will result in significantly lower TAG levels in obese subjects compared to a low fat diet.
- 2.) A low carbohydrate diet will result in significantly lower leptin levels in obese subjects compared to a low fat diet.
- 3.) The change in TAG levels will be related to the change in leptin level concentrations.

# *Chapter 3*

## **Methods**

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### **Experimental Design**

In a balanced randomized design, subjects consumed one of two experimental diets for a 12-wk period, a low fat diet or a low carbohydrate diet. Twenty men and twenty women completed the study, with ten individuals of each gender in the carbohydrate restricted and fat restricted diet groups. Reasonable efforts were made to ensure that assignment to the two dietary groups was balanced in respect to gender, age, and BMI.

Prior to beginning the diet, subjects were provided with food scales and received thorough instructions on how to complete a detailed weighed food record over a 5-day period to assess habitual intake. Two blood draws from fasting subjects were performed at the same time of day to account for diurnal variation in lipids at baseline on day one of the study and again during the last week of the study. An oral fat tolerance test was performed at baseline on day two of the study and again during the last week of the study.

### **Subjects**

Overweight (BMI > 25 kg/m<sup>2</sup>) men and women (n = 40) between the ages of 18 and 55 yr volunteered to participate in this study that was conducted in accordance with

the guidelines of the Institutional Review Board at the University of Connecticut. All subjects also provided written informed consent to participate. Potentially eligible subjects met at least two of the three following criteria after a 12 h fasting blood sample was taken: blood triglycerides between 150 and 500 mg/dL, blood HDL-cholesterol < 40M/50F mg/dL, and blood glucose > 110 mg/dL. Subjects were further excluded on the basis of using any glucose-lowering, lipid-lowering, or vasoactive prescriptions or supplements, having any metabolic or endocrine disorders, having already consumed a low carbohydrate diet within the last 3 months, or achieved a weight loss greater than 5.0 kg in the past 3 months. Subjects were either sedentary or moderately active and maintained the same level of physical activity throughout the study documented by analysis of log sheets on which all exercise sessions were recorded.

### **Diet Interventions**

No explicit instructions were provided regarding caloric intake for either diet, given the goal to permit expression of any non-cognitive aspect of food intake. The low fat diet was designed to contain < 10% saturated fat, and < 300 mg cholesterol per day. Standard diabetic exchange lists were used to ensure approximately 20% energy from protein, 25% energy from fat, and 55% energy from carbohydrate. Food was not provided for the subjects, but educational materials and counseling regarding the specific diet including lists of appropriate foods, recipes, and example meal plans were offered. Subjects were instructed to restrict foods that were high in fat or saturated fat including fatty meats, butter, and desserts. Foods encouraged included whole grains (breads,

cereals, and pastas), fruit/fruit juices, vegetables, vegetable oils, and low fat dairy and meat products.

The low carbohydrate diet was designed to contain approximately 60% fat, 30% protein, and 10% carbohydrate to induce a small level of ketosis. There were no restrictions on the type of fat from saturated and unsaturated sources or cholesterol levels. Food was not provided, but the subjects were instructed to restrict such carbohydrate rich items as breads, pasta, cereals, rice, potatoes, candy, and sweets. Commonly consumed foods included beef, chicken, fish, cheese, nuts, seeds, eggs, certain vegetables, and other specially designed low carbohydrate foods. Subjects were instructed to restrict fruit and fruit juices and avoid all low carbohydrate breads and cereals. Alcohol consumption was limited to one drink per day.

All subjects were provided with a multi-vitamin/mineral complex providing  $\leq$  100% of the RDA and instructed to consume one pill every other day. Subjects were told to cease taking any other nutritional supplement during the course of the study. All subjects received extensive initial instruction and weekly follow-up counseling by registered dietitians to review progress, assess compliance, and provide further dietetic education if necessary. Subjects were also able to contact a dietician twenty-four hours a day via phone or e-mail regarding any questions pertaining to their diet.

Subjects received thorough instructions for completing detailed seven-day weighed food records during weeks one, six, and twelve, and food measuring utensils and scales were provided to ensure accurate reporting of food/beverage amounts consumed. Food diaries were analyzed for energy and macro/micronutrient content using the food software program Nutritionist Pro (Version 3.1, First Databank Inc, The Hearst

Corporation, San Bruno, CA). The program had no missing values for the nutrients reported, and the database was extensively modified internally to incorporate new foods and recipes. To ensure that carbohydrates were restricted throughout the low carbohydrate diet period, subjects tested their urine daily using urine reagent strips (Bayer Corporation, Elkhart, IN). This urine test is specific for acetoacetic acid, which produces a relative color change when reacting with nitroprusside. A sensitive indicator, this test was used because of its past compliance in earlier carbohydrate restricted studies. Compliance during the low fat diet period was assessed through analysis of food records and measurement of the respiratory exchange ratio obtained after the diet.

### **Fasting Blood Collection**

Blood samples were obtained on two separate days, at the beginning and end of the 12-wk experimental diet period. Samples were obtained after an overnight fast and abstinence from alcohol and strenuous exercise for 24 h. Subjects reported to the laboratory between 0700 and 0900 h and rested quietly for 10 minutes in the supine position before a blood sample was obtained from an antecubital vein and collected into a tube coated with a silicone-gel. The blood was then centrifuged and the serum stored at -80° C for subsequent analysis.

### **Oral Fat Tolerance Test**

An oral fat tolerance test was performed after each experimental diet in order to determine the postprandial lipemic response to a fat-rich meal. A flexible catheter was inserted into a forearm vein and blood samples were obtained from a 3-way stopcock

connected to the end of the catheter. The catheter was kept patent with a constant saline drip. Subjects rested in a seated position for 10 min before obtaining a baseline blood sample. A fat-rich test meal (225 mL whipping cream, sugar-free instant pudding, 28.5 g macadamia nuts) was then consumed providing 908 kcal, 13% carbohydrate, 3% protein, and 84% fat. The meal was consumed in approximately 15 min and postprandial blood samples were obtained immediately after the meal (0 hr) and hourly (1, 2, 3, 4, 5, 6 hr) for a total of six hours. Subjects rested quietly in a seated position consuming only water throughout the postprandial period.

### **Determination of Serum Lipids and Leptin**

Approximately 3 mL of serum from each blood draw was sent to a certified medical laboratory (Quest Diagnostics) for determination of fasting and postprandial TAG concentrations using automated enzymatic procedures (Olympus America) with calculated precision values < 3%.

Fasting leptin levels were measured in duplicate in the laboratory through the use of a Human Leptin Enzyme-Linked Immunosorbent Assay (ELISA) kit, an enzymatically amplified two-step sandwich type immunoassay utilizing horseradish peroxidase (HRP) and tetramethylbenzidine (TMB) for detection. Intra-assay coefficient of variation was 1.7%.

### **Statistical Analyses**

All statistical analyses were done with Statistica software, version 5.5 (StatSoft Inc, Tulsa, OK). A 2x2x2 analysis of variance (ANOVA) was used to evaluate the main



effect of diet (low carbohydrate and low fat) and gender (men vs women) over time (week 0 and week 12) on weight loss, plasma triglycerides, and leptin. Significant main or interactive effects were further analyzed using a Fishers LSD post-hoc test.

Relationships among selected variables were examined using Pearson's product-moment correlation coefficient. The alpha level for significance was set at 0.05.

# Chapter 4

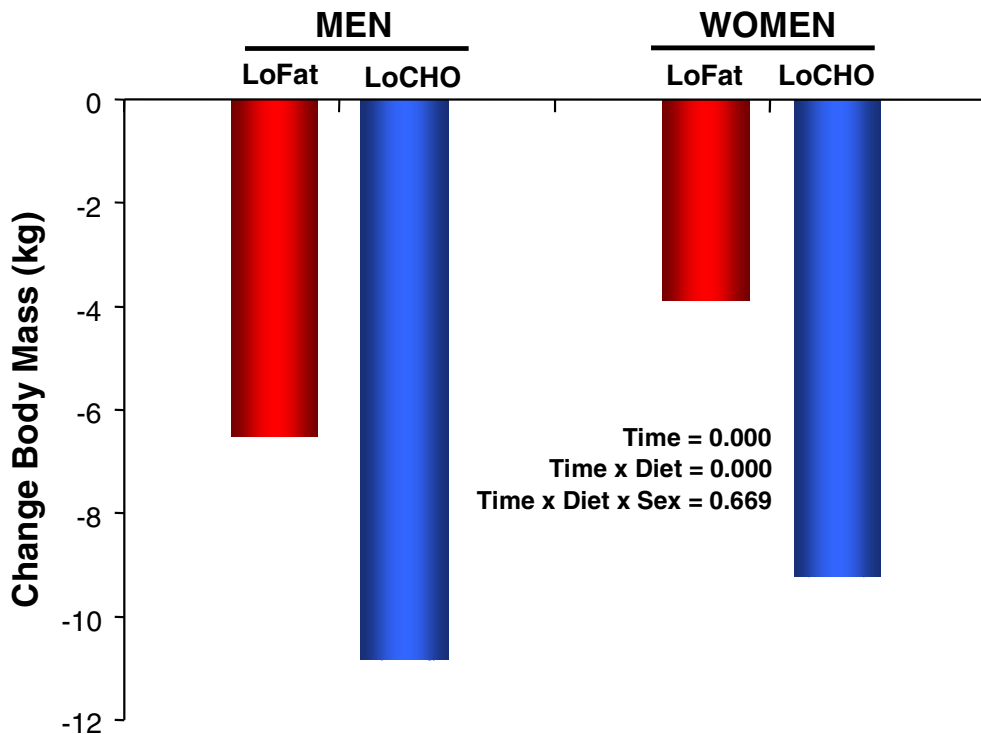
## Results

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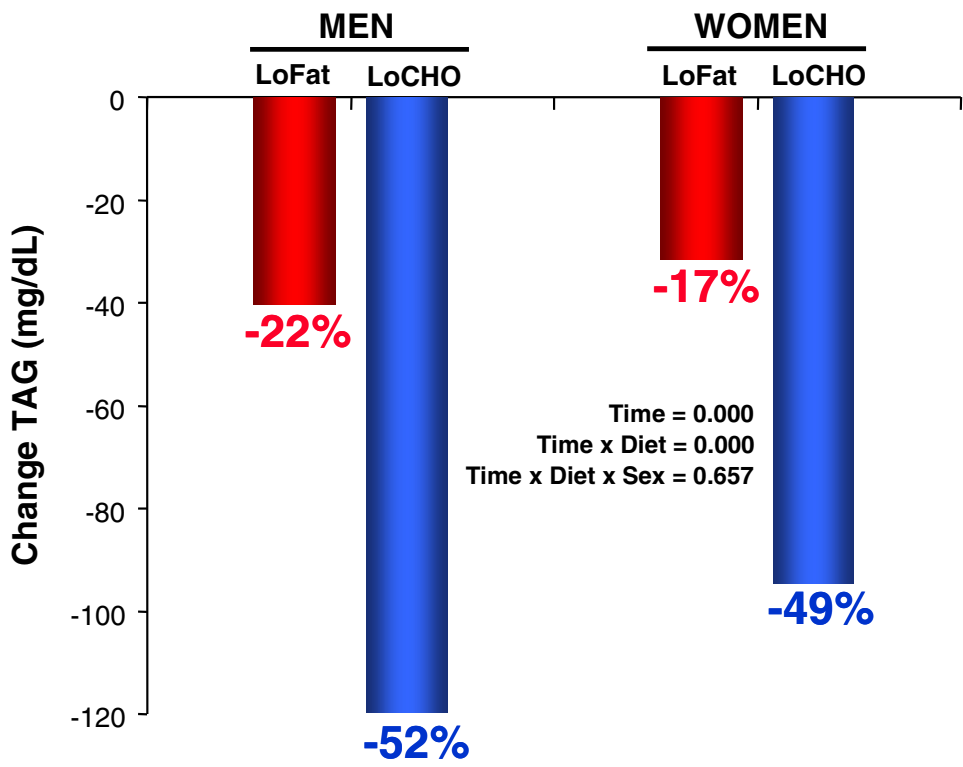
The subject characteristics at the start of the study are shown in Table 1. After 12 weeks, there was a significant reduction in body weight in both diet groups, though the effect was greater with the carbohydrate restricted diet (-10.0 kg) than the fat restricted (-5.2 kg) diet (Figure 1). Similarly, fasting plasma TAG decreased to a greater extent in the low carbohydrate (-51%) than the low fat (-19%) diet group (Figure 2). Further, the area under the postprandial TAG curve during the oral fat tolerance test decreased to a significantly greater extent in the low carbohydrate (-47%) than the low fat (-15%) diet group. As expected, leptin levels decreased with weight loss, though the magnitude of the decrease was much larger in the low carbohydrate (-42%) than the low fat (-18%) diet group (Figure 3). Reductions in leptin levels were greater in the carbohydrate restricted diet group even after normalizing and correcting for the effect of weight loss (data not shown). In all subjects combined, there was a significant relationship between the decrease in fasting TAG and the decrease in leptin (Figure 4). An even stronger association was seen with the decrease in TAG area under the curve and decrease in leptin, with the change in the lipemic response accounting for 23% of the variability in leptin response.

**Table 1.** Subject characteristics at the start of the study.

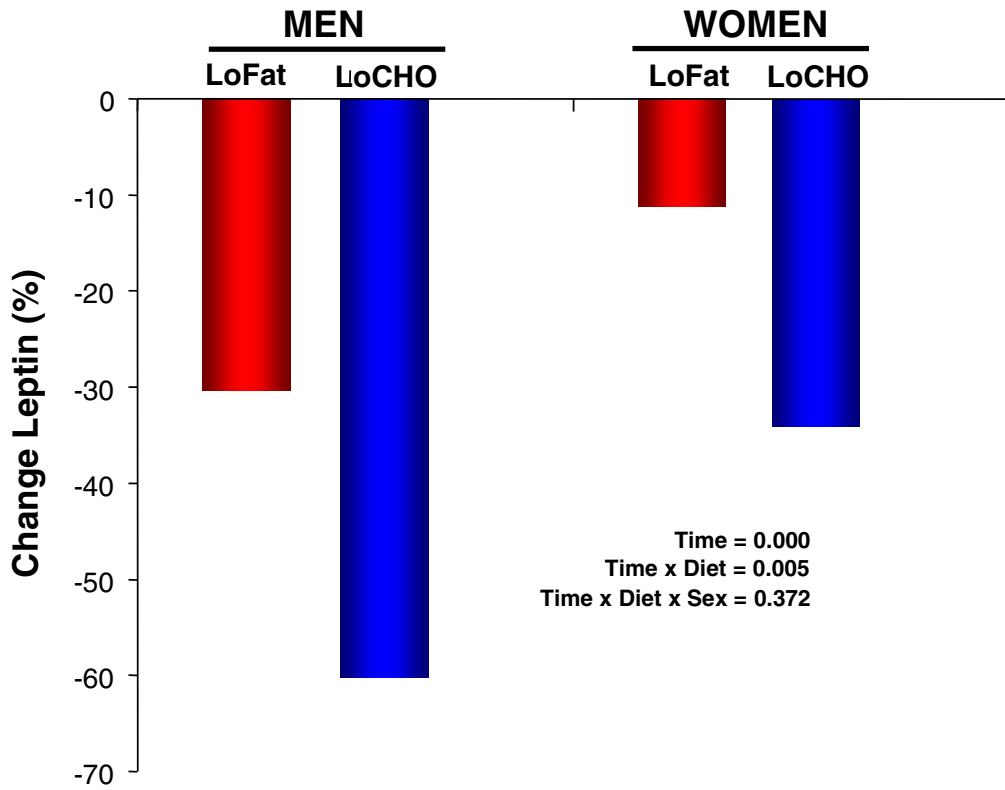
	<b>MEN</b>		<b>WOMEN</b>	
	<b>LoFat</b> <i>(n=10)</i>	<b>LoCHO</b> <i>(n=10)</i>	<b>LoFat</b> <i>(n=10)</i>	<b>LoCHO</b> <i>(n=10)</i>
<b>Age (yr)</b>	38.5 ± 10.9	31.9 ± 12.3	35.2 ± 14.3	33.2 ± 10.8
<b>BM (kg)</b>	101.9 ± 8.3	103.2 ± 13.8	86.9 ± 17.1	89.8 ± 10.4
<b>Body Fat (%)</b>	34.4 ± 6.6	34.7 ± 4.1	44.0 ± 6.2	46.5 ± 4.2
<b>BMI (kg/m<sup>2</sup>)</b>	31.3 ± 3.2	33.3 ± 5.2	32.8 ± 4.9	33.7 ± 5.5
<b>TC (mg/dL)</b>	197 ± 11	202 ± 22	211 ± 32	214 ± 30
<b>LDL (mg/dL)</b>	126 ± 31	124 ± 17	130 ± 33	137 ± 25
<b>HDL (mg/dL)</b>	34.4 ± 4.3	33.4 ± 5.4	43.0 ± 4.6	38.1 ± 7.6
<b>TAG (mg/dL)</b>	185 ± 62	230 ± 60	189 ± 57	192 ± 51
<b>TAG/HDL</b>	5.6 ± 2.4	7.1 ± 2.2	4.5 ± 1.6	5.3 ± 1.8



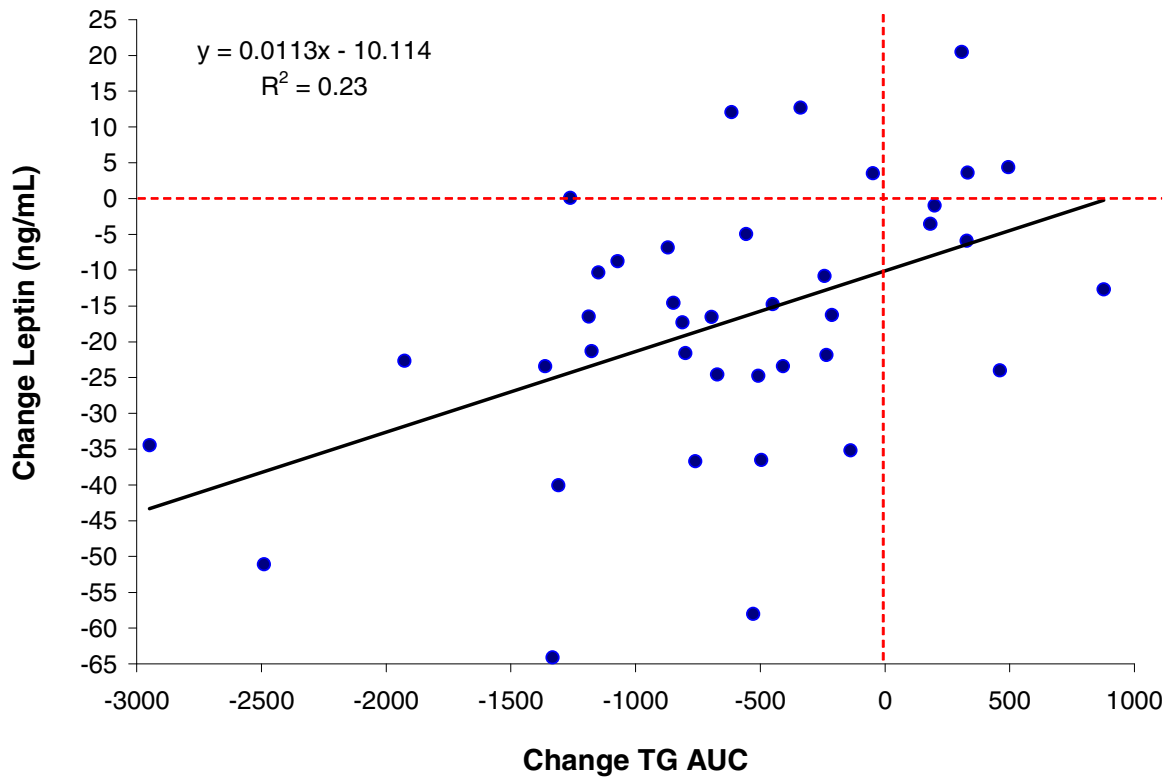
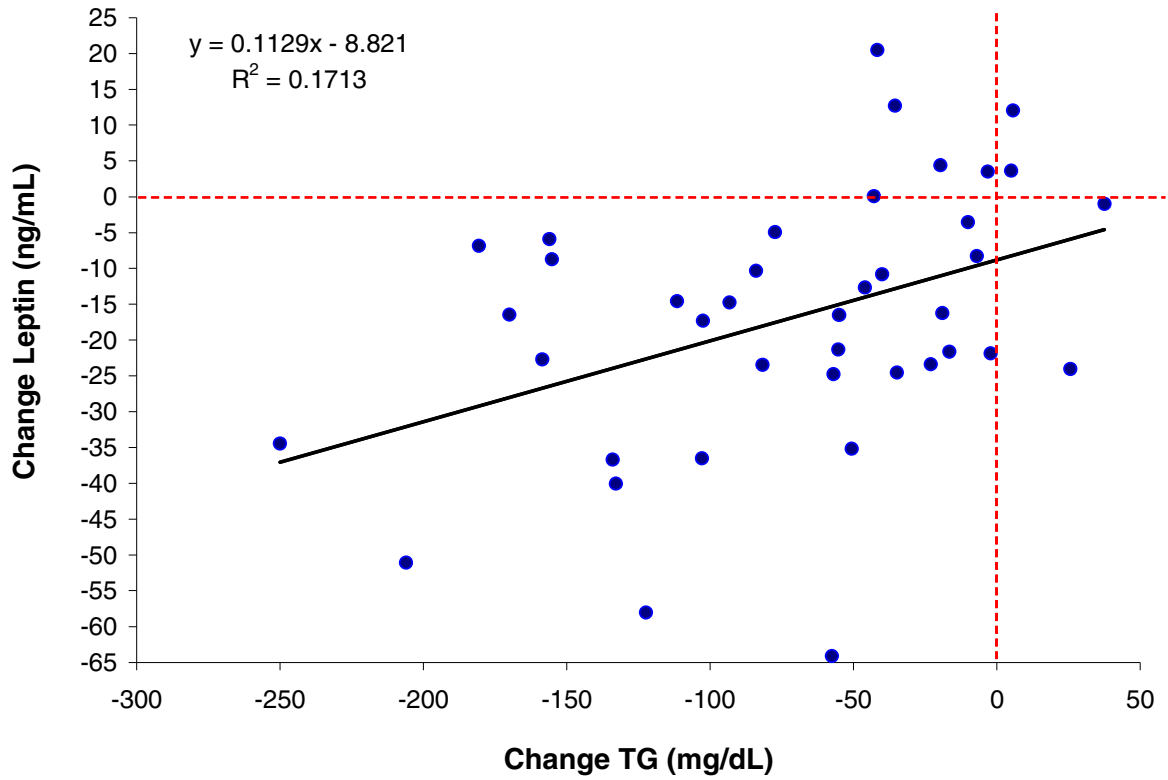
**Figure 1.** Weight loss in men and women that consumed either low carbohydrate (LoCHO) or low fat (LoFat) diets for 12 weeks.



**Figure 2.** Change in fasting triglycerides in men and women that consumed either low carbohydrate (LoCHO) or low fat (LoFat) diets for 12 weeks.



**Figure 3.** Change in fasting leptin in men and women that consumed either low carbohydrate (LoCHO) or low fat (LoFat) diets for 12 weeks.



**Figure 4.** Relation between the change in leptin and the change in fasting triglycerides (upper panel) and the change in triglyceride area under the curve (lower panel). N = 40.

# Chapter 5

## Discussion

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The aim of this study was to directly compare the effects of low carbohydrate and low fat diets on plasma TAG and leptin concentrations in overweight and obese subjects with hypertriglyceridemia. Similar to previous studies that utilized overweight subjects, overweight/obese individuals were found to have high circulating levels of leptin indicative of leptin resistance. Since the gene expression of leptin is related to adiposity, it follows that higher levels of leptin are seen in very overweight and obese individuals<sup>5</sup>. The baseline leptin values of these subjects at the onset of the study were extremely elevated, perhaps more so than would be expected based on their adiposity, which may be explained in part by their metabolic hypertriglyceridemia status. Preliminary studies in animals have shown high levels of circulating TAG, as seen with hypertriglyceridemia, inhibit the transport of leptin across the blood-brain barrier and contribute to leptin resistance<sup>4</sup>. In this current study, both diet intervention groups led to a significant decrease in plasma TAG, though the carbohydrate restricted diet resulted in a significantly greater reduction than the fat restricted diet in both the postabsorptive and postprandial states. Accordingly, the reductions in plasma TAG were found to be directly related to the decreases in circulating levels of leptin, linking TAG as a factor in the etiology of leptin resistance in humans.

The effect of diet composition on the reduction of plasma TAG for this study agreed with results obtained from previous studies. The low carbohydrate diet resulted in much greater reductions in fasting serum TAG, more than doubling the percent decrease when compared to TAG reductions from the low fat diet group. Similar results were obtained even during the postprandial period, with the area under the postprandial TAG curve decreasing to a greater extent in the low carbohydrate than the low fat diet by more than double the percent decrease. With lower levels of TAG, it follows that there would be a significant reduction in leptin production, as leptin is produced predominantly by adipocytes in adipose tissue. Further, lower levels of TAG would reduce the impedance of leptin transport through the cerebrospinal fluid to the hypothalamus. Instead of an increase or accumulation, then, of leptin in the plasma, there would be a resultant decrease as leptin is able to pass through the blood-brain barrier<sup>4</sup>. Therefore, the low carbohydrate diet group would be expected to have lower levels of leptin compared to the low fat diet group due to lower levels of TAG.

As expected, there was a significantly greater reduction in plasma leptin in those individuals following the carbohydrate restricted diet than those adhering to the fat restricted diet. More than double the percent decrease in leptin levels correlated with the carbohydrate restricted diet compared to the fat restricted diet. These results may be partially explained by the greater weight loss achieved on the low carbohydrate diet than the low fat diet. Although significant weight loss was seen in both diet groups, the subjects following low fat diets lost on average approximately half of what the subjects following low carbohydrate diets were able to lose over the twelve week period. With greater weight loss and decreases in body mass, it follows that there would be a greater

reduction in body fat and thus lower levels of leptin. Consequently, a greater decrease in leptin was seen in those individuals following the carbohydrate restricted diet.

However, it is even more likely the significant reduction in plasma leptin is due to the greater decreases in plasma TAG seen with the low carbohydrate diet. Even after normalizing the data to remove the effect of weight loss, the percent decrease in leptin was still greater for those following a carbohydrate restricted diet than those following a fat restricted diet. These results are significant as they provide preliminary evidence of diet induced TAG reductions as an underlying mechanism in lowering plasma leptin and ultimately improving leptin sensitivity. Further, they provide proof that an increase in TAG is at the very least partially responsible for leptin resistance and reduced leptin sensitivity in obese hypertriglyceridemic individuals.

After combining subjects from both diet groups, there was still a significant relationship between the change in TAG and change in leptin levels. Both changes occurred in a directly proportional relationship to one another. Similarly, the change in TAG area under the curve was proportional and directly related to the change in leptin levels. Statistically, both association changes were significant confirming it is highly unlikely these associations were due to chance. The outcomes from this study mirror and further confirm in humans what has already been established by experiments performed on animals with increases in TAG inhibiting the transport of leptin to the hypothalamus and reducing leptin sensitivity. Similarly, greater decreases in body weight were seen in both humans and animals adhering to diets that limited the accumulation of plasma triglycerides <sup>4</sup>.



Results from this study and previously completed studies confirm leptin levels are elevated in overweight and obese individuals. However, documented weight loss and improved metabolic profiles have been associated with exogenous leptin administrations in normal sized animals and leptin-deficient human patients, suggesting the normal route of leptin transport to receptor sites in the hypothalamus is inhibited in obese individuals<sup>3</sup>. Obesity is also associated with high levels of TAG, which may possibly be contributing to leptin resistance at the blood-brain barrier and preventing the hypothalamus from signaling changes in the regulation of appetite and energy expenditure that would decrease the volume of adipocytes in adipose tissue<sup>4</sup>. If a method of lowering plasma TAG could be achieved, it is quite likely that leptin sensitivity would improve by reducing the inhibition of leptin transport through the cerebrospinal fluid. With leptin acting effectively as an anti-obesity hormone at the level of the hypothalamus, overweight and obese individuals would be expected to reverse their metabolic symptoms and lose weight by reducing their food intake and increasing energy expenditure. Because previous studies have already shown carbohydrate restricted diets are effective at lowering plasma TAG more so than fat restricted diets, a greater decrease in leptin would be expected to correlate with a low carbohydrate diet secondary to greater weight loss.

The experimental results obtained from this study agree with these aforementioned hypotheses and by confirming such an association exists between TAG and leptin, the possibility is raised that other methods of lowering TAG may improve the transport of leptin across the blood-brain barrier and ultimately improve fat metabolism in overweight and obese individuals. Clinical studies have already determined leptin

resistance can be reserved through pharmacological treatment that results in the reduction of plasma TAG levels. Gemfibrozil and other drugs used to clinically treat hypertriglyceridemia have been able to improve the transport of leptin to the hypothalamus specifically through TAG reduction<sup>4</sup>. Certainly, a combination of a carbohydrate restricted diet, exercise, and pharmacological intervention would improve the metabolic profiles of overweight individuals and allow the anti-obesity effects of leptin to predominate and improve fat metabolism.

Further studies on the effectiveness of such triglyceride lowering techniques on fat metabolism should be tested in the future to determine which method, or combination of methods, best improves the metabolic profiles of at risk overweight and obese individuals. Because obesity is the second leading cause of preventable death in the United States and an increasing number of children across all races are becoming susceptible, it is critical further research on this area of nutrition in relation to weight loss be completed. Additional, or perhaps follow-up studies, should also be carried out to determine whether weight loss and decreases in TAG and leptin persist over longer periods of time (> 12 wk). Further follow-up studies should also be performed to determine if any negative health effects occur as a result of adhering to carbohydrate or fat restricted diets for extended periods of time. In principle, low carbohydrate diets are more effective at lowering TAG and countering obesity; however, future research is warranted to determine overall health benefits and implications.

# Chapter 6

## Literature Cited

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